When citing an abstract from the 2023 annual meeting, please use the format below.

[Authors]. [Abstract Title]. Program No. XXX.XX. 2023 Neuroscience Meeting Planner. Washington, D.C.: Society for Neuroscience, 2023. Online.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.01/A1

Topic: A.02. Postnatal Neurogenesis

Support: CIHR Grant PJT-173287
HBHL Innovative Ideas grant A57

Title: Characterizing neurogenesis in the adult human hippocampus with spatial transcriptomics

Authors: *S. SIMARD[1], R. RAHIMIAN[1], M. A. DAVOLI[1], S. THÉBERGE[1], N. MATOSIN[2], G. TURECKI[1,3], C. NAGY[1,3], N. MECHAWAR[1,3];
[1]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Mol. Horizons, Sch. of Chem. and Mol. Bioscience, Fac. of Sci., Univ. of Wollongong, Wollongong, Australia; [3]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: The neurogenic potential of the adult human dentate gyrus (DG) has been widely debated in recent years. This study aims to provide new insight on the extent of adult hippocampal neurogenesis (AHN) in the human brain at the transcriptomic level. Using the 10x Genomics Visium Spatial Gene Expression platform on frozen DG sections (Douglas-Bell Canada Brain Bank) from young (n=2, mean age=23.5 years old) and middle-aged neurotypical males (n=2, mean age=42.5 years old), we computationally examined the spatial mapping of various neurogenesis markers within the DG. We also assessed the simultaneous detection of markers specific to neural stem cells (NSC), proliferative cells and immature granule neurons in DG cells from infant (n=1, age=2 years old), adolescent (n=1, age=16 years old) and middle-aged male (n=6, mean age= 43.5 years old) post-mortem hippocampal samples using multiplexed fluorescent *in situ* hybridization (RNAscope; ACD Bio). Our Visium data reveals that neurogenesis markers can map to DG cells and regions outside of the subgranular zone (SGZ) of the DG, the hippocampal neurogenic niche, confirming the importance of using multiple markers to characterize different neurogenic cell types in the human hippocampus. For example, we observed that the NSC-specific marker *NES* is spatially resolved to cells in the DG and in regions enriched for oligodendrocyte precursor cell-specific markers. We also found that the proliferative markers *PCNA* and *MCM2* are very lowly expressed, and the immature neuronal marker *DCX* shows dispersed expression within the DG. Using RNAscope, we found very few cells expressing NSC-specific markers and proliferative cells but detected a stable average number of *DCX*-expressing cells in the SGZ from childhood to middle age. Across ages, the majority of DCX^+ DG cells expressed the inhibitory neuronal marker *GAD1* while the remainder displayed an excitatory phenotype ($SLC17A7^+$) or were non-committed. We also identified $PROX1^+DCX^+CALB2^+$ immature granule neurons in the adult DG. Additionally, *DCX* expression was detected in cells expressing glial markers, such as *TMEM119* and *ALDH1L1*, although rare, and in non-neurogenic brain regions. Our findings reveal that the human brain exhibits very low levels of AHN, due to the lack of NSCs and expression of proliferative markers from childhood

to middle age. However, the small population of *PROX1*$^{+}$*DCX*$^{+}$*CALB2*$^{+}$ immature granule neurons in the adult DG suggests the existence of a local reserve of plasticity for the adult human hippocampus during physiological aging and the identification of inhibitory *DCX*$^{+}$ DG cells across all ages confirms the presence of this subpopulation in the human hippocampus.

Disclosures: **S. Simard:** None. **R. Rahimian:** None. **M.A. Davoli:** None. **S. Théberge:** None. **N. Matosin:** None. **G. Turecki:** None. **C. Nagy:** None. **N. Mechawar:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.02/A2

Topic: A.02. Postnatal Neurogenesis

Support: HU21C0157

Title: The role of ErbB3 binding protein 1 in adult neurogenesis

Authors: *Y. KIM[1], J.-Y. AHN[2];
[1]Mol. Cell Biol., Sungkyunkwan Univ., Suwon, Korea, Republic of; [2]Mol. Cell Biol, Sungkyunkwan Univ. Sch. Med., Suwon, Gyeonggi-do, Korea, Republic of

Abstract: Adult neurogenesis generates new neurons in subventricular zone (SVZ) and subgranular zone (SGZ) throughout life followed by formation of neural plasticity. Aberrant hippocampal neurogenesis contributes cognitive deficits and memory loss in neurodegenerative diease. However, the molecular mechanism by which adult hippocampal neurogenesis decreases by aging is unclear. In this study, we demonstrated that *Ebp1*, which plays critical role in embryonic brain development, contributes to determination neural stem cell (NSC) fate. In the hippocampus of *Ebp1* conditional knock-out mice (*Ebp1*-CKO), in addition to the alterations of neurogenesis- and cell differentiation-related genes, NSC and neuroblast are decreased while astrocytes are increased. The proliferation and differentiation capabilities of neurospheres collected from *Ebp1*-CKO mice are attenuated compared with wild type mice. *Ebp1* depletion induces histone deacetylation disrupting proneural transcription factor ASCL1 and increases *Sox9*, which is expressed on astrocyte progenitor cells. Thus, our findings suggested that *Ebp1* is the key molecular factor to determine NSC fate in adult hippocampus and may provide a new possibility to maintain stem cell population and repress neuroinflammatory response.

Disclosures: **Y. Kim:** None. **J. Ahn:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.03/A3

Topic: A.02. Postnatal Neurogenesis

Support: FPU grant FPU21/02928
Spanish Ministry of Science and Innovation grant PID2020-119236RB-I00
Michael J Fox Foundation grant 000858
María de Maeztu Unit of Excellence grant MDM-2017-0729
María de Maeztu Unit of Excellence grant CEX2021-001159-M

Title: Does neuronal RTP801 modulate adult hippocampal neurogenesis in health and in Alzheimer's disease?

Authors: *P. GARCIA-SEGURA[1,2], G. CAMPOY-CAMPOS[1,2], J. SOLANA-BALAGUER[1,2], A. CHICOTE[1,2], L. PÉREZ-SISQUÉS[1,2,3], J. ALBERCH[1,2,4,5,6], A. GIRALT[1,2,4,5,6], C. MALAGELADA[1,2,4];
[1]Dept. of Biomedicine, Univ. of Barcelona, Barcelona, Spain; [2]Inst. of Neurosciences, Univ. of Barcelona, Barcelona, Spain; [3]King's Col. London, London, United Kingdom; [4]Ctr. de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain; [5]Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [6]Production and Validation Ctr. of Advanced Therapies (Creatio), Fac. of Med. and Hlth. Sci., Barcelona, Spain

Abstract: Introduction: Neurogenesis is the process of new neuron formation in the nervous system. This process is maintained throughout life in specific neurogenic niches. Among them, the dentate gyrus (DG) of the hippocampus is gaining attention given the role that adult hippocampal neurogenesis (AHN) has in cognition and memory. RTP801/REDD1 is a stress-induced protein that inhibits mTOR signaling pathway via the TSC1/TSC2 complex. In addition, RTP801 has been linked to embryonic neurogenesis since selective silencing of RTP801 in radial glia, by *in utero* electroporation, impairs migration and promotes premature differentiation of neural progenitors during cortex development. In addition, neuronal RTP801 regulates neuroinflammation in Alzheimer's disease (AD) and selective silencing of its expression recovers gliosis hallmarks and neuroinflammation in the 5xFAD murine model of AD. Interestingly, neuroinflammation is known to be a key regulator of AHN which dramatically halts the neurogenic process. Aim: To study the role of neuronal RTP801 in AHN in physiological conditions and in the 5xFAD mouse model of AD. Methods: In this study, 6-month-old male WT and 5xFAD mice (B6SJL-Tg(APPSwFlLon, PSEN1*M146L*L286V)6799Vas/Mmjax) were subjected to 1µL bilateral injections of rAAV2/8-H1-shControl-RSV-GFP (shCt) or rAAV2/8-H1shRTP801-RSV-GFP ((shRTP801) at CA1 (AP: −2.0; Lateral + /−1.5, and DV: −1.3) and DG (AP: −2.0; Lateral + /−1.5, and DV: −2.1) (mm). Behavioral tests were performed 4 weeks later and then tissue was prepared for immunofluorescence and biochemical analyses.Results: We found that silencing neuronal RTP801 improves cognition as assessed by behavioral tests. Immunofluorescence analyses of mice hippocampi revealed that RTP801 knockdown in neurons tends to decrease the number of Sox2+ cells in the subgranular zone (SGZ) of the DG. In this line, the number of mature NeuN+ neurons increases, independently of the genotype. Altogether

our results suggest that neuronal silencing of RTP801 increases the differentiation of neural stem cells (NSCs) of the SGZ to mature neurons of the granular cell layer, thereby increasing the number of neurons in both control and AD conditions. Conclusion: This new putative role of RTP801 paves the way for further studies aimed to unravel the significance of such process but already suggests an important role in migration and differentiation of NSCs in AHN.

Disclosures: **P. Garcia-Segura:** None. **G. Campoy-Campos:** None. **J. Solana-Balaguer:** None. **A. Chicote:** None. **L. Pérez-Sisqués:** None. **J. Alberch:** None. **A. Giralt:** None. **C. Malagelada:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.04/A4

Topic: A.02. Postnatal Neurogenesis

Support: NRF Grant RS-2023-00246151
NRF Grant 2021R1A2C1013180

Title: Vildagliptin promotes adult neurogenesis and dopaminergic neuron reinforcement through FNDC5 signaling pathway

Authors: ***Y. BANG**, S. LEE, H. CHOI;
CHA Univ., Seongnam-si, Korea, Republic of

Abstract: Emerging evidence suggests that impaired adult neurogenesis is a common feature in many neurodegenerative diseases. Irisin, a myokine derived from FNDC5, has been implicated in neurogenesis. Vildagliptin, a DPP-4 inhibitor, has shown neuroprotective effects in neurodegenerative disease models. In this study, we investigated the potential of vildagliptin in regulating adult neurogenesis through FNDC5 modulation. Oral administration of vildagliptin led to elevated numbers of neurogenesis markers, including doublecortin (DCX)-positive, and bromodeoxyuridine (BrdU)/DCX-double-labeled cells in the subgranular zone (SGZ) and ventricular-subventricular zone (V-SVZ). Additionally, we explored the impact of vildagliptin on dopaminergic neurons. Vildagliptin administration led to an increase in tyrosine hydroxylase mRNA levels, a marker for dopaminergic neurons, in the olfactory bulb and substantia nigra (SN). Overall, our study suggests that vildagliptin has the potential to enhance adult neurogenesis and may hold promise as a therapeutic approach for neurological disorders.

Disclosures: **Y. Bang:** None. **S. Lee:** None. **H. Choi:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.05/A5

Topic: A.02. Postnatal Neurogenesis

Title: Role of Eph/ephrins in the Regulation of Neuroblast Fate and Topographical Mapping

Authors: *D. YEROSHENKO[1], C. DE SILVA[1], S. BELLIZZI[2], I. LIVINGSTON[3], J. C. CONOVER[4];
[1]Physiol. & Neurobio., [2]Univ. of Connecticut Physiol. & Neurobio., [3]Univ. of Connecticut, Storrs, CT; [4]Univ. Connecticut, Univ. Connecticut, Storrs Manfld, CT

Abstract: We identify Eph-ephrin signaling as a mechanism that supports cell-cell physical interactions to direct migration and topographical mapping in the postnatal forebrain. In postnatal development, the forebrain rostral migratory stream (RMS) is a long-range pathway that consists of fasciculated chains of neuroblasts that migrate through a dense meshwork of astrocytes before dispersal and then integration within the olfactory bulb. However, our understanding of the molecular cues that coordinate this extensive migration and guide new neuron distribution has been limited.Receptor tyrosine kinases Ephs and their ephrin ligands are known for coordinating and directing cell migration through direct cell-cell contact. They are abundantly expressed at the ventricular-subventricular zone (birthplace of migratory neuroblasts), RMS (migratory pathway), and the olfactory bulb (final destination), making them candidates for regulating neuroblast migration and integration. Previously, our group found that EphA4 is a critical player in RMS organization, as EphA4$^{-/-}$ mice show disorganization of the astrocyte meshwork, loss of neuroblast fasciculation, and aberrant neuroblast migration, as a result neuroblasts deviate from the tight confines of the RMS. Immunohistochemistry and single-cell analyses also revealed unique neuroblast and astrocyte subpopulations based on EphA4 and ephrin expression patterns.Here, we address the hypothesis that Ephs/ephrins guide the migratory neuroblasts to their final destination within the olfactory bulb. To confirm that Ephs/ephrins are actively signaling in the neuroblasts, we analyze their protein distribution patterns within the olfactory bulb, including the specific localization of activated (phosphorylated) receptors and ligands. Additionally, we use single-cell expression transcriptomics and proteomics to highlight differential Eph/ephrin expression across different immature inhibitory interneuron subpopulations. In summary, we propose that the differential co-expression of specific Ephs/ephrins in migratory neuroblast subpopulations acts as guidance cues during forebrain migration and olfactory bulb distribution.

Disclosures: D. Yeroshenko: None. **C. de Silva:** None. **S. Bellizzi:** None. **I. Livingston:** None. **J.C. Conover:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.06/A6

Topic: A.02. Postnatal Neurogenesis

Support: CIHR PJT-186046

Title: Integration and decoding of niche signals by adult neural stem cells

Authors: *A. MARYMONCHYK[1], A. WILLIS[2], V. CLAVET-FOURNIER[1], D. JEONG[3], F. LAVOIE-CARDINAL[1], D. KAPLAN[4,3], F. MILLER[2,3,4], A. SAGHATELYAN[1,5];
[1]Univ. Laval, Quebec, QC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Dept. of Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [4]Hosp. Sick Children, Toronto, ON, Canada; [5]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: The adult brain has a remarkable capacity to produce new cells that migrate and integrate into pre-existing neuronal circuits throughout the lifespan of animals. The largest population of adult neural stem cells (NSCs) is located in the subventricular zone (SVZ). They are largely quiescent and their activation is modulated by a number of SVZ niche factors. Calcium (Ca2+) is known to integrate such signals resulting in distinct Ca2+ frequency and amplitude in the cell body of activated and quiescent NSCs. However, NSCs contact most of niche elements via their processes where most of the Ca2+ events occur. The role of Ca2+ activity in NSC processes is unknown. By combining sparse NSCs labeling approach, 2photon ex-vivo Ca2+ imaging and post-hoc immunolabeling for multiple niche elements, we characterized the spatiotemporal dynamics of Ca2+ signals in NSCs processes with event-based analysis tool (AQuA). We found heterogeneous Ca2+ activity patterns in NSCs processes and specialized high activity “hot-spots” where Ca2+ events repeatably occurs. To determine close to which cellular element in the SVZ niche these Ca2+ hot-spots occur, we performed multiple rounds of post-hoc immunolabeling to depict localization of blood vessels, EGFR+ or Ki67+ progenitors, neuroblasts, GFAP+ cells and microglia. By using spatial statistics, we showed that NSCs display Ca2+ signals near rapidly proliferating transit-amplifying precursors (TAPs) that are the direct progeny of NSCs. Using super-resolution microscopy (STED), we revealed that NSCs processes are in tight contact with and in some cases bifurcates to wrap around dividing TAP. Using scRNA-sequencing and cell surface proteome analysis, we generated a communication network model of ligands expressed by TAPs and corresponding receptors expressed by NSCs. This analysis identified EphrinB1 and its receptor EphB2 as a potential pathway of communication between TAP and NSCs. We next used pharmacological approach (Ephrin B1-Fc) and optogenetic stimulation of NSCs electroporated with opto-EphB2 receptor to show that modulation of Efnb1-EphB2 pathway increases Ca2+ frequency in NSC processes. Altogether, our data suggest that NSCs exhibit heterogeneous Ca2+ events in their processes triggered by various niche elements. Furthermore, our data indicate that NSCs receive a constant feedback input from rapidly dividing progeny through Efnb-EphB pathway that maintain a high Ca2+ frequency in NSCs, a hallmark of the quiescent state.

Disclosures: A. Marymonchyk: None. **A. Willis:** None. **V. Clavet-Fournier:** None. **D. Jeong:** None. **F. Lavoie-Cardinal:** None. **D. Kaplan:** None. **F. Miller:** None. **A. Saghatelyan:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.07/A7

Topic: A.02. Postnatal Neurogenesis

Title: The Role of Interleukin 1 Receptor 1 in Adult Hippocampal Neurogenesis: Insights from Voluntary Running Paradigm in IL-1R1 Knockout Mice

Authors: *C. MORAIS LOSS, C. ALVAREZ, M. SMIRNOVA, D. NEMETH, S. MCGOVERN, N. QUAN, H. VAN PRAAG;
Stiles-Nicholson Brain Inst., Florida Atlantic Univ., Jupiter, FL

Abstract: Inflammatory related processes (such as stress, aging, infections) disrupt adult hippocampal neurogenesis, a process important for regulation of memory and mood. The role of the pro-inflammatory cytokine Interleukin 1 (IL-1) in adult neurogenesis is unclear. In the present exploratory study the function of this cytokine was examined under basal or under voluntary wheel running condition (a strong pro-neurogenic stimulus). Specifically, we have begun to investigate if the suppression of IL-1 signaling (through the ablation of IL-1 receptor, IL-1R1) affects adult neurogenesis and behavior. Young adult female mice under the C57Bl/J6 background were allocated to either control sedentary (SED) or running (RUN) conditions through a Randomized Block Design considering Genotype (IL-1R1 knockout - KO; and WT-like control - WT) and litters as factors. Mice were injected with bromodeoxyuridine (BrdU; 50 mg/5 ml/kg) to label dividing cells. After one month in their respective housing conditions mice underwent behavioral tests (Y-Maze and Activity Box). Upon completion of testing mice were anesthetized and perfused transcardially with 4% paraformaldehyde prior to dissection of brain tissue. To minimize experimental bias, computer generated randomization was applied for the allocation of mice to the groups (SED or RUN) and to set the order in which the mice were subjected to any of the procedures in the experiment (from housing location up to perfusion order). The cages were coded to assure experimenters were blinded for the Genotype during the whole experiment. Wheel running distance was monitored continuously using a Tecniplast DVC rack system. Our preliminary data indicate that IL-1R1 KO mice have reduced activity levels. BrdU+ cell numbers were similar under sedentary conditions for IL-1R1 KO and WT mice suggesting that the IL-1 receptor does not regulate adult cell genesis under basal conditions. Under running conditions the IL-1R1 KO displayed reduced BrdU labeling than WT mice. It remains unclear whether blunted cell genesis in the IL-1R1 KO mice is a result of reduced activity or due to the loss of the IL-1R1. Research is in progress to address this question.

Disclosures: C. Morais Loss: None. **C. Alvarez:** None. **M. Smirnova:** None. **D. Nemeth:** None. **S. McGovern:** None. **N. Quan:** None. **H. van Praag:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.08/A8

Topic: A.02. Postnatal Neurogenesis

Title: Gpr161 controls adult neural stem cell proliferation and neurogenesis through the retinoic acid signaling pathway

Authors: *T. KASZA[1], M. ABABON[1], P. G. MATTESON[2], J. H. MILLONIG[3];
[1]Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ; [3]Neurosci. and Cell Biol., [2]Rutgers Univ., Piscataway, NJ

Abstract: Adult neurogenesis is the process by which new neurons are generated and integrate into existing neural networks. Adult neurogenesis requires the presence of adult neural stem cells (aNSCs) which occupy specific neurogenic regions in the adult mammalian brain. We have discovered a role for Gpr161, an orphan GPCR in adult neurogenesis. Gpr161 is expressed in neurogenic zones in the adult mouse including the sub-granular zone (SGZ) of the hippocampus. To investigate the role of Gpr161 in aNSC mediated molecular signaling pathways we knocked down and overexpressed Gpr161 in hippocampal derived neurospheres in-vitro. Knockdown and overexpression of Gpr161 decreases and increases neurosphere size and self-renewal potential respectively. Knockdown and overexpression of Gpr161 results in a decrease and increase in the proliferation rate of aNSCs. Knockdown of Gpr161 decreases survivability while overexpression had no effect. Gpr161 signaling is linked to the retinoic acid (RA) signaling pathway through the phosphorylation of the retinoic acid receptor α (RARα) which leads to expression of genes involved in RA signaling. Overexpression of Gpr161 increases the cAMP and RA production in neurospheres while knockdown increases cAMP but has no significant effect on RA production. This effect is independent of Gpr161's role in the Shh signaling pathway. Overexpression of Gpr161 in the SGZ increases new neuron production in-vivo. Further exploration of Gpr161's molecular signaling pathways will elucidate the RA mediated signaling pathway responsible for the effects of Gpr161 on aNSCs.

Disclosures: T. Kasza: None. **M. Ababon:** None. **P.G. Matteson:** None. **J.H. Millonig:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.09/A9

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01 NS116914-03

Title: Activation of interleukin 1 receptor type 1 signaling promotes neural stem cell proliferation in the adult dentate gyrus in a dosage-dependent and cell type-specific manner

Authors: *M. I. SMIRNOVA[1,2], N. KOCAK[1,2,4], D. P. NEMETH[2], H. VAN PRAAG[2,3,4], N. QUAN[3,4,2];
[1]Charles E. Schmidt Col. of Sci., [3]Charles E. Schmidt Col. of Med., [2]FAU Stiles-Nicholson Brain Inst., Jupiter, FL; [4]Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: Adult neurogenesis is the process by which neural stem cells (NSC) proliferate and differentiate to produce new mature neurons in the mammalian brain. Specifically, adult neurogenesis in the granule cell layer of the dentate gyrus (DG) of the hippocampus functions in learning and the formation of new memories. Loss of adult DG neurogenesis is implicated in the progression of neurodegenerative disorders, thus understanding the mechanisms that modulate adult DG neurogenesis can open new avenues for the design of targeted therapeutics. Interleukin-1 (IL-1) is a proinflammatory cytokine and a master regulator of neuroinflammation. Most studies suggest that IL-1 signaling via its receptor interleukin 1 receptor type 1 (IL-1R1) decreases adult neurogenesis in the DG; however, the exact mechanisms are unknown. Conversely, other studies provide evidence for a pro-neurogenic effect of IL-1 signaling. Our lab has developed a unique transgenic mouse model where IL-1R1 can be expressed in certain cell types under control of cell type-specific promoters, allowing for the identification of the cell type(s) that facilitate IL-1 signaling. We have genetic lines with IL-1R1 expression in neurons, endothelial cells, myeloid cells, and astrocytes. Ten-week-old wildtype female mice were injected with various dosages of a viral vector containing IL-1B unilaterally. Phosphate buffered saline (PBS) was injected into the other DG as a control. A week later, the mice were injected intraperitoneally with 5-Ethynyl-2'-deoxyuridine (EdU), a proliferation marker, and perfusion-fixed four hours later. EdU was labeled using ClickIT Cell Proliferation Kit and the DG sections were imaged on a confocal microscope. Our preliminary results indicate that at low doses of IL-1B (5 x10^5 PFU/uL), the number of proliferating NSCs in the DG increases, possibly suggesting increased neurogenesis. At higher doses of IL-1B (8.39 x10^7 PFU/uL), there is a suppression of neurogenesis and induction of neuroinflammation. A ten week-old female mouse expressing IL-1R1 only in astrocytes (Aldh1CreER-Il1r1 r/r) was injected with 5 x10^5 PFU/uL dosage of IL-1B unilaterally. The number of proliferating NSC on the IL-1B injection side was twice as much as on the PBS injection side, supporting that astrocytes may have a critical role in promoting adult neurogenesis. Altogether, our preliminary data suggest that (1) low dose IL-1 is important for NSC proliferation while (2) excess amount of IL-1 can cause suppression of neurogenesis and induction of inflammation. Additionally, (3) astrocytic IL-1R1 promotes NSC proliferation.

Disclosures: M.I. Smirnova: None. **N. Kocak:** None. **D.P. Nemeth:** None. **H. van Praag:** None. **N. Quan:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.10/A10

Topic: A.02. Postnatal Neurogenesis

Support: GR123141

Title: Glutamate transported through Excitatory Amino Acid Transporter 1 induces adult hippocampal neural stem cell self-renewal by altering metabolism and intracellular calcium

Authors: *I. ROSADO-BURGOS[1,2], J. D. RIESKAMP[1,2], E. KIRBY[2,3,4];
[1]The Ohio State Univ. Neurosci. Grad. Program, Columbus, OH; [2]Dept. of Psychology, [3]Dept. of Neurosci., [4]Chronic Brain Injury Program, The Ohio State Univ., Columbus, OH

Abstract: Neural stem cells (NSCs) are responsible for the generation of new neurons in several select niches throughout adulthood in a wide variety of mammals. Neurogenesis in one of these niches, the mammalian hippocampus, has potential behavioral and clinical relevance through support of memory function and emotional regulation. To preserve these hippocampal functions, NSCs must balance their sustained maintenance with proliferation over time to prevent NSC and neurogenesis depletion. Our lab recently found that glutamate, an abundant neurotransmitter in the hippocampus and known inducer of NSC proliferation, requires transport into NSCs via Excitatory Amino-Acid Transporter 1 (EAAT1) to induce NSC self-renewal in the adult mouse hippocampus. This receptor-independent action of glutamate was unexpected and the mechanism by which glutamate transport acts as a signaling event remained unclear, though we found lipogenesis is essential for this process. Here, we aimed to understand how EAAT1 activity induces adult hippocampal NSC self-renewing proliferation and lipogenesis. EAAT1 activity includes the co-transport of glutamate and ions into the cell. This ionic flux provides net depolarization, which can increase intracellular calcium signaling. Transported glutamate can be used independent of ionic influx as a substrate in metabolism, such as via its conversion to tricarboxylic acid cycle (TCA) intermediates by glutamate dehydrogenase 1 (GluD1). We treated adult hippocampal NSCs in-vitro with 0 or 100uM of glutamate along with GluD1 inhibitor epigallocatechin gallate (EGCG) and/or endoplasmic reticulum-expressed RyR calcium channel antagonist, dantrolene. We found that neither EGCG nor dantrolene treatment alone blocked glutamate-induced NSC proliferation, but a combination of the two did. These findings imply that both the intracellular calcium release and glutamate metabolism by GluD1 are needed for NSCs to proliferate in response to glutamate. Ongoing research aims to understand which intracellular mechanisms are downstream of EAAT1-mediated ionic influx or glutamate influx and whether these are necessary or sufficient on their own or in combination to increase lipogenesis and NSC proliferation. Answering these questions will advance our understanding of how transported glutamate induces cell-autonomous self-renewing proliferation, which could bring us closer to understanding NSC behavior in disease and provide a target for maintaining neurogenesis throughout life.

Disclosures: I. Rosado-Burgos: None. **J.D. Rieskamp:** None. **E. Kirby:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.11/A11

Topic: A.02. Postnatal Neurogenesis

Support: NIH NIAAA 1R01AA027462-01A1
NIGMS COBRE P20GM109089
NIH NIAA 5T32AA014127

Title: The neurogenic response to enriched environment is impaired in ventral hippocampus in a mouse model of prenatal alcohol exposure

Authors: *A. RODRIGUEZ, L. CUNNINGHAM, L. LI;
Univ. of New Mexico Med. School, Dept. of Neurosciences, Albuquerque, NM

Abstract: Fetal alcohol spectrum disorder (FASD) is a leading cause of preventable intellectual disability and neural developmental disorders. Depression and anxiety are the most common mental illnesses in people with FASD. These disorders are related to ventral hippocampal function. The hippocampus is a unique structure that is capable of producing neurons during adulthood. This ability is stimulated by an enriched environment (EE). Additionally, part of the therapeutic effect of common antidepressants is increased neurogenesis. Prior research conducted by our lab has shown that prenatal alcohol exposure (PAE) impairs EE-mediated neurogenesis in the dorsal hippocampus (Gustus et al., 2020). However, the impact of PAE on neurogenesis in the ventral hippocampus has not been studied. Here, we tested the hypothesis that EE-mediated neurogenesis is impaired in the ventral hippocampus in PAE mice. Nestin-CreER^{T2}:tdTomato mice were used to label adult-generated hippocampal dentate gyrus cells (DGC) after PAE and EE. Moderate PAE was accomplished using a well-characterized limited access drinking in the dark paradigm (Brady, Allan, & Caldwell, 2012) in which female mice were offered 10% EtOH in 0.066% saccharin for 4 hr beginning 2hrs into awake cycle, throughout pregnancy (estimated BECs 80-90 mg/dl; Gustus et al., 2020). Mice offered saccharin alone served as controls (SAC). Offspring were gender segregated at birth, administered tamoxifen (180 mg/kg daily for 5 days) 2 weeks post-weaning and reared in standard housing (SH) or EE living conditions for 6 weeks until sacrifice. Data for males only is reported here (female analysis is underway). We found that PAE had no significant (Tukey Post-Hoc, p=0.97) impact on neurogenesis (+tdTom DGCs) under SH conditions (SAC-SH: M=93.0, SD = 56.91, N=5; PAE-SH: M=81.75, SD = 33.45, N=4), but significantly impaired (p= 0.01) the neurogenic response to 6 weeks of EE (SAC-EE: M=173.9, SD = 21.44, N=5; PAE-EE: M=76.55, SD = 24.77, N=4). As ventral and dorsal EE-mediated neurogenesis is impaired in PAE mice, we are currently investigating whether treatment with the antidepressant, fluoxetine, will reinstate this neurogenesis.

Disclosures: A. Rodriguez: None. **L. Cunningham:** None. **L. Li:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.12/A12

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant NS086965
NIH Grant NS085171

Title: Ablation of Follistatin mimics the biphasic changes in hippocampal neurogenesis observed after recurrent seizures

Authors: *Y. FURUTA, M. AL FAISAL, C.-H. FU, W. YU, J. CHIN;
Baylor Col. of Med., Houston, TX

Abstract: Adult neurogenesis is associated with cognition and mood, both of which are altered in conditions with recurrent seizures, such as Alzheimer's disease (AD) and epilepsy. Using transgenic mice that express mutant human amyloid precursor protein (APP mice), we previously showed that spontaneous seizure activity in APP mice drives aberrant neural stem cell (NSC) division and neurogenesis, and accelerates the depletion of a finite pool of NSCs in the dentate gyrus (DG), resulting in chronic reductions in neurogenesis. Such alterations in neurogenesis may contribute to cognitive decline in AD, highlighting the importance of studying the mechanisms that regulate neurogenesis in a seizure-dependent manner. To this end, we used RNA-sequencing to identify regulators of neurogenesis that are differentially expressed in the DG of APP mice compared to nontransgenic (NTG) littermates. One of the molecules of interest that we identified was Follistatin (FST), a secreted protein that antagonizes TGF-β superfamily proteins, which was markedly downregulated in APP mice. We found that FST expression in APP mice was restored after treatment with an anti-seizure drug, and that FST expression is suppressed in a pharmacological model of epilepsy, indicating that seizures are necessary and sufficient to drive changes in FST expression. To examine whether a reduction in FST expression is sufficient to drive downstream alterations in neurogenesis such as those observed after recurrent seizures, we used FST conditional knockout (KO) mice in which ablation of FST is under control of Camk2a-CRE. We first validated that FST expression is absent in the DG of FST KO mice. We also verified that ablation of FST did not induce seizures, as indicated by the lack of abnormal expression of the seizure-induced transcription factor ΔFosB. Finally, we demonstrated that compared to their wild-type littermates, FST KO mice exhibited an increase in neurogenesis at 1 month of age that returned to baseline by 3 months of age. These findings suggest that a reduction of FST expression is sufficient to induce aberrant neurogenesis dynamics, and may play a critical role in the biphasic modulation of neurogenesis in conditions with recurrent seizures.

Disclosures: **Y. Furuta:** None. **M. Al Faisal:** None. **C. Fu:** None. **W. Yu:** None. **J. Chin:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.13/A13

Topic: A.02. Postnatal Neurogenesis

Support: NIH/NINDS R01 N5124775

Title: Diffusion characteristics of tagged vascular endothelial growth factor in mouse dentate gyrus.

Authors: L. MILLER, A. WALTERS, *E. KIRBY;
Ohio State Univ., Columbus, OH

Abstract: Neural stem cells (NSCs) have the potential to support hippocampal plasticity and learning and memory in both healthy conditions and with disease/injury. One mechanism by which they can provide this support is via secretion of soluble growth factors. Our lab has shown that endogenous NSCs in the adult mammalian hippocampus express and secrete the pleiotropic growth factor vascular endothelial growth factor (VEGF). We have also found that endogenous NSC VEGF suppresses neuronal hyperexcitability in the dentate gyrus (DG) of the hippocampus (where NSCs reside) and supports memory function. However, it is unclear why NSC-derived VEGF has such functional importance when VEGF is also secreted by astrocytes, which are more numerous and make more VEGF than NSCs. Given that NSCs and astrocytes exist in somewhat separate layers of the DG, the diffusion properties of soluble VEGF could dictate the functional effect of VEGF derived from the different cell types. Little is known about the half-life or diffusion characteristics of VEGF within the hippocampus. To identify and investigate VEGF diffusion, we used genetic code expansion coupled with bioorthogonal non-canonical amino acid tagging to visualize VEGF diffusion in live hippocampal tissue. We performed unilateral injections of bioorthogonally tagged VEGF (either VEGF120 or VEGF164) or vehicle into DG of both male and female mice. Animals were then perfused at 10 min, 1 h, 3 h, or 6 h post-injection. Click-reacted tagged VEGF was detected with infrared imaging and diffusion away from the infusion point was quantified over time and space. Ultimately, we observed no significant difference in diffusion distance between sexes or VEGF isoform. VEGF spread peaked at 1-3 h post-injection, and there was no significant spread to the hemisphere contralateral to the infusion site. These data also suggest an unexpectedly wide radius of VEGF diffusion, suggesting that VEGF from NSCs could reach quite far within the DG. These findings provide insight on how VEGF behaves *in vivo* in DG, improving understand of how NSC-derived VEGF may influence hippocampal function. Understanding soluble factor diffusion away from cellular sources is also informative for regenerative medicine approaches that rely on paracrine signaling.

Disclosures: L. Miller: None. **A. Walters:** None. **E. Kirby:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.14/A14

Topic: A.02. Postnatal Neurogenesis

Support: NIH R21NS123797 to EDK

Title: Bioorthogonal noncanonical amino acid tagging allows for visualization of the cellular location of excitatory amino acid transporter 1 expressed in individual cells

Authors: *N. DEVASTHALI[1], E. D. KIRBY[2,3];
[1]Neurosci. Grad. Program, [2]Dept. of Psychology, [3]Chronic Brain Injury Program, The Ohio State Univ., Columbus, OH

Abstract: The subgranular zone of the hippocampus is one of the few regions of the adult mammalian brain where neural stem cells (NSCs) exist and can proliferate to yield functional neurons throughout life. These newly-born neurons integrate into existing hippocampal circuitry to support learning, memory and affect regulation. Our lab has previously shown that the neurotransmitter glutamate drives self-renewing proliferation of adult NSCs via its transporter, excitatory amino acid transporter 1 (EAAT1). However, it is not known where in NSCs EAAT1 protein is located. NSCs have a complex morphology with the cell body in the subgranular zone plus a radial process that extends through the granule cell layer to the inner molecular layer. The location of the EAAT1 protein in an NSC will determine which of several sources of glutamate could act most potently on NSC-expressed EAAT1. Unfortunately, the widespread expression of EAAT1 in astrocytic processes makes traditional immunolabelling approaches unable to resolve EAAT1 expressed by NSCs versus the EAAT1 in closely apposed astrocytic processes. To resolve this, we adapted a bioorthogonal noncanonical amino acid tagging system in combination with genetic code expansion and amber stop codon suppression to visualize the cellular location of EAAT1 expressed in individual cells. We designed a plasmid with an in-frame amber stop codon (TAG) inserted into the coding sequence for EAAT1 ($EAAT1^{TAG}$), as well as a T2A-linked mCherry reporter. We transiently expressed this plasmid in astrocytic C8D1A cells along with a bacteria-derived pyrrolysyl/tRNApyl pair which suppresses the amber stop codon by catalyzing incorporation of the noncanonical amino acid trans-Cyclooct-2-en - L - Lysine (TCO*A) into TAG sites. TCO*A tagged proteins were then identified by click reacting with tetrazine bound to biotin (tet-biotin). Western blotting of cell lysates revealed TCO*A incorporation (reacted with a streptavidin IR dye) in EAAT1 protein at the anticipated molecular weight only in cells treated with TCO*A and both plasmids. Similarly, in fixed cells, mCherry+ cells but not mCherry- cells treated with TCO*A and both plasmids showed robust tagging signal compared to cells that did not receive TCO*A. These findings reveal that expression of our constructs in live cells led to incorporation of TCO*A in EAAT1, that this tagged EAAT1 is present only in cells expressing mCherry, and that the tagged EAAT1 can be visualized *in situ* and in lysates by click reacting with tet-biotin. Ongoing work seeks to apply this system *in vivo* to sparsely express $EAAT1^{TAG}$ and visualize the tagged protein in mCherry-expressing NSCs.

Disclosures: N. Devasthali: None. **E.D. Kirby:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.15/A15

Topic: A.02. Postnatal Neurogenesis

Support: NIH R01NS124775 to EDK

Title: Hippocampal neural stem cell proximity to vasculature emerges during postnatal development in mice

Authors: *I. S. CARTER, N. DEVASTHALI, A. I. SAULSBERY, E. D. KIRBY; Psychology, The Ohio State Univ., Columbus, OH

Abstract: Adult neurogenesis, the process of creating new neurons throughout life, occurs in two main areas of the mammalian brain; the subventricular zone and the dentate gyrus (DG) of the hippocampus. The surrounding vasculature within each of these niches is an important source of support for adult neurogenic processes. Within the DG, the vasculature is especially dense and neural stem cells (NSCs) and their immediate progeny exist in especially close proximity to local blood vessels in adulthood. This unique arrangement is hypothesized to support adult neurogenesis in several ways, such as by providing scaffolding for progenitors and neuroblasts to migrate tangentially through the DG, as well as by providing NSCs access to circulating support molecules such as growth factors. Though the proximity of the adult NSCs to vessels is well established, little is known about how it develops. To characterize the development of NSC proximity to blood vessels, we quantified the distance from radial glia-like NSC bodies to the nearest blood vessel in mice between 2 and 9 weeks of age, a time period covering from early formation of the major DG cell layers to adulthood. We identified NSCs and endothelial cells in wildtype mice perfused at 2, 3, 5 and 9 weeks of age using immunofluorescent phenotypic markers in fixed tissue slices. We found that from 2 weeks to 9 weeks of age, there was a progressive reduction in the distance between NSC bodies and the nearest blood vessel. These findings suggest that the association of RGL NSCs with vasculature is not a preserved feature from early development, but rather one that arises de novo during postnatal maturation. They further imply that the development of the RGL NSC neurogenic vascular niche is not complete until adulthood. Further characterization of the development of the neural stem cell niche and surrounding vasculature will provide insight into the unique mechanisms that ensure preservation of neurogenesis in the adult DG.

Disclosures: I.S. Carter: None. **N. Devasthali:** None. **A.I. Saulsbery:** None. **E.D. Kirby:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.16/A16

Topic: A.02. Postnatal Neurogenesis

Support: PSC-CUNY Research Award TRADA-50-540

Title: Soma size of newborn hippocampal neurons corresponds to behavior in the aging mouse

Authors: S. CASTONGUAY[1], A. HERNANDEZ NUNEZ[2], R. INIRIO[2], T. BHUIYAN[2], F. GNAZZO[2], *C. PYTTE[2];
[1]Boston Univ., Boston, MA; [2]Queens Col. CUNY, Flushing, NY

Abstract: Aging is associated with characteristic changes in neural substrates and corresponding cognitive decline. A comprehensive catalog of age-related changes in neural substrates is critical to identifying therapeutic targets for delaying or mitigating age-related cognitive decline in both the healthy and diseased aging brain. However, our understanding of features and mechanisms of the aging brain is still incomplete. In the rodent hippocampus, hippocampal volume, total cell number, granule cell size and granule cell dendritic spine density exhibit steep declines between young adulthood and old age, which have been linked to changes in cell firing properties and functional deficits in hippocampal-dependent learning and memory. Aging is also associated with declining neurogenesis in the hippocampal dentate gyrus and slowed dendritic maturation in newborn neurons. These neurogenic impairments have also been linked to changes in hippocampal-mediated behaviors, including increased anxiety-like behavior and declining performance in learning and memory tasks. Because new granule cells are added to the dentate gyrus over the lifetime of the animal, it is important to determine whether age-related changes in cellular structure and properties are due to the age of the animal or the age of the cell. For instance, it is established that granule neurons in the mouse hippocampus become smaller as the animal ages. It has been assumed that this is due to the progressive shrinking of mature neurons as the cells themselves age, perhaps due to diminishing inputs associated with dendritic degeneration, physiological deterioration, and/or accumulation of structural damage leading to cellular senescence. However, here we demonstrate that new granule neurons in older brains are in fact born smaller. We labeled newborn neurons in the granule cell layer of the hippocampus using immunohistochemistry for doublecortin (DCX) in male and female C57/Bl6 mice aged 98-621 days. DCX is expressed in neuronal-committed progenitor cells and persists until neurons are about 30-40 days of age. We found that DCX+ soma size decreased with increasing age of the animal in the same cell-age cohorts (0~40 days). Further, new neuron soma size was positively correlated with short-term spatial memory performance in the Y-maze, and inversely correlated with measures of anxiety-like behavior in the elevated plus maze, independent of numbers of new neurons, with mouse age held constant. These findings identify a novel feature of neurogenesis that may contribute to age-related deficits in cognition and emotional processing.

Disclosures: S. Castonguay: None. **A. Hernandez Nunez:** None. **R. Inirio:** None. **T. Bhuiyan:** None. **F. Gnazzo:** None. **C. Pytte:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.17/A17

Topic: A.02. Postnatal Neurogenesis

Title: Testing the environmental sculpting hypothesis of postnatal hippocampal neurogenesis

Authors: *G. MODARA[1], I. SCHWEIN[2], M. S. MADHAV[1], J. S. SNYDER[2];
[1]Sch. of Biomed. Engin., [2]Dept. of Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Neurogenesis in the dentate gyrus generates new granule cells (GCs) that gradually integrate into the hippocampal network and are required for hippocampal functions such as learning, memory and pattern separation. Typically, hippocampal neurogenesis peaks around birth and then declines dramatically with age. During their development, immature GCs undergo a critical period characterized by high experience-dependent plasticity. According to the environmental sculpting hypothesis, GCs in this highly plastic state become attuned to features present in their environment, enabling enhanced learning of situations containing those features later in adulthood. To test this hypothesis, we will use home cage LCD screens to passively expose adolescent mice to one of two simple visual stimuli patterns (A or B). In adult mice, we will then assess their ability to distinguish between complex stimuli that are composed of combinations of the previously experienced elementary stimuli. We anticipate that mice exposed to simple stimuli A during adolescence will exhibit better discrimination of the complex version of stimuli A, compared to discrimination of complex stimuli B. Additionally, to investigate whether experience-dependent activity in developing GCs is essential for learning in adulthood, we will use transgenic AsclCreER - Hm4di mice to chemogenetically inhibit newborn neurons during stimuli exposure in adolescence. We expect that neuronal inhibition will prevent cells from becoming tuned to the environmental stimuli, thereby eliminating any experience-dependent improvements in discrimination in adulthood. In another condition, neurons will be allowed to become tuned during passive exposure to simple stimuli in adolescence, but they will be silenced when mice are trained to discriminate the complex stimuli. We predict that the activation of tuned neurons during the discrimination task is necessary, and we anticipate the elimination of any experience-dependent improvements in discrimination when these neurons are silenced. Through this work, we seek to establish a behavioral paradigm for environmental sculpting using visual cues, and establish a lifelong functional role for the large number of immature GCs present as a juvenile first experiences their environment.

Disclosures: G. Modara: None. **I. Schwein:** None. **M.S. Madhav:** None. **J.S. Snyder:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.18/A18

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant ZIAMH002784

Title: Investigating the role of adult-born granule cells in the dorsal versus ventral hippocampus

Authors: *N. FREEDGOOD, H. CAMERON;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: The dentate gyrus (DG) of the hippocampus is one of the few regions in the mammalian brain known to produce new neurons throughout adulthood. These new neurons contribute to various hippocampal-related processes, including difficult discriminations, cognitive flexibility, attention, motivation, and stress response. Although the hippocampus maintains the same intrinsic circuitry throughout its longitudinal axis, the dorsal and ventral regions are proposed to have different functions. The dorsal hippocampus is thought to play a crucial role in spatial navigation and memory, whereas the ventral hippocampus is thought to be involved in anxiety-like behaviors. Therefore, it is expected that the neurogenesis that occurs at each pole of the hippocampus would also be implicated in these different behaviors. However, few studies have tested these ideas, because it is difficult to specifically target adult-born neurons in a spatially specific manner in mice with commonly-used methods. We are using two different methods to target new neurons in the dorsal and ventral hippocampus of rats. First, we used an opsin-expressing retrovirus that is specifically incorporated into dividing cells and can be injected into either the dorsal or ventral DG to optogenetically inhibit new neurons selectively in a portion of the DG. In addition, we are using radioactive ablation to ablate new neurons in a spatially-specific manner. We are using immunohistochemistry for immediate-early genes and doublecortin to assess the specificity of inhibition and ablation, respectively, and investigating the functional contributions of the adult-born neurons in each region in various behavioral tasks. This study contributes to the understanding of the differences in functional roles across the longitudinal axis of the hippocampus.

Disclosures: N. Freedgood: None. **H. Cameron:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.19/A19

Topic: H.08. Learning and Memory

Support: National Institute for Translational Neuroscience (INNT/Brazil) (465346/2014-6)
Canadian Institutes of Health Research (CIHR; 202104PJT-461851-NSB-CEDA-217167)
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 467546/2014-2)

Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; 202.944/2015)
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; 467546/2014-2)

Title: Reactivation of leptin receptors restores brain volume, neurogenesis and memory impairment caused by the absence of leptin signaling in early life

Authors: *C. FERNANDES DA SILVA[1], L. FORNY-GERMANO[2], M. M. DE ANDRADE[2], A. M. RAMOS-LOBO[3], F. TOVAR-MOLL[4], J. HOUZEL[2], J. DONATO, JR[5], F. DE FELICE[6]; [1]Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil; [2]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [3]Sao Paulo Univ., São Paulo, Brazil; [4]D'Or Inst. for Res. and Educ., Rio de Janeiro, Brazil; [5]Dept. of Physiol. and Biophysics, Univ. De Sao Paulo, Sao Paulo, Brazil; [6]Queen's Univ., Kingston, ON, Canada

Abstract: Defects in adipocyte hormone leptin signaling affect energetic homeostasis and are associated with obesity, a condition increasingly linked to cognitive decline and a higher risk of dementia in aging. Evidence shows the ability of leptin to improve metabolic function in leptin-deficient mice and humans. However, it remains to be demonstrated whether restoring leptin signaling may alleviate the deleterious consequences of obesity on the brain. Here, we first assessed the effects of impaired leptin production on brain morphology, neurogenesis and cognition in Lep^{Ob} mice, which carry a mutation in the leptin gene. Next, we investigated the importance of leptin signaling during development, by analyzing the same parameters in LepRNull mice, which do not express leptin receptor and are hyperphagic and morbidly obese. Compared to wild-type mice, LepRNull display reduced brain volume, decreased neurogenesis and impaired cognition. When LepR expression was restored in adult LepRNull mice, brain atrophy, neurogenesis reduction and impairment of cognition were restored. Our findings reveal the importance of stimulating leptin signaling early in life to protect the brain from obesity.

Disclosures: C. Fernandes Da Silva: None. **L. Forny-Germano:** None. **M.M. de Andrade:** None. **A.M. Ramos-Lobo:** None. **F. Tovar-Moll:** None. **J. Houzel:** None. **J. Donato:** None. **F. De Felice:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.20/A20

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI Grant Number: 17H04754

Title: Exercise type influences the promoting effect of exercise on hippocampal neurogenesis in mice

Authors: *R. TSUCHIDA[1], T. YAMAGUCHI[1], D. FUNABASHI[2], I. KITA[1], T. NISHIJIMA[1]; [1]Tokyo Metropolitan Univ., Tokyo, Japan; [2]Tsukuba Univ., Tsukuba, Japan

Abstract: [Background] A growing number of studies have revealed the beneficial effects of exercise on brain function, which are most pronounced in the hippocampus. Long-term exercise induces various structural changes in the hippocampus, including neurogenesis, contributing to improved cognitive function, dementia, and depression. The effects on the hippocampus depend on exercise conditions. Although the intensity of exercise has been widely recognized as a dominant factor, we recently found that exercise type is another factor affecting the effects of exercise on the hippocampus (Tsuchida et al., Neurosci Lett, 2022). Briefly, we compared the treadmill exercise, one of the most used types of exercise in rodents, and rotarod exercise, used to assess motor learning and coordination, at matched intensities. Although the treadmill activated hippocampal neural activity in mice, the rotarod did not, suggesting that the effects on hippocampal neural activity depend on the type of exercise. However, it is unclear whether the effects of long-term exercise on hippocampal function also depend on the type of exercise. [Purpose] This study aimed to examine whether the effects of long-term exercise on hippocampus in mice differ depending on the type of exercise. [Methods] The exercise period was 5 weeks. The exercise intensity of the treadmill was 15 m/min, and that of the rotarod was 30 rpm, which were almost equivalent at just below the lactate threshold. In the 5th week of exercise intervention, the hippocampus-dependent spatial learning and memory were examined by Morris water maze. Although depression-like behavior is partially regulated by the hippocampus, it is a non-hippocampus-dependent variable and was examined by a forced swim test (FST). [Results] The density of Doublecortin (DCX)-positive immature neurons was significantly increased by the treadmill, but not by the rotarod exercise, compared to that of the respective control groups. In addition, the density of DCX-positive neurons in treadmill runners was significantly higher than that in rotarod runners. Contrary to our hypothesis, spatial learning and memory were not improved by either type of exercise. FST showed that depression-like behavior improved in both types of exercise, suggesting that rotarod exercise, although cannot activate hippocampal neurons, has an antidepressant effect. [Conclusion] These results demonstrate that long-term treadmill exercise enhances hippocampal neurogenesis more efficiently than does the intensity-matched rotarod exercise. These results strengthen our claim that exercise type is another important factor influencing the effects of exercise on the hippocampus.

Disclosures: R. Tsuchida: None. **T. Yamaguchi:** None. **D. Funabashi:** None. **I. Kita:** None. **T. Nishijima:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.21/A21

Topic: H.08. Learning and Memory

Title: Long-term spatial memory resistance to exercise-enhanced neurogenesis in rats

Authors: *E. R. BOLTON, N. M. FOURNIER, J. WEBB, B. OBRIEN, H. LEHMANN; Psychology, Trent Univ., Peterborough, ON, Canada

Abstract: Neurogenesis, the generation of new neurons, occurs continuously in the hippocampus throughout life. As these new neurons are added, they alter hippocampal circuitry, may compete with existing neurons for inputs and outputs, and potentially impact access to information already stored within these circuits. A potent stimulator of hippocampal neurogenesis, running exercise enhances the acquisition of new memories, including spatial memories. However, this exercise-increased neurogenesis has also been shown to interfere with recalling a previously acquired spatial memory in mice and rats. This suggests that neurogenesis may induce "forgetting" of old memories. In the present study, we replicate and expand upon these previous observations to investigate whether maintaining an elevated level of neurogenesis also induces the forgetting of a previously acquired memory. In our study, male rats were trained to learn the location of a hidden platform in the Morris Water Task (MWT) and then assigned to either sedentary (SED) or running exercise (RE) groups. The RE group was given access to running wheels in their home cages for five weeks, while the SED group was not. After this period, both groups underwent training in a second version of the MWT before being tested for their recall (memory) of the platform location from the first MWT. A different pool, room, and distinct cues were used in the second MWT to create a separate spatial memory. The results showed that the groups similarly learned the first MWT prior to any experimental manipulation and that the RE group remembered it just as well as the SED control group after a month of continued exercise-enhanced neurogenesis. Learning and retention of the second MWT that both occurred under enhanced-neurogenesis conditions for the RE group was again like that of the SED group. Preliminary analysis of doublecortin-positive cells confirms that the RE group had increased hippocampal neurogenesis. These findings suggest that suddenly increasing and maintaining increased levels of hippocampal neurogenesis using running exercise does not interfere with successfully retaining a previously formed spatial memory.

Disclosures: E.R. Bolton: None. **N.M. Fournier:** None. **J. Webb:** None. **B. Obrien:** None. **H. Lehmann:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.22/A22

Topic: H.08. Learning and Memory

Support: NIH Grant ZIAMH002784

Title: Decision-making under conflict is influenced by adult-born neurons in the rat hippocampus

Authors: A. P. SWIERCZ[1], H. R. MARTIN[1], R.-M. KARLSSON[1], *H. CAMERON[2,1];
[1]Section on Neuroplasticity, NIMH, [2]NIH, Bethesda, MD

Abstract: When difficult decisions must be made, the hippocampus contributes to conflict-resolution by modulating the inhibition of dominant behavioral responses. The dentate gyrus, unique for its ability to produce new neurons into adulthood, plays a key role in this process. Whether or not neurogenesis is directly involved in difficult decision-making, however, remains unclear. Here we ablated neurogenesis in adult male rats to investigate the role of adult-born neurons in conflict decision making using a platform-mediated avoidance task (PMA). Neurogenesis was eliminated by 8 weeks of treatment with valganciclovir in male transgenic Long Evans rats expressing the herpes simplex virus thymidine kinase (TK) under control of the glial fibrillary acidic protein (GFAP) promoter. In the PMA task, animals were trained to associate two distinct conditioned stimuli (auditory and light cues) with negative or positive outcomes. During the conflict phase, rats were presented with both stimuli and forced to choose between avoiding a negative outcome (foot shock) and approaching a positive outcome (food reward lever). The decision to avoid comes at the cost of losing food rewards, while the decision to approach increases the likelihood of receiving a foot shock. We examined decision-making under increasing levels of conflict and alternating patterns of cue presentation. Animals lacking adult-born neurons learned cue associations and displayed normal levels of approach-avoidance behavior in the absence of conflict. During high conflict situations, however, animals without neurogenesis exhibited a significant reduction in avoidance behavior and increased lever pressing relative to wildtype controls. We are currently using immediate-early gene expression analyses to assess neuronal activity during conflict in several brain regions associated with approach-avoidance behavior.

Disclosures: A.P. Swiercz: None. **H.R. Martin:** None. **R. Karlsson:** None. **H. Cameron:** None.

Poster

PSTR447. Postnatal Neurogenesis: Molecular Mechanisms and Regulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR447.23/A23

Topic: H.08. Learning and Memory

Support: NIH grant: ZIAMH002784

Title: Involvement of adult hippocampal neurogenesis in the decision to engage in intermale aggression in mice

Authors: *M. C. TSUDA, T. AKOH-ARREY, J. MERCURIO, D. LUKASZ, A. RUCKER, M. AIREY, H. JACOBS, H. A. CAMERON;
Section on Neuroplasticity, NIMH, Bethesda, MD

Abstract: Adult neurogenesis affects many types of rodent behavior, but few studies elucidate its role in social interactions. The present study investigated the role of adult hippocampal neurogenesis in the expression of aggressive behavior in male mice. We inhibited adult hippocampal neurogenesis by treating male transgenic mice expressing the herpes simplex virus thymidine kinase (TK) under a GFAP promoter with the anti-viral drug valganciclovir. After 8 weeks of drug treatment, TK and wild-type (WT) littermate controls were single housed for one week and then tested for intermale aggression in the resident-intruder paradigm. TK mice showed fewer aggressive bouts, shorter cumulative duration of aggression, and longer latency to exhibit aggression towards a CD-1 male intruder compared to WT mice, suggesting reduced levels of offensive aggression in TK mice. Interestingly, when TK mice were paired instead with a smaller, nonaggressive male (olfactory bulbectomized C57BL/6J mouse), aggression levels were comparable to WT mice. Additionally, we evaluated the loss of hippocampal neurogenesis on defensive aggressive behaviors by presenting TK and WT mice as the intruder, rather than the resident in the resident-intruder test. WT mice showed both defensive attack and avoidance responses to a resident attack, but TK mice predominantly avoided fighting when attacked by the resident. Furthermore, using the tube test to assess social dominance in WT and TK mice, we found that when WT and TK mice opposed each other in the tube, TK mice lost 75% of the trials, suggesting that ablation of adult hippocampal newborn neurons contributes to submissive-like behaviors. Our findings suggest that TK mice avoid social confrontations unless they perceive they can clearly win the fight, such as a nonaggressive smaller mouse. Finally, we examined whether ablation of adult hippocampal neurogenesis affected social investigation and discrimination behaviors, which could contribute to changes in aggression levels. TK mice exhibited normal social investigation and recognition towards novel mice, suggesting that TK mice have normal olfaction, social memory, and social interest. Taken together, adult hippocampal neurogenesis plays an essential role in the instigation of intermale aggression in mice.

Disclosures: M.C. Tsuda: None. **T. Akoh-Arrey:** None. **J. Mercurio:** None. **D. Lukasz:** None. **A. Rucker:** None. **M. Airey:** None. **H. Jacobs:** None. **H.A. Cameron:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.01/B1

Topic: A.03. Stem Cells and Reprogramming

Title: Development and Characterization of three novel Patient-Derived iPSCs and NPCs as Disease Models for Maternally inherited Leigh syndrome(MILS)

Authors: *M. HASCHKE[1], M. SCHÜLKE[2];
[1]Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Dept. of Pediatric Neurol., Charité – Universitätsmedizin, Berlin, Germany

Abstract: Maternally inherited Leigh syndrome (MILS) is a neurodevelopmental disorder caused by mitochondrial DNA (mtDNA) mutations. Generally, MILS is characterized by a wide range of clinical manifestations, including neurodevelopmental delay, muscle weakness, hypotonia, and stroke-like episodes. To date, there is no treatment or cure available. The development of treatment options has been difficult due to the lack of disease models specific to mtDNA mutations.Recently, neural progenitors differentiated from patient-derived pluripotent stem cells (iPSCs) have been shown to be an effective modeling tool for neuronal disease associated with mtDNA mutations. This study aimed to develop and characterize neural progenitor cells (NPCs) and iPSCs derived from MILS patients carrying the mtATP-6 mutation.We generated six human iPSC lines from dermal fibroblasts of three patients (two clones per patient) affected by MILS. The patients carry homoplasmic or heteroplasmic mutations of the maternally inherited mtATP-6 gene: patient A (heteroplasmic m.8570T>C, male, 4 y), patient B (homoplasmic m.8993T>G, female, 5 y), patient C (heteroplasmic m.8993T>G, female, 5 y). To generate iPSCs, a non-integrative reprogramming method introducing OCT4, SOX2, KLF4, and c-MYC with a Sendai reprogramming kit was used. The established cell lines were assayed for pluripotency via immunofluorescence staining, RT-qPCR analysis, and embryoid body (EB) formation.Additionally, the integrity of the karyotype was assessed using a single nucleotide polymorphism (SNP) array. The degree of heteroplasmy was evaluated to ascertain that the mutation level was retained.Following a small molecule-based protocol by Reinhardt et al., iPSCs were further differentiated into NPCs. All iPSC lines showed the expression of pluripotency markers and were capable of forming the three germ layers during EB development. After differentiation into NPCs, the expression of neuronal markers was upregulated while pluripotency markers were downregulated.The iPSC lines exhibited a normal karyotype. We showed that the generated iPSCs and NPCs of patient B and C retained the same degree of heteroplasmy as their parental fibroblasts, indicating the preservation of the mtDNA mutation during reprogramming. In patient line A, the heteroplasmy dropped from 96.2% (fibroblasts) to 66.9% (iPSCs) and to 60.9% (NPCs).This study provides a valuable disease model for patient-specific investigation of the pathogenesis of MILS and paves the way for the development of potential therapeutic interventions targeting mtDNA mutations and associated neurological disorders.

Disclosures: **M. Haschke:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); MDC Berlin. **M. Schülke:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.02/B2

Topic: A.03. Stem Cells and Reprogramming

Title: Cerebral organoids from patients with multiple sclerosis reveal innate defect in oligodendrocyte maturation and myelination capacity

Authors: T. MEHTA, A. MCDERMOTT, ***N. DAVIAUD**, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. Of New York, New York, NY

Abstract: Multiple sclerosis (MS) is an autoimmune neurological disorder characterized by inflammation, demyelination, and neural degeneration. Although environmental and genetic factors both contribute to the development of MS, the etiology of this condition remains unknown due to the lack of accurate animal models and relative inaccessibility to human brain tissue. Unlike relapsing-remitting MS, it has been proposed that progressive forms of MS exhibit impaired remyelination of lesions due to a defect in the differentiation and migration of oligodendrocyte precursor cells. Thus, it is crucial to further elucidate this mechanism in order to develop novel treatments for myelin repair in progressive MS subtypes. Cerebral organoids recapitulate early human neurodevelopment, including the generation, proliferation, and differentiation of oligodendrocyte precursor cells into myelinating oligodendrocytes. The aim of this study was to use cerebral organoids derived from induced pluripotent stem cells of patients with MS to investigate the genetic contribution of MS on oligodendrocyte differentiation and maturation, as well as myelination. Immunofluorescence analyses were conducted on organoids derived from patients with MS as well as healthy controls at three timepoints (42, 120, and 200 days) to assess oligodendrocyte differentiation along its lineage and myelination capacity. Our findings revealed a significant decrease of oligodendrocyte lineage marker Olig2 in MS organoids compared to control at all three timepoints highlighting a lower number of oligodendrocytes lineage cells. Moreover, at day 120 we detected a decrease of $Olig2^{+}/APC^{+}$ mature oligodendrocyte number as well as a strong decrease of $O4^{+}$ mature oligodendrocyte number in MS organoids, particularly PPMS, compared to control. These results were further confirmed by RT-qPCR. After 200 days of culture, myelin formation was observed in the organoids by immunostaining for the myelin marker MBP. MS organoids displayed a decreased expression of MBP compared to controls. Taken together, these results suggest a defect in oligodendrocyte maturation and a lack of myelin formation in MS organoids. Mature cerebral organoids developed from MS patient is a valuable tool to study MS. Our findings revealed a deficiency of oligodendrocyte precursors differentiation and of oligodendrocyte maturation, which could be the underlying mechanism for impaired remyelination in MS patients, particularly in progressive forms. These insights may inform potential treatment approaches, such as strategies aimed at oligodendrocyte protection, for promoting myelin repair in progressive forms of MS.

Disclosures: T. Mehta: None. **A. McDermott:** None. **N. Daviaud:** None. **S.A. Sadiq:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.03/B3

Topic: A.03. Stem Cells and Reprogramming

Support: NIH/ NINDS K08NS102526
2020 Doris Duke Charitable Foundation Clinical Scientist Career Development Award

Title: Altered neuronal development and connectivity in a model of 15q11.2 related neurodevelopmental disorders

Authors: *C. W. HABELA[1], S. LIU[1], A. TAGA[1], R. DASTGHEYB[2], D. E. BERGLES[4], G.-L. MING[5], H. SONG[6], N. J. MARAGAKIS[3];
[1]Neurol., Johns Hopkins Univ., Baltimore, MD; [3]Neurol., [2]Johns Hopkins Univ. SOM, Baltimore, MD; [4]Johns Hopkins Univ. Sch. Med., Baltimore, MD; [5]Dept Neurosci, [6]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The 15q11.2 locus is deleted in 1.5% of patients with genetic epilepsy and it confers risk for intellectual disability and schizophrenia, suggesting that this region is important for neuronal development. One of the 4 genes at this locus, CYFIP1, is a regulator of fragile X mental retardation protein and cytoskeleton remodeling, has been shown to regulate early neurogenesis and late synaptogenesis in mice. We examined whether there were differences in neural development in neurons derived from induced pluripotent stem cells (iPSCs) from patients with 15q11.2 deletion and mice deficient for Cyfip1. A forebrain specific neuronal differentiation protocol was used to generate primarily glutamatergic cortical neurons from iPSCs from control and 15q11.2 deletion patients. There was a reduction in electrophysiological spike rate, bursting and synchronization of culture networks in the 15q11.2 deleted human neurons compared to controls. This was associated with decreased single cell dendritic complexity, decreased dendritic length and altered structural connectivity across culture networks. There was decreased response to GABA agonists and antagonists, indicating decreased inhibitory control over network activity despite an increase in the relative proportion of inhibitory neurons in the 15q11.2 Del cultures. Inhibitory synapse quantification demonstrated an increase in the number of inhibitory synapses onto inhibitory neurons. Cyfip1 deficient mice demonstrated decreased latency to seizure in response to disinhibition by flurothyl but not kainic acid. These data suggest that deletion of the 15q11.2 region results in cell autonomous changes in neurons and synaptic maturation that contribute to pathologic changes in network excitability. Inhibition of inhibitory neurons is one possible explanation for the paradox of decreased baseline activity and synchronization as well as a predisposition to hyperexcitability *in vivo*. Together, these studies indicate that 15q11.2 deletion results in impaired early neuronal development that may contribute to increased seizure susceptibility in humans with the deletion.

Disclosures: C.W. Habela: None. **S. Liu:** None. **A. Taga:** None. **R. Dastgheyb:** None. **D.E. Bergles:** None. **G. Ming:** None. **H. Song:** None. **N.J. Maragakis:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.04/B4

Topic: A.03. Stem Cells and Reprogramming

Title: Characterization of a Systems Biology Platform Incorporating Human Microglia with Rodent Neurons and Astrocytes

Authors: C. NODIN[1], *J. PIHL[1], B. MA[2], M. KARLSSON[1], J. LEVENSON[2];
[1]Cellectricon AB, Mölndal, Sweden; [2]FireCyte Therapeutics, Inc., Beverly, MA

Abstract: Microglia, the innate immune cell of the CNS, are increasingly recognized as a critical nexus for the promotion and maintenance of CNS function. Dysfunction in microglia often manifests as a neurotoxic and inflammatory microenvironment that if not resolved, cascades to a chronic neuroinflammation associated with many pathological conditions including neurodevelopmental, neuropsychiatric and neurodegenerative diseases. Despite the longstanding recognition of their centrality in chronic and degenerative diseases of the CNS, there is still a critical need for assay platforms that recapitulate the systems biology of microglia in a way that meaningfully facilitates neuroinflammatory drug discovery. Here we describe the initial development and qualification of a rodent-human chimeric assay platform that permits quantification of endpoints relevant to neuronal and microglial function in the presence and absence of neuroinflammation. The platform is built upon rodent primary cortical neurons and astrocytes. After the initial culture period, ≥90% of the rodent microglia die and are replaced with IPSC-derived human microglia. The hIPSC microglia distribute and intercalate into the base rodent culture, and exhibit morphological and molecular signatures indicative of homeostatic microglia. Stimulation of cultures with LPS results in a dramatic shift of hIPSC microglia to an amoeboid morphology and a robust increase in secretion of pro-inflammatory cytokines. hIPSC microglia exhibit phagocytosis as measured with zymosan bioparticles. Exposure to LPS and zymosan particles is associated with robust additional increase in pro-inflammatory cytokine secretion, compared with LPS treatment alone. Additionally, chronic (5 d) stimulation with LPS induces morphological shifts in astrocytes indicative of the reactive state. Induction of reactive astrocytosis requires hIPSC microglia as chronic stimulation of rodent neuron-astrocyte cocultures with LPS fails to induce morphological changes in astrocytes. These initial observations indicate that human microglial disease-relevant inflammatory biology can be measured in the context of rodent neurons and astrocytes in culture. Future studies will interrogate neuronal function and establish methods for compound screening to support an integrated, systems biology approach to neuroinflammatory drug discovery.

Disclosures: **C. Nodin:** A. Employment/Salary (full or part-time):; Cellectricon AB, Neongatan 4B, 431 53 Mölndal, Sweden. **J. Pihl:** A. Employment/Salary (full or part-time):; Cellectricon AB, Neongatan 4B, 431 53 Mölndal, Sweden. **B. Ma:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics, Inc., 100 Cummings Ctr, Suite 451-C, Beverly, MA 01915, USA. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB, Neongatan 4B, 431 53 Mölndal, Sweden. **J. Levenson:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics, Inc., 100 Cummings Ctr, Suite 451-C, Beverly, MA 01915, USA.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.05/B5

Topic: A.03. Stem Cells and Reprogramming

Support: INSTITUTE BLOCK GRANT
NMRC OF YIRG

Title: Ivns1abp mutation disrupts actin filament organization and results in cellular senescence

Authors: *F. YUAN[1], C. BONNARD[2], B. REVERSADE[2], S.-C. ZHANG[3];
[1]Duke-Nus Grad. Med. Sch. Singapore, Singapore, Singapore; [2]Inst. of Mol. and Cell. Biology, A*STAR, Singapore, Singapore; [3]Univ. of Wisconsin - Madison, Univ. of Wisconsin, Madison, WI

Abstract: We have recently encountered a family whose teenagers display symptoms of premature aging and severe neuropathy, including dyschromatosis, gray hairs, progressive motor deficits, and intellectual developmental delay. Whole genome sequencing revealed a homozygous mutation in the IVNS1ABP gene, an influenza virus nonstructural protein-1 binding protein belonging to the kelch protein family. What does IVNS1ABP do and how its mutation results in premature aging and neuropathy are unknown. We acquired dermal fibroblasts from the patients and their family members, generated isogenic induced pluripotent stem cells (iPSCs) from the fibroblasts and then differentiated the isogenic iPSCs to neural progenitor cells (NPCs). The mutant fibroblasts, iPSCs, and NPCs grow more slowly with extended cell cycle duration. In particular, the cytokinesis is disrupted, leading to mislocalized centrosomes and dysregulated spindle orientation and consequently mitotic failure. Correspondingly, mutant NPCs display increased DNA damage and genome instability as well as senescence markers like p16. Proteomics and transcriptomics revealed alteration of actin and actin-binding proteins by IVNS1ABP mutation. Indeed, mutant IVNS1ABP impairs its interaction with and polymerization of actin, explaining the disrupted cytokinesis and cellular senescence. Our findings thus identify dysregulated actin polymerization and organization as a mediator of proliferative cellular senescence in this undiagnosed disease.

Disclosures: F. Yuan: None. **C. Bonnard:** None. **B. Reversade:** None. **S. Zhang:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.06/B6

Topic: A.03. Stem Cells and Reprogramming

Support: NIH NIA RF1AG080636
DoD W81XWH2010368

DoD W81XWH2010371
Harold and Ronna Cooper Post-Doctoral Fellowship for Parkinson's Disease Research
Orchard Foundation
Consolidated Anti-Aging Foundation

Title: Apoe genotype modulates lipids and inflammation in human ipsc-derived neuron and glia cultures

Authors: E. DI BIASE, P. ROWICKA, O. COOPER, *P. J. HALLETT, O. ISACSON; Neuroregeneration Inst., Harvard Med. Sch. / McLean Hos., Belmont, MA

Abstract: Appropriate lipid transfer between neurons and glia via lipid transporters such as apolipoproteins, is essential for maintaining metabolic and structural integrity of neurons, with dysfunction leading to synaptic and cellular damage. Apolipoprotein E (APOE) is the most ubiquitous brain lipoprotein and facilitates lipid transport between cells and is also involved in cellular responses to inflammation. The APOE4 allele of APOE is the strongest genetic risk factor for late-onset Alzheimer's disease (AD) as well as a genetic risk factor, along with glucocerebrosidase, GBA1, for Lewy body and Parkinson's disease dementia (LBD/PDD).
An advanced *in vitro* platform of human induced pluripotent stem cell (iPSC) derived neurons and astrocytes has been established to test how APOE modulates neuron-glia mechanisms induced by elevated glycolipids, cholesterol and inflammatory processes. Human isogenic iPSCs carrying APOE3/3 and APOE4/4 were differentiated into excitatory cortical neurons (expressing βIII-tubulin and MAP2) and astrocytes (expressing S100β, GFAP, and APOE). Pharmacological treatment of human APOE3/3 and APOE4/4 neurons with U18666A (an inhibitor of the cholesterol NPC1 transporter), to increase cellular lipid load, induced accumulation of cholesterol, and of filipin and Nile-Red labeled lipid particles. Upon co-culture of neurons with corresponding APOE3/3 or APOE4/4 human astrocytes for 7 days, APOE3/3 neurons accumulated fewer Nile Red-labeled lipid particles than APOE4/4 neurons following lipid challenge with NPC1-inhibition. Ongoing analyses will determine cellular mechanisms of APOE release, lipidation of APOE, and readouts of cell function and health.
Critical human data shows that lipid dyshomeostasis can significantly contribute to the etiology and onset of age-related neurodegenerative disorders. Identifying the mechanisms by which human APOE functionally interacts with increased lipid load in human neurons and glia will provide an impactful understanding of the pathogenic pathways that lead to AD and LBD/PDD.

Disclosures: E. Di Biase: None. **P. Rowicka:** None. **O. Cooper:** None. **P.J. Hallett:** None. **O. Isacson:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.07/Web Only

Topic: A.03. Stem Cells and Reprogramming

Support: S Lee is the recipient of a DeNardo Education and Research Foundation scholarship.
We thank G. Chinnadura and S. Vijalingam for their helpful discussions.
We thank the Genome Engineering and iPSC Center (GEiC) at Washington University in St. Louis for iPSC engineering services.

Title: CTBP1 mutant allele affects neurodevelopment by dysregulating the Wnt pathway

Authors: *U. EZEKIEL[1], **S. LEE**[2];
[1]Clin. Hlth. Sci., St. Louis Univ., Saint Louis, MO; [2]Hlth. Mgmt., St. Louis Univ., St. Louis, MO

Abstract: C terminal binding proteins (CtBP1 and CtBP2) are highly conserved and are expressed in different vertebrate tissues. CtBP1 and CtBP2 (CtBPs) are transcriptional co-repressors and perform genetically related and unique transcriptional functions during vertebrate development. CtBPs mediate transcriptional repression by binding to various chromatin-modifying factors and DNA-binding repressors at the promoter regions of target genes through a high-affinity protein interaction interface (PXDLS-binding cleft). There are 14 cases that have been identified as with pathogenic de novo CTBP1 mutations that were found in a heterozygous condition: 13 of the patients have a missense mutation in *CTBP1* (*CTBP1*p.R342W), and one has a deletion mutation (*CTBP1*p.A56_S58del), and both variants map to the same domain of the protein. All patients exhibited intellectual disability and HADDTS syndrome (hypotonia, ataxia, developmental delay, and tooth enamel defects). A hallmark of patients with *CTBP1* mutations is cerebellar atrophy. The mechanism of pathogenesis leading to the neurodevelopmental and multiple pathological phenotypes due to *CTBP1* heterozygous mutation is unknown. Neurons differentiated from induced pluripotent cells derived from patients and age-matched controls indicated that neurites derived from patient cells were thinner compared to neurites derived from control cells. To facilitate an accurate assessment of transcriptional activity in neurons with *CTBP1*p.R342W mutation, we made isogenic iPSC cell lines that were then differentiated into neuronal stem cells (NSC). We generated neurons from NSC, and total RNA isolated from 21-day neurons was subjected to RNA sequence and analyzed. The transcriptome analysis indicated changes encompassing adhesion, neuronal development, and the Wnt signaling pathway. During neuronal development, Wnt signaling plays significant roles in axon, dendrite, and dendritic spine formation. We observed that genes involved in the Wnt pathway, calcium regulation, adhesion, and neuronal morphogenesis were downregulated. Our preliminary studies indicate that when neural stem cells are differentiated into neurons, the addition of Wnt inhibitor (XAV939) prevented neurite formation only in heterozygous mutant cells. These results suggest that the pathogenic *CTPB1* allele dysregulates the Wnt pathway and thereby inhibits neuron differentiation.

Disclosures: U. Ezekiel: None. **S. Lee:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.08/B7

Topic: A.03. Stem Cells and Reprogramming

Support: CIHR grant #PJT-159586
CIHR grant #CRU1126
NSERC CGSD

Title: Modelling Prenatal Cannabinoid Exposure as a Risk Factor for Schizophrenia Vulnerability in Patient-Derived Cerebral Brain Organoids

Authors: *M. H. SARIKAHYA[1], M. RODRIGUEZ RUIZ[1], D. GUMMERSON[1], E. PROUD[1], S. R. V. MORENO[1], S. COUSINEAU[1], J. P. G. LAZO[2], K. YEUNG[1], D. B. HARDY[1], W. J. RUSHLOW[1], S. R. LAVIOLETTE[1];
[1]Univ. of Western Ontario, London, ON, Canada; [2]Western Univ., London, ON, Canada

Abstract: Clinical and preclinical studies have shown that prenatal cannabinoid exposure (PCE) can disrupt fetal brain development and increase susceptibility to neuropsychiatric disorders, including schizophrenia (SZ), cognitive, and mood/anxiety disorders. However, the underlying mechanisms of these effects remain poorly understood. Our research aims to elucidate these mechanisms using human-derived cerebral organoids. We generated human cerebral organoids from induced pluripotent stem cells to mimic the in vivo development of the prenatal human brain. Organoids were derived from four healthy control and four SZ patient cell lines. The differentiated organoids were exposed to Δ9-tetrahydrocannabinol (THC; 100nM), cannabidiol (CBD; 500nM), and a combination of THC and CBD (100nM THC/500nM CBD) over 27 days (days 3-30) which resembles the early stages of cortical growth in the human fetus. We employed various techniques including electrophysiological characterization using a 3D brain microelectrode array, lipidomic and metabolomic analyses using matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI IMS), gene expression analyses through immunofluorescence, western blot protein analyses, quantitative PCR, and transcriptomics via RNA sequencing (RNAseq). Preliminary characterization revealed expected neuronal molecular markers expressed in the organoids at different developmental stages. THC-CBD combined treatment and THC-exposed organoids showed distinct lipidomic and metabolomic profiles compared to CBD-exposed organoids in control cell lines, with more pronounced differences in SZ cell lines. Notably, these lipidomic changes resemble findings from our rodent models. PCE in rodents and humans leads to fetal growth restriction and reduced availability of polyunsaturated fatty acids (PUFAs), resulting in lifelong cognitive and emotional abnormalities resembling SZ. Severe reductions in PUFAs were observed following THC-CBD combined treatment and THC exposure. We are investigating electrophysiological abnormalities associated with these PUFA deficits using microelectrode array and immunofluorescence techniques. Differential expression of neuronal markers between control and SZ organoids was observed through qPCR, with higher mRNA levels of neuronal markers in the control group. Comprehensive RNAseq data comparing organoid treatment groups will be presented. Our study utilizing human-derived cerebral organoids has begun to unravel the cellular-level effects of gestational exposure to THC on human brain development and its association with neuropsychiatric disorders.

Disclosures: **M.H. Sarikahya:** None. **M. Rodriguez Ruiz:** None. **D. Gummerson:** None. **E. Proud:** None. **S.R.V. Moreno:** None. **S. Cousineau:** None. **J.P.G. Lazo:** None. **K. Yeung:** None. **D.B. Hardy:** None. **W.J. Rushlow:** None. **S.R. Laviolette:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.09/B8

Topic: A.03. Stem Cells and Reprogramming

Title: In vitro Rett syndrome model based on MeCP2 knockdown in iPSC-derived neurons

Authors: *R. YAMOTO, M. NAKAO, T. HAZAMA, T. HOSOYA;
RICOH COMPANY, Ltd., Kawasaki, Kanagawa, Japan

Abstract: Rett Syndrome (RTT) is a progressive and pervasive X-linked neurodevelopmental disorder that predominantly affects girls by the early childhood. The detailed disease mechanisms remain unclear and fundamental treatments are yet to be established. The vast majority of typical RTT cases is triggered by sporadic mutations in the methyl CpG-binding protein 2 (MeCP2) gene. Among many different symptoms, MeCP2 mutations affect multiple stages of the brain development. For the analyze of the disease mechanisms and drug development, in vitro modes based on iPSC-derived cells with diseased MeCP2 are being investigated. In addition to MeCP2 mutations RTT is also affected by the genetic background, and thus it is necessary to analyze the effect of MeCP2 modifications in cells generated from multiple iPSC strains. Although the genome editing technology is often used for this purpose, it tends to be time-consuming and costly. We therefore developed an in vitro RTT model using shRNA knockdown of MeCP2. iPSC-derived neurons and human primary astrocytes were co-cultured and transduced with lentiviral vectors encoding MeCP2 shRNA. The neurons in the co-culture exhibited neurite atrophy defined as the reduction in the complexity of neural arborization, which is a hallmark of RTT. This neurite atrophy was rescued by overexpression of MeCp2, while the phenotype was absent in co-cultures transduced with control shRNA. Furthermore, brain-derived neurotrophic factor (BDNF), an enhancer of neurite arborization in RTT, significantly increased the neurite density in the MeCP2 knockdown co-cultures. Therefore, MeCp2 knockdown induces neurite atrophy that is rescued by a drug for RTT, suggesting that the RTT model exhibits a disease-related neurite phenotype. Because the method can be easily applied to multiple iPSC strains, it is suited to generate RTT models with various genetic backgrounds and therefor will contribute to the RTT research and high-throughput drug screening.

Disclosures: **R. Yamoto:** None. **M. Nakao:** None. **T. Hazama:** None. **T. Hosoya:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.10/B9

Topic: A.03. Stem Cells and Reprogramming

Support: Wellcome Leap 1kD Program

Title: Constructing human cortico-basal ganglia-thalamic circuits from pluripotent stem cells

Authors: *Y. MIURA, J.-I. KIM, O. REVAH, X. YANG, M. V. THETE, B. CUI, S. P. PASCA;
Stanford Univ., Stanford, CA

Abstract: Abnormalities in the activity of the neural circuits connecting the cerebral cortex, parts of the basal ganglia, and thalamic nuclei have been strongly associated with neuropsychiatric disorders, including autism, schizophrenia, and obsessive-compulsive disorder. These neural circuits have been extensively manipulated in rodents and studied by imaging in patients, but how these pathways are formed during human neural development and how this assembly goes awry in disease remains unknown. Here, we describe a novel approach to construct from human pluripotent stem cells the cortico-basal ganglia-thalamic pathway by building loop assembloids. More specifically, we generated three-dimensional (3D) self-organizing, regionalized organoids that resemble the human cerebral cortex, the basal ganglia, the mesencephalon, and the diencephalon, and functionally integrated them into four-part assembloids that include glutamatergic, GABAergic, and dopaminergic neurons. Live calcium imaging using a custom wide-field microscope and extracellular recordings from individual parts of the loop assembloids revealed synchronized patterns of neuronal activity that are altered by the loss of disease risk gene. We anticipate that this approach will enable functional, *ex vivo* studies of the cortico-basal ganglia-thalamic neural circuits during human development and in disease states.

Disclosures: Y. Miura: None. **J. Kim:** None. **O. Revah:** None. **X. Yang:** None. **M.V. Thete:** None. **B. Cui:** None. **S.P. Pasca:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.11/B10

Topic: A.03. Stem Cells and Reprogramming

Support: Aust. Nat. Health & Med Res Council (NHMRC), Project Grant APP1188169 (PGN)
MRFF AR LEUKODYSTROPHIES FLAGSHIP The Massimo Mission

(EJW, MRS)
SM and BA are supported by a UQ PhD scholarships.

Title: Generation of grey-white matter spinal cord organoids to model HBSL-hypomyelination, lumbar spasticity and enable drug screening

Authors: *S. D. MORRISON[1,2], B. AL-MHANAWI[1], G. PIETROGRANDE[1], E. J. WOLVETANG[1], P. G. NOAKES[2], M. R. SHAKER[1];
[1]Stem Cell Engin. group, Australian Inst. for Bioengineering and Nanotechnology, St Lucia, Australia; [2]Sch. of Biomed. Sci., The Univ. of Queensland, St. Lucia, Brisbane, Australia

Abstract: Current human spinal cord organoid models fail to replicate correct co-specification of motoneurons and myelinating oligodendrocytes. Consequently, these models do not exhibit grey-white matter polarity; achieving polarity is important since defects in these structures are hallmarks of neurological diseases including leukodystrophies such as Hypomyelination with Brainstem and Spinal cord involvement and Leg spasticity (HBSL). We employed HBSL patient iPSCs and controls, including an isogenic control carrying the HBSL-associated gene correction, to generate spinal cord organoids with lumbar cellular identity. We used immunohistochemistry to assess and compare the cellular organisation of (n=8-12) lumbar spinal cord organoids across groups. Specifically, we selected multiple markers to identify distinct cell populations: oligodendrocytes (SOX10, PDGFR1a, CNPase, PLP1, MBP), neurons (NeuN), astrocytes (GFAP) and motor neurons (ISL1, ChAT). In non-HSBL spinal cord organoids, we observed SOX10+ oligodendrocytes at peripheral locations, which encompassed central populations of neurons including motor neurons (NeuN and/or ChAT+). Closer inspection revealed that peripherally located motor neurons (ISL1+, ChAT+) were enveloped by myelinating (PLP1+, MBP+) oligodendrocytes. By contrast, HBSL spinal cord organoids failed to show peripheral distribution of SOX10+ oligodendrocytes and demonstrated delayed neuronal differentiation. Next, we used multi-electrode array to compare spontaneous, and acetylcholine evoked neural activity, in HBSL-3 (chr2:136664933, c.1459C>T16p.Arg487Cys) and its isogenic corrected control HBSL-3C spinal cord organoids. Spontaneous mean firing rates (MFRs) and burst frequency (BF) did not differ between HBSL-3 and HBSL-3C. By contrast, HBSL-3 demonstrated acetylcholine evoked periodic hyperactivity, reminiscent of the lumbar spasticity observed in HBSL. Altogether, our lumbar spinal cord model recapitulates the cellular make-up and nascent architecture of the developing human spinal cord and further exhibits pathological features of that will enable evaluation of drug and gene therapy interventions for HBSL patients.

Disclosures: S.D. Morrison: None. **B. Al-mhanawi:** None. **G. Pietrogrande:** None. **E.J. Wolvetang:** None. **P.G. Noakes:** None. **M.R. Shaker:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.12/B11

Topic: A.03. Stem Cells and Reprogramming

Title: Modeling serotonergic signaling in the developing human nervous system with neuromodulatory assembloids

Authors: *S. KANTON[1], X. MENG[1], F. BIREY[1,2], M.-Y. LI[1], N. REIS[1], P. MCQUEEN[1], J.-I. KIM[1], M. THETE[1], N. SAKAI[1], S. P. PASCA[1];
[1]Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA; [2]Emory Univ., Atlanta, GA

Abstract: Neuromodulators such as serotonin, dopamine or acetylcholine regulate axon guidance and progenitor proliferation, influence intrinsic firing properties and synaptic strength, and have been strongly associated with neuropsychiatric disorders. Recent advances in stem cell biology and self-organizing 3D cultures have made possible the derivation of diverse human neural cell types and circuits in organoids and assembloids. However, current in vitro models do not capture neuromodulatory input, which restrict broader applications in neuroscience and modeling of disease. To address this, we have developed a novel cellular platform by integrating human brainstem organoids that contain serotonin-producing, raphe nucleus-like neurons with cortical organoids to generate neuromodulatory assembloids. Using live imaging, pharmacology, viral tracing and electrophysiological methods, we discovered reciprocal projections between TPH2 serotonergic neurons and cortical glutamatergic neurons to hRnOs, and changes in cortical network activity following assembly. We then leveraged this model to study 16p11.2 deletion syndrome, which has been linked to serotonin dysfunction. Taken together, this novel platform has the potential to capture neuromodulatory cross-talk in human neural development and to bring insights into genetic forms of neurodevelopmental disease.

Disclosures: S. Kanton: None. **X. Meng:** None. **F. Birey:** None. **M. Li:** None. **N. Reis:** None. **P. McQueen:** None. **J. Kim:** None. **M. Thete:** None. **N. Sakai:** None. **S.P. Pasca:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.13/B12

Topic: A.03. Stem Cells and Reprogramming

Support: Lieber Institute for Brain Development
BBRF Young Investigator Grants

Title: Setd1a haploinsufficiency and common schizophrenia risk converge on neurodevelopmental perturbations of chromatin modification and genomic stability

Authors: *T. SAWADA[1], A. S. FELTRIN[1], Y. WANG[1], B. ARAUJO[1], A. E. MCCORD[1], H. GILES[1], S. HAN[1], E. RADULESCU[1], Q. CHEN[1], B. QAMAR[1], A. R. BARBOSA[1], R. S. JACOMINI[1], V. DIMITROVA[2], R. KANEVA[3], V. VLADIMIROV[4], T. M. HYDE[1], D. R. WEINBERGER[1], A. C. M. PAQUOLA[1], J. A. ERWIN[1];

[1]Lieber Inst. for Brain Develop., Baltimore, MD; [2]Univ. Obstetrics and Gynecology Hosp. "Maichin Dom", [3]Dept. of Med. Chem. and Biochem., Med. Univ. of Sofia, Sofia, Bulgaria; [4]Dept. of Psychiatry, Univ. of Arizona, Col. of Med., Phoenix, AZ

Abstract: Heterozygous loss-of-function (LoF) mutations in *SETD1A*, a histone H3 lysine 4 (H3K4) methyltransferase, is one of handful of single gene variants that are robustly associated with risk for schizophrenia (SCZ). Histone methylation is among the most enriched pathways in common SCZ variants. However, like most rare variants, the *SETD1A* LoF variants are pleiotropic and are also genetic risks for early onset neurodevelopmental disorders. Here, to understand how SETD1A LoF leads to SCZ-associated phenotypes, and the relevance to SCZ patients without these rare mutations, we investigate SETD1A-regulated substrates in human prenatal brain using CUT&Tag and investigated biological substrates disrupted in isogenic neuronal models carrying *SETD1A* LoF variants, including the most common pathogenic mutation (*c.4582-2delAG>-*). SETD1A preferentially acts at promoters at common SCZ risk loci regulating histone modification, DNA repair and synaptic function. SETD1A LoF causes a decrease of H3K4me3 and increased genomic instability, via failures in preventing replication stress by binding fragile genes and coordinating transcription of DNA repair. Inhibition of KDM5 H3K4me3 demethylases rescues the phenotype of SETD1A-deficiency. Our findings indicate that SETD1A-dependent maintenance of genomic stability and chromatin modification are a convergent mechanism of risk underlying SCZ pathogenesis and highlight H3K4me3 as a therapeutic target for SETD1A haploinsufficiency and to normalize common variant-associated SCZ risk.

Disclosures: T. Sawada: None. **A.S. Feltrin:** None. **Y. Wang:** None. **B. Araujo:** None. **A.E. McCord:** None. **H. Giles:** None. **S. Han:** None. **E. Radulescu:** None. **Q. Chen:** None. **B. Qamar:** None. **A.R. Barbosa:** None. **R.S. Jacomini:** None. **V. Dimitrova:** None. **R. Kaneva:** None. **V. Vladimirov:** None. **T.M. Hyde:** None. **D.R. Weinberger:** None. **A.C.M. Paquola:** None. **J.A. Erwin:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.14/B13

Topic: A.03. Stem Cells and Reprogramming

Title: Development of an ASO-based therapy targeting UBE3A for a patient with Dup15q Syndrome

Authors: B. TORROBA[1], B. GURKAN[2], M. HILLER[3], O. FEDORENKO[5], L. BUTLER[6], M. BSIBSI[1], E. PERLSTEIN[7], D. MAGNANI[4], D. F. FISCHER[4], R. REDIS[1], ***L. BUTI**[1]; [2]Discovery Sci. NL, [3]Advanced Modalities, [1]Charles River, Leiden, Netherlands; [4]Discovery UK, Charles River, Saffron Walden, United Kingdom; [5]Discovery UK, Charles River Labs.,

Saffron Walden, United Kingdom; [6]discovery UK, Charles river, Saffron Walden, United Kingdom; [7]Perlara, San Francisco, CA

Abstract: Maternal 15q duplication (Dup15q) syndrome is a developmental disorder caused by the presence of at least one extra maternally derived copy of the Prader-Willi/Angelman critical region (PWACR). This region is approximately 5 Mb long and within chromosome region 15q11.2-q13.3. Maternal Dup15q syndrome is characterized by hypotonia and motor delays, variable intellectual disability, autism spectrum disorder and epilepsy. Although ~40 genes are located in the PWACR, evidence supports the overexpression of the ubiquitin-protein E3A ligase (*UBE3A*) gene as the predominant molecular cause of the phenotypes observed in Dup15q syndrome. Therefore, the lowering of UBE3A expression by antisense oligonucleotides (ASOs) might be able to reduce the severity of the symptoms. The goal of this project was to develop an ASO-based therapy for a single Dup15q patient. Non-allele specific ASOs targeting *UBE3A* transcripts were screened in control human fibroblasts using transfection. ASOs with 50% to 96% *UBE3A* knockdown efficiencies were selected for potency determination in fibroblasts, followed by immunotoxicity studies in human peripheral blood mononuclear cells (PBMCs) to minimize the risk of potential inflammatory related adverse events upon administration in the patient. ASOs were further selected based on *UBE3A* mRNA knockdown efficacy and potency in Dup15q iPSC-derived cortical neurons. Ongoing studies are focused on characterizing the functional phenotypes of control and Dup15q patient neurons using Microelectrode Arrays (MEAs), followed by phenotype rescue for lead selection

Disclosures: B. Torroba: None. **B. Gurkan:** None. **M. Hiller:** None. **O. Fedorenko:** None. **L. Butler:** None. **M. Bsibsi:** None. **E. Perlstein:** None. **D. Magnani:** None. **D.F. Fischer:** None. **R. Redis:** None. **L. Buti:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.15/B14

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01 NS111986
Eagles Autism Foundation

Title: Elucidating the role of non-imprinted genes in Dup15q syndrome neuronal phenotypes

Authors: *D. ANJAN KUMAR, T. M. ROBINSON, M. ELAMIN, E. S. LEVINE; Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Dup15q syndrome is caused by maternal duplication or triplication of the chromosome 15q11-q13 region and is characterized by developmental delay, motor deficits, seizures, and autism. Of the duplicated genes in this region, *UBE3A*, which encodes a ubiquitin ligase, is exclusively expressed from the maternal allele in neurons and is believed to be the

major driver for Dup15q syndrome phenotypes. However, overexpression of *UBE3A* alone in mouse models fails to fully recapitulate behavioral phenotypes, suggesting a contributing role of other genes in the region. To gain a better understanding of the pathophysiology underlying this syndrome, we use patient-specific neurons derived from induced pluripotent stem cell lines as well as isogenic CRISPR-corrected lines. Using patch clamp electrophysiology, we have shown that human Dup15q neurons exhibit increased intrinsic excitability, altered action potential properties, and elevated levels of excitatory synaptic activity. We have also found that overexpression of *UBE3A* alone is insufficient to mimic all cellular phenotypes, which suggests a role for other non-imprinted genes in the duplicated region. Non-imprinted genes in this region include a cluster of $GABA_A$ receptor subunit genes (*GABRB3, GARBA5,* and *GABRG3),* and *HERC2*, another ubiquitin ligase, all of which are associated with neurodevelopmental disorders. To evaluate the roles of these genes, we have normalized the expression of *GABRB3* and *HERC2* in Dup15q neurons using antisense oligonucleotides and performed electrophysiological recordings at various developmental time points. Preliminary results suggest that *GABRB3* overexpression is not required for intrinsic hyperexcitability and altered action potential properties. However, normalizing *GABRB3* expression prevented some of the synaptic phenotypes in Dup15q neurons early in neural development, indicating a potential role for this gene in Dup15q. Ongoing experiments will determine if *HERC2* overexpression also contributes to hyperexcitable phenotypes in this syndrome. Identifying the roles played by non-imprinted genes in this region is important for developing more effective therapies and for generating improved mouse models of Dup15q syndrome.

Disclosures: D. Anjan Kumar: None. **T.M. Robinson:** None. **M. Elamin:** None. **E.S. Levine:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.16/B15

Topic: A.03. Stem Cells and Reprogramming

Support: 2021YFF1200800

Title: Induced pluripotent stem cells: human models for studying neurological disorders

Authors: *M. TAO[1], W. ZHU[2], Y. LIU[2,1];
[1]Southeast Univ., Nanjing, China; [2]Nanjing Med. Univ., Nanjing, China

Abstract: Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disease with movement disorder. At present, the causes of PD are still poorly understood and whether it is related to the number of neurotransmitters in a single vesicle remains to be clarified. Here, we performed electrochemical cytometry based on nano-tip microelectrodes to quantify the vesicular storage at the single-cell level in human neurons and midbrain organoids (MOs) which derived

from an induced pluripotent stem cell line from one youngonset Parkinson's disease (YOPD) patient. We show a significant deficiency in vesicular catecholamine storage and a slower pore forming process on the surface of the microelectrode in the DA neurons derived from the YOPD patient. The upregulation of a-synuclein in both neurons and organoids derived from the YOPD patient is associated with vesicular storage dysfunction, revealing a correlation between the pathogenesis of YOPD and vesicular chemical storage deficiency, a novel chemical insight into the potential pathology of YOPD. Notably, efficacy evaluation and drug testing were performed with our platform to demonstrate that both amantadine, a clinical drug for PD, and phorbol 12-myristate 13-acetate, an attractive candidate, ameliorate the dysfunction of vesicular storage in DA neurons derived from the YOPD patient. Our platform offers promising avenues for new drug discovery for PD and other neurodegenerative disorders.In addition to neurodegenerative disorders, the employment of hiPSCs to study mood disorders continues to increase. It may be attributed to hiPSCs can be reprogrammed from patients' samples and can maintain entire genetic information of patients. Major depressive disorder (MDD), one of the mood disorders, is a leading cause of disability, with a global prevalence of 4.4-5.0%. With ever-increasing prevalence of MDD worldwide, a comprehensive exploration of pathogenesis is urgently needed to strengthen the knowledge base of MDD. Several reports have revealed individuals with MDD often display pathological change in midbrain. Hence, we established MDD patients derived MOs and found abnormal sodium and potassium currents, as well as amplitudes of action potentials. Also, increased calcium signaling occurred in MOs derived from MDD. In conclusion, identifying the pathological mechanisms underlying these disorders by hiPSCs plays a key role in discovering novel therapeutic strategies.
Keywords: Human brain organoids, Parkinson disease, Electrochemical cytometry, Major depressive disorder, Midbrain organoids.

Disclosures: **M. Tao:** None. **W. Zhu:** None. **Y. Liu:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.17/B16

Topic: A.03. Stem Cells and Reprogramming

Title: Organoid and cortico-motor assembloid technologies as models for Amyotrophic Lateral Sclerosis.

Authors: *A. MANSFIELD, T. PIO, N. SMITH, R. YU, S. WARIYAR, J. ANDERSEN; Emory Univ., Atlanta, GA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron disease characterized by degeneration of both upper and lower motor neurons. Patient-derived induced pluripotent stem cell (iPSC) technology has enabled the *in vitro* study of non-accessible human cells whilst maintaining complex genetic backgrounds. Here, we show iPSC-derived three-dimensional

organoid and assembloid technologies as models for ALS. We generated and validated region-specific organoids resembling the cortex and spinal cord from human iPSCs derived from ALS patients carrying a hexanucleotide repeat expansion in C9orf72, and compared them to organoids derived from isogenic controls in which the repeat expansion was removed. Transcriptional and molecular characterization revealed cell type-specific differences. In addition, assembly of cortical, spinal cord and muscle organoids to form cortico-motor assembloids allowed us to investigate functional differences between C9orf72 and isogenic motor neurons. Altogether, organoids and assembloids provide a unique opportunity to study cell type-specific phenotypes in a complex system. In the future, this system will allow for the formation of 'hybrid' assembloids, comprising control and disease organoids, to investigate disease progression.

Disclosures: A. Mansfield: None. **T. Pio:** None. **N. Smith:** None. **R. Yu:** None. **S. Wariyar:** None. **J. Andersen:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.18/B17

Topic: A.03. Stem Cells and Reprogramming

Support: 5R00MH119319-04

Title: Investigating the molecular landscape of TCF4-mediated cortical development using region-specific human brain organoids

Authors: *R. C. SIMAMORA[1], X. CHEN[2], S. P. PASCA[2], F. BIREY[1];
[1]Human Genet., Emory Univ. Sch. of Med. Neurosci. Grad. Program, Atlanta, GA; [2]Psychiatry and Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: The assembly of fetal cortical networks represents an essential period during which early principles of circuit formation and function are established. This elaborate process follows a highly orchestrated series of events such as the migration of GABAergic interneurons, their functional integration with glutamatergic counterparts, and activity-dependent maturation of physiological properties. Genetic perturbations that adversely affect any of these processes can lead to aberrant wiring of cortical circuits and various neuropsychiatric disorders, such as autism spectrum disorder, schizophrenia, and epilepsy. Despite its significance, the molecular mechanisms underlying cortical circuit formation remain largely unexplored, primarily due to limited access to human tissue for functional investigations. To address this need, we previously developed a forebrain assembloid system, an in vitro model of the human developing cortex where region-specific forebrain cultures derived from human induced pluripotent stem cells (hiPSCs) are assembled to enable a functional analysis of mid-late gestation phenomena that underlie human cortical assembly. Using forebrain assembloids, we have previously identified the transcription factor TCF4 to be a potential driver of activity-dependent human cortical

interneuron development. TCF4 is a member of the basic helix-loop-helix family of transcription factors (TFs) that interacts with other proneuronal TF to mediate several neurodevelopmental processes. In humans, TCF4 expression in the brain is highest during embryonic development. The human TCF4 gene is heavily spliced with a diverse isoform repertoire and a predicted number of 18 different proteins with unique N-terminal sequences; however, the full extent of functional specialization across different isoforms remains unknown. Here, using Cleavage Under Targets & Release Using Nuclease (CUT&RUN), we first identify the gene targets of TCF4 before and after chemical depolarization. We then examined the differential recruitment of activity-responsive TCF4 isoforms at distinct stages of development. Next, through co-immunoprecipitation, we investigated the repertoire of binding partners that interact with TCF4 to regulate gene expression at different stages of development. Collectively, the present study elucidates the different levels of regulation through which TCF4 dictates human forebrain development at the molecular level and paves the way to better understanding how TCF4-related neurodevelopmental disorders, such as schizophrenia and Pitt-Hopkins Syndrome, might arise in development.

Disclosures: **R.C. Simamora:** None. **X. Chen:** None. **S.P. Pasca:** None. **F. Birey:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.19/B18

Topic: A.03. Stem Cells and Reprogramming

Support: NINDS Grant T32 NS96050

Title: Determining Activity-Dependent Effects in Human Spinal Cord Neurons and Glia Using Organoid Systems

Authors: *T. A. PIO[1], S. S. WARIYAR[1], A. MANSFIELD[1], M. BETTAIAH[2], J. ANSDERSEN[2];
[2]Dept. of Human Genet., [1]Emory Univ., Atlanta, GA

Abstract: Neural activity plays a pivotal role in the development and maintenance of neuronal and glial cell populations in the nervous system. Altered levels of activity within the spinal cord have been associated with conditions such as amyotrophic lateral sclerosis, spinal muscular atrophy, chronic pain, and spinal cord injury. Understanding cell type-specific responses to changes in neuronal activity are essential to identifying novel and effective therapeutic targets in these conditions. Due to lack of access to human tissue for functional studies, the precise role that activity plays on the specification and functional maturation of cell types in the human spinal cord has not been well defined. Here, we aim to investigate the cell type-specific effects of altered neuronal activity in the human spinal cord. Spinal cord organoids, derived from human induced pluripotent stem cell (hiPSCs), are three-dimensional spheroids which contain a

diversity of cell types including motor neurons, interneurons, astrocytes, and myelinating oligodendrocytes. These can be kept in culture long-term and allow for the interrogation of complex human cell-cell interactions. We used optogenetics to chronically stimulate neurons within these organoids, and then we monitored changes in gene expression, morphology, and function with a focus on motor neurons and glia. Chronic stimulation of spinal cord organoids followed by single cell RNA sequencing revealed cell type-specific responses, as well as an upregulation of markers associated with maturity. Understanding the interplay between neural activity and developing cellular populations is crucial for unraveling the complexities of spinal cord formation, function, and pathology. Overall, our findings highlight the utility of using hiPSC-derived spinal cord organoids as a model system to investigate the complex interplay between diverse neuronal and glial populations.

Disclosures: **T.A. Pio:** None. **S.S. Wariyar:** None. **A. Mansfield:** None. **M. Bettaiah:** None. **J. Ansdersen:** None.

Poster

PSTR448. Modeling Development and Disease with Human Pluripotent Stem Cells

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR448.20/B19

Topic: A.03. Stem Cells and Reprogramming

Support: MOST 111-2314-B-007-002-MY3

Title: Characterizing the effect of neuron's magnetic field by using magnetic nanoparticles in human neural iPSCs

Authors: *T.-Y. CHU[1], T.-C. YANG[2], C.-C. CHIAO[3];
[1]Natl. Tsing Hua Univ., Hsinchu County, Taiwan; [2]Taipei Med. Univ., Taipei, Taiwan; [3]Natl. Tsing Hua Universtiy, Hsinchu, Taiwan

Abstract: Neuron’s magnetic field is a fascinating topic that is still being explored by scientists, researchers, and medical professionals. It is the magnetic field generated by the electrical activity of neurons in the brain and other areas of the body. The magnetic field can be detected by sensitive instruments, and it provides valuable insights into how the brain functions and communicates with other parts of the body. In the present study, we showed that neural organoids developed from human iPSCs also exhibit neural magnetic field. When magnetic nanoparticles (MNPs, 1 mg/ml) were put on the retinal organoids derived from human iPSCs for 2 mins, it was observed that the particles started to move toward to the strong magnetic field that generated by the organoid. In addition, when those organoids were at better and heathier condition, they attracted more particles on them. After 30 mins, those particles were arranged in a certain pattern that surrounded the organoid periphery. To clarify if the arrangement was due to the magnetic field, we used a different kind of nanoparticles which have no magnetism but with very similar characteristics and particle size as the previous one. The results showed that those

particles with no magnetism did not surround the organoid periphery but only scattered randomly in the medium after 2 mins. These findings demonstrated that the particles were indeed arranged by the magnetic field generated by the neural organoid.

Disclosures: T. Chu: None. **T. Yang:** None. **C. Chiao:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.01/B20

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH122485
NIH Grant F31 MH131259
NIH Grant T32 MH019113

Title: Single-cell RNA sequencing and behavioral assessment show altered dorsal striatal development in a prenatal stress mouse model

Authors: *M. EVANS[1,2], S. V. MAURER[2], B. W. Q. HING[2,3], M. A. WEBER[4], N. S. NARAYANAN[4], H. E. STEVENS[2,5];
[1]The Univ. Of Iowa Neurosci. Grad. Program, Iowa City, IA; [2]Psychiatry, [3]Mol. Physiol. and Biophysics, [4]Neurol., [5]Hawkeye Intellectual and Developmental Disabilities Res. Ctr., Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: Background: Many people with autism spectrum disorder (ASD) show abnormalities of the dorsal striatum, a forebrain structure known for its roles in motor skill and learning. These abnormalities may contribute to restricted, repetitive behavior, a diagnostic criterion for ASD. The current work investigates striatal-dependent, ASD-relevant behaviors, as well as cellular and transcriptomic phenotypes of the dorsal striatum in adult mice in a prenatal stress (PS) model, as PS is a risk factor for neurodevelopmental disorders including ASD. Methods: CD-1 female mice were time-mated with GAD67-GFP+ CD-1 males. From embryonic days 12-18, pregnant dams underwent repetitive restraint stress under bright light (PS; n = 15) or saline injections (SAL; n = 13) three times daily. 8-12-week-old GFP+ offspring were tested on open field and rotarod (n = 10-11/condition and sex), and some then trained and tested on interval timing (n = 8-9/condition and sex). 8-12-week-old littermate GFP- male offspring dorsal striatum was used for single-cell RNA sequencing (scRNAseq) (n = 4/condition) using the 10x Genomics Chromium Single-Cell System with processing by Cell Ranger, Seurat in RStudio, and Nebula. 8-12-week-old littermate GFP- female offspring dorsal striatum was used for bulk RNA sequencing (RNAseq). Pathway analysis was done using DAVID and PANTHER. Immunohistochemistry for substance P, enkephalin, and GFP was used to label and stereologically count adult littermate dorsal striatal medium spiny neurons (MSNs). Results: PS offspring showed no differences in open field distance traveled (ANOVA PS main effect; p = 0.35) but showed higher rotarod motor skill

compared to controls (ANOVA PS main effect; $p = 0.04$). PS offspring also showed faster interval timing (ANOVA PS main effect; $p = 0.02$). In males, scRNAseq showed differential gene expression in multiple striatal cell types, implicating changes in ribosome structure and function, synaptic regulation, and more. There was significant overlap between SFARI ASD-risk genes and differentially expressed genes in both Drd1 (Fisher's exact test; $p = 2.01^{-19}$) and Drd2 (Fisher's exact test; $p = 1.05^{-12}$) MSNs after PS. Transcriptomic analysis of female striatum is ongoing. Pilot studies suggested no group differences in adult dorsal striatal volume or in GAD67GFP+ or substance P+ cell density. Enkephalin analysis is ongoing. Conclusions: Striatal outcomes, particularly in ribosomal and synaptic changes, are critical aspects here and across models of ASD. Future studies will investigate PS effects on ribosomes in specific striatal cell types as potential mechanisms to target with novel interventions.

Disclosures: **M. Evans:** None. **S.V. Maurer:** None. **B.W.Q. Hing:** None. **M.A. Weber:** None. **N.S. Narayanan:** None. **H.E. Stevens:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.02/B21

Topic: A.07. Developmental Disorders

Support: T32MH019113
R01MH122485

Title: Threshold effects of prenatal stress on inhibitory systems and relevant behaviors

Authors: *S. V. MAURER[1,2], M. M. EVANS[1,3,2], B. W. Q. HING[1,4,2], J. L. INMAN[1,2], H. E. STEVENS[1,5,2];
[1]Psychiatry, [2]Iowa Neurosci. Inst., [3]Interdisciplinary Grad. Program in Neurosci., [4]Mol. Physiol. and Biophysics, [5]Intellectual and Developmental Disabilities Res. Ctr., Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: Prenatal stress (PS), a risk factor for neurodevelopmental disorders (NDDs), leads to alterations in neuronal inhibitory systems which may influence behavior. However, there are multiple PS models with various impacts. Our previous work showed that different PS models lead to varied inhibitory system outcomes which may arise from different "thresholds" of stress effects. Therefore, we sought to determine thresholds for PS model impacts on inhibitory systems and behavioral outcomes using "low," "high," and single component "immune" PS. CD1 female mice mated to CD1 GAD67GFP+/- males were assigned to four groups (each: N=6): no PS, saline injections (i.p., "low" PS), restraint stress + saline injections ("high" PS), and IL6 injections to simulate increased maternal IL6 as a PS component ("immune" stress; 100 ng/injection). 45 min restraint stress in a plexiglass tube under bright light and/or injections occurred 3x daily beginning embryonic day 12 (E12) until E13 or birth. Immunocytochemistry,

H&E staining, and stereology were used to assess brain and placental outcomes. Inhibitory system-dependent behaviors were assessed: stereotypy, rotarod motor learning, and water T-maze habit learning and cognitive flexibility. All three PS models decreased GAD67GFP+ cell migration into dorsal forebrain, indicating a low threshold for this effect of PS. In contrast, only "high" PS (not "low" or "immune") increased Ki67+ cell density in ganglionic eminence as previously shown. These effects were only observed in male, not female, offspring. Though few effects were found in adolescent, female, or "low" PS behavior, "high" PS significantly changed adult stereotyped behaviors, rotarod learning, and water T-maze cognitive flexibility in males. "Immune" PS recapitulated stereotypy and rotarod effects of "high" PS. Interestingly, we saw similar thresholds of effect in placenta, with no morphology effects of "low" PS but some similarity in labyrinth zone reduction from "high" and "immune" PS, suggesting decreased nutrient exchange. Placental macrophage and brain microglia (which critically alter inhibitory systems) analysis is ongoing. In conclusion, all models, including "low" PS, impacted inhibitory progenitor migration- indicating a low threshold for this effect. Only "high" PS altered inhibitory progenitor proliferation, indicating a high threshold for this effect. Inhibitory system-dependent behavioral differences were observed with "high" and "immune" but not "low" PS, suggesting that some initial "low" threshold inhibitory system changes may be compensated for later. Distinct levels and aspects of PS may therefore underlie varied links with NDDs.

Disclosures: S.V. Maurer: None. **M.M. Evans:** None. **B.W.Q. Hing:** None. **J.L. Inman:** None. **H.E. Stevens:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.03/B22

Topic: A.07. Developmental Disorders

Support: NJ Governor's Council for Medical Research and Treatment of Autism CAUT17BSP011 [to VS & TST]
NJ Governor's Council for Medical Research and Treatment of Autism CAUT17BSP022 [to TST & MS]
Rutgers BHI-CAHBIR [to TST & VS]
NIH R01 NS069861 [to VS]
NSF/IOS: 1556968 & 2034864 [to TST]
AES 957615 [to AH]

Title: Autism-epilepsy phenotype in mice with developmental interneurons specific deletion of neuropilin-2.

Authors: *C. EISENBERG[1,2], D. SUBRAMANIAN[4], A. HUANG[4], J. BAEK[2], H. NAVEED[2], M. SHIFLETT[3], V. SANTHAKUMAR[4], T. S. TRAN[2];

[1]Biol. Sci., Rutgers University, Newark, Newark, NJ; [2]Biol. Sci., [3]Psychology, Rutgers Univ., Newark, NJ; [4]UC Riverside, Riverside, CA

Abstract: Early developmental perturbations in circuit formation have been proposed to underlie autism spectrum disorders (ASDs) and epilepsy. We previously demonstrated that mice with global constitutive knockout (KO) of Neuropilin 2 (Nrp2), a secreted semaphorin receptor essential for neural circuit formation and synapse maintenance, exhibit abnormal social interactions and increased seizure susceptibility. Developmentally, Nrp2 is expressed in inhibitory neuron precursors in the median ganglionic eminence and regulates interneuron migration to the pallium, including the hippocampus. Here we examined the specific effect of developmental deletion of Nrp2 in inhibitory neuron progenitors on hippocampal circuit function. Consistent with the role for Nrp2 in interneuron migration, *Nrp2f/f::Nkx2.1-CreERT2* (iCKO) mice, where Nrp2 is specifically deleted in inhibitory neurons precursors during the period of migration to the pallium, showed significant reduction in parvalbumin, neuropeptide Y, and somatostatin positive neurons in the CA1 of the hippocampus compared to littermate controls. Although CA1 neurons from WT and iCKO mice did not differ in intrinsic passive and active membrane properties, sIPSC and mIPSC frequency was reduced in iCKO mice. Interestingly, sEPSC but not mEPSC frequency was reduced in CA1 neurons from iCKO animals. At the functional level, the circuit changes in excitation and inhibition contributed to a reduction in latency to kainic acid evoked seizures in the iCKO mice. Importantly, iCKO mice exhibited behavioral deficits compared to littermate control in both social preferences assessed with the 3-chambered area test and goal-directed learning with an instrumental conditioning task. Taken together, our data demonstrates that Nrp2 signaling is required cell-autonomously during interneuron migration, and that when perturbed results in the reduction of specific interneuron populations in the hippocampus. Decreased interneuron numbers altered synaptic inhibition to hippocampal CA1 pyramidal neurons without impacting their intrinsic physiology. Our findings identify that early disruption of processes critical for interneuron circuit establishment can lead to social deficits, impaired learning, and increased seizure susceptibility consistent with ASD/epilepsy phenotype.

Disclosures: C. Eisenberg: None. **D. Subramanian:** None. **A. Huang:** None. **J. Baek:** None. **H. Naveed:** None. **M. Shiflett:** None. **V. Santhakumar:** None. **T.S. Tran:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.04/B23

Topic: A.07. Developmental Disorders

Support: NINDS R00NS114166
NINDS R01NS133434
Brain & Behavior Research Foundation Young Investigator Award
GR114536

Title: Neural Mechanisms of Tactile Sensory Processing Deficits in a mTORopathy

Authors: *M. EDWARD[1], S. A. GETZ[2], J. A. VARGAS ORTIZ[4], A. CHE[3], A. F. BORDEY[5]; [1]Neurosurg. and Psychiatry, Yale Univ., New Haven, CT; [2]Yale, [3]Psychiatry, Yale, New Haven, CT; [4]Psychiatry, Yale Med. Sch., New Haven, CT; [5]Dept Neurosurg, Yale Sch. Med., New Haven, CT

Abstract: Abnormal sensitivity to sensory stimuli is one of the most prevalent features in individuals with autism spectrum disorders (ASD). Dysfunction in tactile processing, in particular, has been closely linked to emotional and social distress, exacerbating social deficits associated in ASD. Despite their impact and prevalence, little is known about the neural mechanisms underlying tactile processing deficits. In this study, we examined the impact of constitutively active (CA) Rheb, an ASD risk gene encoding a small GTPase that directly activates mTOR complex 1 (mTORC1), on circuit connectivity and tactile sensory responses in mouse primary somatosensory cortex (S1). mTORC1 signaling pathway is well-conserved for all aspects of brain development and is dysregulated in individuals with idiopathic ASD. Using in utero electroporation (IUE), we selectively expressed RhebCA in S1 layer (L) 2/3 pyramidal neurons resulting in increased mTORC1 activity. We examined how this manipulation impacted the circuitry and network activity and ultimately tactile sensory encoding in S1 using a combination of approaches including in vivo two-photon imaging in awake mice. At postnatal day (P) 28, we identified a set of cellular changes including cytomegaly, neuronal misplacement, increased local axonal and dendritic complexities associated with increased dendritic coverage of neighboring barrel columns, but no change in barrel formation. RhebCA neurons also displayed increased rheobase and loss of spines but increased spine head area.These cellular changes were accompanied with altered spontaneous network activity and whisker-evoked responses in vivo. These findings suggest that RhebCA-mediated mTORC1 overactivation leads to S1 mis-wiring and compromised sensory function, providing a potential neural mechanism underlying sensory processing deficits in ASD.

Disclosures: M. Edward: None. **S.A. Getz:** None. **J.A. Vargas Ortiz:** None. **A. Che:** None. **A.F. Bordey:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.05/B24

Topic: A.07. Developmental Disorders

Support: BBRF Young Investigator Grant 30131

Title: Effect of Chd7 Haploinsufficiency in Lineage-specific Mouse Neocortical Porgenitor and Progenies

Authors: *Z. LI, A. HORVATH, L. MCCARTHY, T. F. HAYDAR;
Children's Natl. Med. Ctr., Washington, DC

Abstract: CHD7 haploinsufficiency is the leading cause of CHARGE Syndrome and has been associated with Autism Spectrum Disorders. However, the pathological mechanism and the function of CHD7 in neurodevelopment is still elusive. CHD7 is highly conserved across vertebrates and shows a temporally and spatially restricted expression pattern in the proliferative zone of the developing mouse neocortex, indicating its fundamental importance in neurodevelopment. Bulk tissue ATACseq and ChIPseq on cultured neural progenitor cells indicate that CHD7 knockout leads to a reduction in chromatin accessibility, particularly around genes critical to neurogenesis and differentiation. My published and unpublished data demonstrated that Chd7 is expressed in the ventricular zone (VZ) and subventricular zone (SVZ) of the embryonic mouse neocortex, as well as in post-migratory neurons. Intermediate progenitor cells (IPCs) show an elevated level of Chd7 expression compared to other cell types and knocking down Chd7 in VZ/SVZ alters the distribution of progenitor cell types and may lead to neuronal migratory defects. Previous studies from the Haydar and other labs have shown that neurons produced by IPCs are primarily cortico-cortical projection neurons (CPNs) in layer 2/3 with distinct morphology and a tonic firing pattern.

Disclosures: Z. Li: None. **A. Horvath:** None. **L. McCarthy:** None. **T.F. Haydar:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.06/B25

Topic: A.07. Developmental Disorders

Support: FRAXA Research Foundation
NIH: NS112706-01

Title: C-subunit mitochondrial leak channel affects synaptic plasticity and behavior

Authors: *P. LICZNERSKI, V. K. GRIBKOFF, L. SHEN, E. A. JONAS;
Yale Univ., New Haven, CT

Abstract: Dysfunction of the gene (*Fmr1*) encoding Fragile X mental retardation protein (FMRP) triggers high levels of mRNA translation, aberrant synaptic development, behavioral disorders and neuronal hyperexcitability in Fragile X patients. We have recently discovered that a leak channel in the inner mitochondrial membrane formed by the ATP synthase c-subunit octamer drives key characteristics of the Fragile X phenotype. We showed that inhibition of leak channel activity restores stimulus-induced and constitutive mRNA translation rates, decreases lactate and key glycolytic and tricarboxylic acid (TCA) cycle enzyme levels, normalizes autistic behaviors, and promotes synapse maturation. Now, we would like to further characterize the role of the leak channel in the pathophysiology of Fragile X. In the current study, we test how

increased neonatal overexpression (P0-P1) of the leak channel in the mouse brain affects phosphorylation of synaptic proteins, and consequently synaptic development, synaptic plasticity and behavior.

Disclosures: P. Licznerski: None. **V.K. Gribkoff:** None. **L. Shen:** None. **E.A. Jonas:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.07/B26

Topic: A.07. Developmental Disorders

Title: Genetic deletion of soluble epoxide hydrolase restores behavioraland synaptic abnormalities in an animal model of autism

Authors: *M. CHU[1,2], C.-W. LEE[2], C.-C. WU[2], H. CHI[2], H.-C. LIN[2,3];
[2]Dept. and Inst. of Physiol., [3]Brain Res. Ctr., [1]Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social defects often accompanied with emotional comorbidities. Aberrations in synaptic function and plasticity are the core feature in the pathophysiology of ASD. Targeting soluble epoxide hydrolase (sEH) has been found to exert protection in a wide-range of pathological conditions. However, the regulation of sEH deficiency on the synaptic deficits of ASD and the underlying mechanisms remain unclear. The valproate (VPA)-induced ASD animal model with genetic sEH knockout was applied in the present study. The results showed that the sEH expression and activity were significantly increased in the prefrontal cortex of VPA-induced ASD animals. Although no effect was found on tail malformation and body weight loss, genetic sEH deletion alleviated social deficits, and fear learning and memory extinction in the VPA-induced ASD mice. After a series of electrophysiological assessments, we found that the beneficial effects of sEH deletion focused on the long-term synaptic plasticity, rather than presynaptic efficiency, in the VPA-induced ASD mice. Furthermore, we observed that the dysregulated AMPK-mTOR pathway was restored under genetic sEH deletion in VPA-induced ASD mice. Taken together, these findings uncovered an important role of sEH deficiency in the synaptic dysfunctions of ASD mediated by AMPK-mTOR pathway, providing a novel therapeutic target for ASD.

Disclosures: M. Chu: None. **C. Lee:** None. **C. Wu:** None. **H. Chi:** None. **H. Lin:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.08/B27

Topic: A.07. Developmental Disorders

Support: Action Potential Grant FamilieSCN2A Foundation
BioMarin Pharmaceutical Inc.
Weill Neurohub Investigator Program
SFARI Grant 629287
NIH Grant MH125978

Title: Impaired cerebellar plasticity hypersensitizes sensory reflexes in SCN2A-associated ASD

Authors: *C. WANG[1], K. J. BENDER[2], G. BOUVIER[3], K. DERDERIAN[1], E. HAMADA[1], X. ZHOU[1], A. NELSON[4], H. KYOUNG[5], N. AHITUV[1];
[2]Dept. of Neurol., [1]UCSF, San Francisco, CA; [3]Physiol., Univ. of California, San Francsico, San Francisco, CA; [4]Neurol., Univ. of California San Francisco, San Francisco, CA; [5]Harvard Univ., Cambridge, MA

Abstract: Children diagnosed with autism spectrum disorder (ASD) commonly present with sensory hypersensitivity, or abnormally strong reactions to sensory stimuli. Such hypersensitivity can be overwhelming, causing high levels of distress that contribute markedly to the negative aspects of the disorder. Here, we identify the mechanisms that underlie hypersensitivity in a sensorimotor reflex found to be altered in humans and in mice with loss-of-function in the ASD risk-factor gene *SCN2A*. The cerebellum-dependent vestibulo-ocular reflex (VOR), which helps maintain one's gaze during movement, was hypersensitized due to deficits in cerebellar synaptic plasticity. Heterozygous loss of *SCN2A*-encoded $Na_V1.2$ sodium channels in granule cells impaired high-frequency transmission to Purkinje cells and long-term potentiation, a form of synaptic plasticity important for modulating VOR gain. VOR plasticity could be rescued in adolescent mice via a CRISPR-activator approach that increases *Scn2a* expression, highlighting how evaluation of simple reflexes can be used as quantitative readout of therapeutic interventions.

Disclosures: C. Wang: None. **K.J. Bender:** 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.; BioMarin Pharmaceutical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regal Therapeutics. **G. Bouvier:** None. **K. Derderian:** None. **E. Hamada:** None. **X. Zhou:** None. **A. Nelson:** None. **H. Kyoung:** None. **N. Ahituv:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regal Therapeutics.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.09/B28

Topic: A.07. Developmental Disorders

Support: 2018SHZDZX05
#82021001

Title: The neural circuit mechanism underlying Cntnap3 regulation of social behavior in autism disease model

Authors: *Z. LI[1], X. XU[1], Z. QIU[2];
[1]Ctr. for Excellence in Brain Sci. and Intelligence Technol. (Institute of Neuroscience), Chinese Acad. of Sci., Shanghai, China; [2]Shanghai Jiaotong Univ. Sch. of Med. Songjiang Inst., Shanghai, China

Abstract: Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental disorder characterized by social communication impairments and repetitive behaviors. Neurobiological research into ASD has largely centered on changes in cortical development, neuronal circuitry, and synaptic function. Our prior research revealed that CNTNAP3, an ASD-related gene and member of the contactin associated protein-like (CNTNAP) family, is instrumental to normal synaptic development and transmission. Notably, Cntnap3-deficient mice displayed social behavior deficiencies, stereotypic behaviors, and cognitive task challenges, further supporting its involvement in ASD. In this study, we investigated the expression pattern of Cntnap3 and its functional role in specific brain regions. We found that Cntnap3 is abundantly expressed in the claustrum (CLA), medial thalamus, and ventral hippocampus. We demonstrated that Cntnap3 in the CLA is essential for normal social behavior, as evidenced by selectively knocking out Cntnap3 in the CLA of wild-type mice and re-introducing human Cntnap3 in the CLA of Cntnap3-deficient mice. Additionally, we discovered that suppressing neuronal activity in the claustrum lessens social behavior. Interestingly, neurons in the CLA projecting to the retrosplenial cortex (RSC), primarily express Cntnap3, and chemogenetic manipulation of the CLA-RSC connectivity also influenced social behavior. Together, these findings provide valuable insights into the role of the claustrum circuitry in an ASD mouse model, shedding light on the mechanisms underlying social behavior regulation.

Disclosures: Z. Li: None. **X. Xu:** None. **Z. Qiu:** None.

Poster

PSTR449. Synaptic and Cellular Mechanisms of Autism II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR449.10/B29

Topic: A.07. Developmental Disorders

Support: CONACyT Grant CB-2016-281617

Title: Maternal immune activation disturbs spike generation temporal fidelity and triggers social and anxiety-like abnormalities in the offspring

Authors: *E. GRIEGO[1], C. CERNA[2], I. SOLLOZO-DUPONT[3], M. FUENZALIDA[2], E. J. GALVAN[1];
[1]Farmacobiología, CINVESTAV, Mexico City, Mexico; [2]Inst. de Fisiología, Univ. De Valparaíso, Valparaiso, Chile; [3]Inst. Nacional de Cancerología, Mexico, Mexico

Abstract: Maternal immune activation (MIA) is considered a risk factor for the development of neuropsychiatric disorders associated with neurodevelopmental alterations, such as autism and schizophrenia. A growing body of evidence in rodents and non-human primates has shown that MIA caused by either viral or bacterial infections results in several neurobiological alterations in the offspring. These changes may play an important role in the pathophysiology of neuropsychiatric disorders like schizophrenia and autism spectrum disorders, which have clinical features that include changes in cognitive processing and social performance. Such alterations are causally associated with the maternal inflammatory response to infection rather than with the infection itself. However, potential neurophysiological alterations at the cellular and synaptic levels in areas of critical relevance in the pathophysiology of MIA-associated disorders such as the hippocampus, have not been fully characterized. In this studie we aim to identify electrophysiological, morphological, and molecular alterations of pyramidal neurons of the CA1 region of the mouse dorsal hippocampus in a model of bacterial LPS-induced MIA. Our data provide evidence that MIA increases neuronal excitability and reduces the morphological complexity of pyramidal neurons of the offspring. Likewise, LPS-induced MIA reshapes the excitation-inhibition balance, by decreasing the perisomatic GABAergic inhibition impinging on CA1 pyramidal neurons. These alterations yield to a dysregulated amplification of the temporal and spatial synaptic integration. In addition, MIA-exposed offspring displayed social and anxiety-like abnormalities. Taken together, the findings reported in this work help to explain the cellular and synaptic underpinnings underlying the behavioral symptoms present in neurodevelopmental disorders associated with MIA.

Disclosures: E. Griego: None. **C. Cerna:** None. **I. Sollozo-Dupont:** None. **M. Fuenzalida:** None. **E.J. Galvan:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.01/B30

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: K02NS112456-01A1

Title: Tbck, a regulator of the endolysosomal pathway

Authors: *M. FLORES-MENDEZ[1,2], J. A. TINTOS-HERNÁNDEZ[1,2], L. RAMOS-RODRÍGUEZ[3], X. R. ORTIZ-GONZÁLEZ[1,2,4,5];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Ctr. for Mitochondrial and Epigenomic Med., Philadelphia, PA; [3]Dept. of Biomed. Grad. Studies, Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Pediatrics, Div. of Neurology, The Children's Hosp. of Philadelphia, Philadelphia, PA; [5]Dept. of Neurology, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Congenital disorders of autophagy (CDA) are an emerging group of inborn errors of metabolism that predominantly affect the central nervous system, leading to pediatric neurodegeneration. Our previous work identified TBCK as a human recessive disorder that disrupts autophagy and lysosomal function in patient's fibroblasts. Since the physiologic function of TBCK is unclear, we used patient derived fibroblasts with biallelic null variants (p.R126X), as well as iPSC-derived neurons, to investigate the role of TBCK. We performed mass spectrometry of immunoprecipitated TBCK to reveal its interacting partners and elucidate its potential cellular function. Our interactome data show that TBCK interacts with PPP1R21, C12ORF4, CRYZL1, consistent with a recently reported FERRY complex that mediated mRNA transport on early endosomes. We also find novel interactors JIP4 and TRIM27. We found loss of TBCK is associated with significant reduction of C12ORF4 protein levels, which is ameliorated by proteosome inhibitors. We found TBCK colocalizing with early endosomes (Rab5 and EEA1), retromer complex proteins (VPS35), late endosomes (Rab7) and lysosomes (Lamp1/2). This suggests TBCK interacts with multiple endolysosomal compartments. Moreover, TBCK-deficient cells showed aberrant size and distribution of early endosomes, with large vesicles accumulating around the nucleus. Vesicles expressing markers associated with the retromer complex (TRIM27 and VPS35) have similar alterations. This suggest TBCK may play a role in endolyosomal maturation and/or trafficking. We also observed that alterations in the endolysosomal flux impair RNA transport, which is stalled around the nucleus. Our data suggest that TBCK is involved in maturation, distribution and/or trafficking of endolysosomal compartments, which may impair RNA transport along these organelles.

Disclosures: M. Flores-Mendez: None. **J.A. Tintos-Hernández:** None. **L. Ramos-Rodríguez:** None. **X.R. Ortiz-González:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.02/B31

Topic: A.07. Developmental Disorders

Support: INBRE Nemours Foundation Funding

Title: Cell death, microglial changes, and behavioral deficits in a two-hit mouse model of hypoxic encephalopathy

Authors: *E. LEMANSKI[1], V. LANGDON[2], B. COLLINS[2], E. WRIGHT-JIN[2];
[1]Univ. of Delaware, Newark, DE; [2]Nemours Children's Hosp., Wilmington, DE

Abstract: Hypoxic ischemic encephalopathy (HIE) is one of the most common and serious causes of neurological deficits in children born at term. HIE can lead to diffuse brain injury often impacting white matter and deep brain regions such as the basal ganglia. This injury results in severe, lifelong motor, cognitive, and memory deficits, and increases the risk of developmental disorders. The standard animal model of HIE, the Rice-Vannucci model, combines postnatal unilateral carotid artery ligation with moderate hypoxia which results in a phenotype similar to a large unilateral ischemic stroke that is not representative of HIE in humans. Our new model of HIE pairs maternal immune activation (MIA) via LPS administration on gestational day (GD) 18 with a progressive hypoxia from 21% to 0% oxygen for 8 total minutes on postnatal day 6 (P6). Control animals underwent saline injection and normoxia (21% oxygen). As sex differences have been reported in both humans and in animal models of HIE with worse outcomes reported in males, sex is statistically fully powered and analyzed as a biological variable. This two-hit model leads to delays in the acquisition of neonatal behaviors including spontaneous locomotion and hindlimb splay as well as changes in adulthood including motor deficits, cognitive deficits, and changes in anxiety-like behaviors. In order to establish the timeline of cellular changes within the brain that may lead to these long-lasting deficits, we are analyzing neonatal brains collected 24-, 48-, and 72- hours following hypoxia on P6. Apoptosis in the basal ganglia and hippocampus is assessed through cresyl violet staining. We hypothesize that immune activation following the two hits of our model will lead to an increase cell death that will peak within this time. As a preliminary measure of time dependent neuroimmune changes within our model, microglia density will additionally be analyzed at these three timepoints. These results will facilitate the further validation of our new model. The development and characterization of translationally relevant models is important for progress in understanding the cellular and molecular mechanisms underlying disease as well as in the development of therapeutic interventions.

Disclosures: E. Lemanski: None. **V. Langdon:** None. **B. Collins:** None. **E. Wright-Jin:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.03/B32

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01-ES025549
NIH Grant 1F31NS130757
Cure Alzheimer’s Fund

Title: Neuron-microglia crosstalk in development: A new role for the neuron-derived cytokine IL34 in microglial function

Authors: *B. DEVLIN, D. NGUYEN, G. GRULLON, M. CLARK, A. CEASRINE, M. DEJA, A. SHAH, S. BILBO;
Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: Crosstalk between neurons and microglia is critical for the proper development of the central nervous system. Interleukin-34 (IL34) is a neuron-derived cytokine that signals through the colony-stimulating factor 1 receptor on microglia. While it is known that IL34 KO mice lack most cortical microglia in adulthood, nothing is known about the role of IL34 signaling in development, or if it may influence microglia-neuron interactions such as synaptic pruning. Using qPCR, immunohistochemistry, and ELISA techniques, we measured IL34 mRNA and protein across mouse brain development. Additionally, we used an IL34 knock-out mouse and function-blocking antibodies to determine the role of IL34 in the development of microglia. Finally, to investigate whether IL34 expression is dependent on neuronal activity, we used designer receptors exclusively activated by designer drugs (DREADDs) to activate and inhibit neurons and measured IL34 mRNA and protein levels with in situ hybridization and ELISA. First, we found that IL34 mRNA and protein levels increase between P8 and P15 in the mouse cortex. Additionally, we demonstrated that IL34 is necessary for the developmental upregulation of microglial homeostatic protein TMEM119. IL34 KO mice exhibit decreased USVs compared to controls at postnatal day 15 (P15), and their microglia are less ramified and have a higher phagocytic capacity compared to controls. Administering a function-blocking antibody against IL34 at P15 phenocopies these effects. Neuronal activation with DREADDs at P10 increased Fos+ cell number in the cortex, and we found that Fos+ neurons have more IL34 compared to Fos- neurons. My current data supports the working hypothesis that IL34 is a signal that instructs microglia to mature, potentially acting as a “brake” to prevent over-pruning of synapses. Ongoing work is examining the functional consequences of IL34 signaling for microglia/neuron interactions, including synaptic engulfment.

Disclosures: **B. Devlin:** None. **D. Nguyen:** None. **G. Grullon:** None. **M. Clark:** None. **A. Ceasrine:** None. **M. Deja:** None. **A. Shah:** None. **S. Bilbo:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.04/B33

Topic: A.07. Developmental Disorders

Support: NIH NINDS 1F99NS130926
NIH TL1TR003019
NJGOV CAUT22AFP011

NJHF PC 124-22
Busch Biomedical Grant
Lurie Family Foundation
Mindworks Charitable Lead Trust
Emch Fund for Microbial Diversity
C&D Fund
NCI-CCSG P30CA072720-5923

Title: The interplay between genetic vulnerability and environmental exposures on the infant gut microbiome-brain axis: Implications for neurodevelopmental disorders

Authors: *C. R. MCDERMOTT[1], Z. GAO[2], A. MIRMAJLESI[3], K. KIMBARK[5], C. NTIM[3], Z. MUGHAL[3], X.-S. ZHANG[2], X. ZHOU[1], J. H. MILLONIG[1], B. A. SAMUELS[4], M. J. BLASER[2], E. M. DICICCO-BLOOM[6];
[1]Neurosci. & Cell Biol., Rutgers Univ. Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Ctr. for Advanced Biotech. & Med., Rutgers Univ., Piscataway, NJ; [3]Cell Biol. & Neurosci., Rutgers Univ., New Brunswick, NJ; [4]Psychology, Rutgers Univ., Piscataway, NJ; [5]Biol., Lebanon Valley Col., Annville, PA; [6]Dept Neurosci & Cell Biol/ Pediatrics (Neurology & Developmental Disabilities), Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Background: Infancy is a critical period of development in which the gut microbiome-brain axis is exquisitely sensitive to both genetic risk and environmental exposures. Such alterations during early life can lead to adverse health outcomes and neuropsychiatric conditions. In fact, exposure to cephalosporin antibiotics during infancy has been recently linked to increased incidence of autism spectrum disorder (ASD). Significantly, not all infants exposed to antibiotics are later diagnosed with ASD, suggesting a contributing role of genetic vulnerability. To examine a gene by environment (GxE) basis for this observation, we studied the 16p11.2 copy number variation deletion (16pDel) mouse, which contributes to ~1% of ASD cases. We hypothesized that early life cephalosporin exposure would alter the gut microbiome-brain axis, with an exacerbated phenotype in genetically vulnerable 16pDel mice. **Methods:** Wildtype (WT) and 16pDel littermates were exposed to saline (control) or the cephalosporin, cefdinir, from P5-9. Alterations in gut microbial composition, metabolomics, hippocampal neurogenesis and gene expression were assessed at P13, in addition to juvenile sociability at P21. **Results:** Oral administration of cefdinir perturbed the gut microbiome (dysbiosis) in all offspring, evidenced by decreased microbial richness. Intriguingly, dysbiosis in the cefdinir-exposed 16pDel males was accompanied by reduced hippocampal proliferation and altered gene expression. These phenotypes were not detected in 16pDel females or in the WT male and female groups. 11 of the 118 differentially expressed genes in the hippocampus overlapped with human ASD genes, with ingenuity pathway analysis predicting changes in EIF2, mTOR, and P70S6K/EIF4 signaling. Pathway analysis also revealed mitochondrial dysfunction and altered oxidative phosphorylation, complementing the detected increased serum pyruvate and uridine metabolite levels. Reductions in sociability at P21 were observed in all cefdinir-exposed mice, with an exacerbated phenotype in the 16pDel group. **Conclusions:** Together, our data supports a GxE model, suggesting a role of early life cefdinir exposure in 16pDel mice by inducing altered gut microbiome signaling to the brain, mediating altered neurodevelopmental pathways with functional consequences.

Disclosures: **C.R. McDermott:** None. **Z. Gao:** None. **A. Mirmajlesi:** None. **K. Kimbark:** None. **C. Ntim:** None. **Z. Mughal:** None. **X. Zhang:** None. **X. Zhou:** None. **J.H. Millonig:** None. **B.A. Samuels:** None. **M.J. Blaser:** None. **E.M. DiCicco-Bloom:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.05/B34

Topic: C.01. Brain Wellness and Aging

Title: The effect of gut microbiome on hippocampal neurogenesis and behavior in a murine model for autism spectrum disorders

Authors: *Y. WILLINGER;
Ariel Univ. Ctr., Tel Aviv, Israel

Abstract: Autism spectrum disorders (ASD) is a neurodevelopmental disorder that features impairment of neurogenesis and changes in the composition of the individual's gut microbiome. The gut microbiome interacts with the brain via several pathways, including immune modulation, and the secretion of various inflammatory mediators. We thus hypothesize that neurogenesis and behavior can be improved in a murine model for autism spectrum disorder, by altering the composition of the gut microbiome. We apply the murine model for ASD, induced by developmental exposure to valproic acid (VPA). Behavior and molecular analysis were performed for characterization of microbiome manipulation and QIIME2 analysis for gut microbiome composition. We observed that naïve microbiome transplantation decreased overall ASD score composed of social interaction, grooming and day three of the water T maze paradigm in both males and females following VPA exposure and improved early and advanced stages of neurogenesis. VPA exposure altered the beta diversity of the gut microbiome and changed the flora of bacteria in females. Serological analysis revealed a decrease of Th17-rlated cytokines mostly in females after exposure to VPA. Considering the results, we concluded that naïve microbiome transplantation alleviates general ASD symptoms and improves overall neurogenesis in both males and females. Immunomodulation is a key factor in understanding the effect of the gut microbiome and ASD.

Disclosures: **Y. Willinger:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.06/B35

Topic: A.07. Developmental Disorders

Support: Wellcome Grant 218662/Z/19/Z

Title: Mechanisms of fetal brain sparing during maternal malnutrition

Authors: *Y. ZHANG, P. SERPENTE, A. GOULD;
Francis Crick Inst., London, United Kingdom

Abstract: Maternal malnutrition substantially restricts weight gain of the fetal body but growth of the brain is selectively protected. This phenomenon, known as brain sparing, is critical for fetal survival but the underlying mechanisms are poorly understood. Using a maternal low protein (LP) dietary model of brain sparing in mice, we observed that LP diet decreased male and female fetal body weight by ~30%, fetal liver weight by ~60%, yet it did not alter the number of $Pax6^+$ radial glia in the neocortex. In contrast, LP decreased the number of $Tbr2^+$ intermediate progenitors at E15.5 - E16.5, although this cortical deficit was rescued by E17.5 - E18.5. Surprisingly, LP increased not decreased the mitotic index of neural stem and progenitor cells at E18.5, despite reducing it in the fetal liver. To identify the molecular basis for this, we examined mammalian target of rapamycin (mTOR) signalling. We found that LP decreased mTOR complex 1 (mTORC1) activity in the fetal brain in post-mitotic neurons but not in proliferating stem and progenitor cells. Blocking mTORC1 activity via rapamycin decreased mitoses in LP but not control brains at E18.5. Hence, mTORC1 activity is selectively required for cell division during brain sparing but not normal brain development. Transcriptomics of LP versus control brains identified Activating Transcription Factor 4 (*Atf4*), as one of the most upregulated genes in the brain but not the liver. *Atf4* expression was decreased by rapamycin treatment, indicating that it is an mTORC1 target in the fetal LP brain. Together, these findings demonstrate that fetal neural stem cells are selectively spared during maternal malnutrition and that the underlying mechanism requires mTORC1 signalling, potentially via its target Atf4.

Disclosures: Y. Zhang: None. **P. Serpente:** None. **A. Gould:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.07/B36

Topic: A.07. Developmental Disorders

Support: NIH grant R01Es033056-03

Title: Maternal high-fat diet promotes aberrant microglia phagocytosis in the dorsal raphe nucleus

Authors: *M. S. PATTON, L. A. GREEN, S. BILBO;
Duke Univ., Durham, NC

Abstract: High maternal weight produces a state of chronic, low-grade inflammation that can have detrimental effects on the developing fetal brain. As our society becomes increasingly sedentary and continues to consume calorically dense processed foods, the projected consequence on human health is alarming. Our lab recently demonstrated that maternal high-fat diet causes endotoxin accumulation in fetal tissue that acts on toll-like receptor 4 (TLR-4) of microglia. This results in excessive phagocytosis of serotonin (5-HT) in the developing dorsal raphe nucleus. Notably, this effect and the resulting anhedonic behavior were specific to male offspring, reflecting the male-bias seen in neurodevelopmental disorders. Continuing this work, here we investigate microglia-neuron interactions using live imaging from embryonic brain slices to determine the signaling mechanism(s) promoting 5H-T phagocytosis in the dorsal raphe. Additionally, using viral tracing and patch-clamp electrophysiology we will characterize the effect of aberrant microglia phagocytosis on serotonin neuron activity and further reveal whether specific serotonergic circuits are sensitive to maternal high-fat diet. The results from this work will shed light on the important neural-immune interactions that shape developing serotonergic circuits and may provide novel circuit-specific therapeutic strategies for neurodevelopmental disorders.

Disclosures: **M.S. Patton:** None. **L.A. Green:** None. **S. Bilbo:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.08/B37

Topic: A.07. Developmental Disorders

Support: ORWH-U54-MH118919

Title: Selective Timing of Maternal Immune Activation Alters Juvenile Developmental Milestones

Authors: *J. A. SHENG[1], S. A. TOBET[1,2,3,4];
[1]Biomed. Sci., [2]Sch. of Biomed. Engin., Colorado State Univ., Fort Collins, CO; [3]Dept. of Psychiatry, [4]Innovation Ctr. on Sex Differences in Med., Harvard Med. Sch., Boston, MA

Abstract: Infections during pregnancy have been associated with increased risk for neuropsychiatric disease later in life. Mouse models of maternal immune activation (MIA) activate different toll-like receptors (TLRs) to initiate innate inflammatory responses. The goal

of the current study is to determine sex-dependent effects of MIA using a TLR7 agonist, resiquimod, on neurodevelopment. Resiquimod (RQ) was administered to timed pregnant mice on embryonic day (E) 12.5. At E15, we measured maternal/fetal cytokines in plasma by ELISA. We found maternal pro-inflammatory cytokines IL-12p40 ($p < 0.001$), IL-6 ($p < 0.05$), and IL-10 ($p < 0.01$) were increased while anti-inflammatory cytokine IL-1B ($p < 0.05$) was decreased in mice exposed to RQ. Fetal cytokines (E15) were also altered by MIA. In both sexes, pro-inflammatory cytokines IL-6 ($p < 0.05$) and IL-17 ($p < 0.0001$) were increased, while IL-10 was increased only in males ($p < 0.001$). Males exposed to MIA exhibited a decrease in cytokines TNFα ($p < 0.05$) and IL-1β ($p < 0.01$). A second group of timed-pregnant dams were allowed to give birth. MIA did not alter the female to male ratio of offspring born per litter. Body weights were reduced significantly in both sexes ($p < 0.0001$) at birth, and over the next 5 weeks. MIA-exposed offspring opened their eyes 5 days later than controls. Similarly, resiquimod-exposed females exhibited pubertal delay with vaginal openings occurring 2-3 days later than control females. On the behavioral side, juvenile and adult male and female offspring exposed to MIA exhibited reduced social-like behavior in a social interaction test ($p < 0.0001$ for all groups). Anhedonia-like behavior was increased in MIA adult female mice ($p < 0.001$). To determine brain bases for behavioral changes, sections through the hypothalamus of juvenile offspring were immunolabeled for ionized calcium binding adhesion molecule-1 (IBA-1), a protein expressed by microglia activated in response to neuroinflammation. IBA-1 labeled cells in the hypothalamus were analyzed using Imaris image analysis software (Andor Technologies, Inc) and showed increased immunoreactive area selectively in MIA females compared to controls ($p < 0.01$). The current findings suggest a significant activation of the maternal immune system by RQ, which further altered peripheral developmental milestones, and microglial responses in juvenile offspring that may provide context for underlying sex differences in behavior. This study provides further support for sex-dependent influences of fetal antecedents for altered development and increased susceptibility for adult disorders through immune mechanisms. Supported by ORWH-U54-MH118919.

Disclosures: **J.A. Sheng:** None. **S.A. Tobet:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.09/B38

Topic: A.07. Developmental Disorders

Support: NIH F31AA030712
NIH 1R01-ES025549
NIH 1U01-AA029969
Charles Lafitte Foundation Program for Research

Title: Immune signaling modulation impacts developmental microglial-parvalbumin interactions

Authors: *J. E. DZIABIS[1], I. O. JONATHAN[1], B. HORVATH[1], C. J. SMITH[2], D. M. NGUYEN[1], N. ROGERS[1], G. ZHANG[1], M. A. KINGSBURY[3], S. D. BILBO[1];
[1]Duke Univ., Durham, NC; [2]Psychology and Neurosci., Boston Col., Chestnut Hill, MA; [3]Pediatrics, Harvard Med. School/MGH, Charlestown, MA

Abstract: Parvalbumin+ interneurons (PVIs) are fast-spiking, GABAergic cells critical for coordinating firing activity to maintain normal brain function. PVIs are a common cell type impacted in disorders with neurodevelopmental origins and immune etiology, such as schizophrenia and autism spectrum disorder, due to their high susceptibility to inflammation during development. Microglia, the brain's resident macrophages, respond to and are affected by perinatal inflammation, impacting synapses and behavior in disease-relevant ways. Critically, microglia influence the maturation and survival of developing neurons, but little is known about the developmental interactions between microglia and interneurons. In these experiments, we were interested in microglia-PVI interactions in the early postnatal period. We hypothesized that broad ablation of microglial inflammatory signaling would be protective for developing PVIs in the face of an early life immune challenge. Our lab developed a mouse in which MyD88, an adaptor protein for nearly all toll-like receptors, is ablated specifically from microglia (Cx3cr1-CreBT-MyD88f/f), blunting microglial proinflammatory signaling. We found that loss of microglial-MyD88 resulted in an increased density of PVIs across the male brain at baseline, an effect exacerbated by early life immune challenge. These mice exhibit normal sociability, but males without microglial-MyD88 show reduced spatial reference memory. These data suggest that microglia regulate developing PVIs in the male brain at baseline in a MyD88-dependent manner, with consequences for cognition. To investigate the role of microglial-MyD88 signaling in early PVI development, we used RNAscope to identify future PVIs prior to the expression of the parvalbumin protein. Microglial-MyD88 loss does not impact the early postnatal cell death period of PVI development in the male dorsal hippocampus, suggesting microglial regulation of the adult male PVI population size happens after the first postnatal week. Timecourse characterization of PVI synaptic density across 6 ages between postnatal day (P)12-P28 in the male MyD88 intact and deficient hippocampus identified P15 as an age of interest, as there was a reduction in PVI synaptic density when microglial-MyD88 is removed. Interestingly, 3D reconstructions of P15 male hippocampal microglia showed an increase in phagocytic capacity in MyD88 deficient microglia. Ongoing work will characterize the involvement of microglial-MyD88 signaling in excitatory synapse refinement between P12-P18, and the potential preference of MyD88 deficient microglia for engulfment of excitatory synaptic material.

Disclosures: J.E. Dziabis: None. **I.O. Jonathan:** None. **B. Horvath:** None. **C.J. Smith:** None. **D.M. Nguyen:** None. **N. Rogers:** None. **G. Zhang:** None. **M.A. Kingsbury:** None. **S.D. Bilbo:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.10/B39

Topic: A.07. Developmental Disorders

Support: The University of Texas Medical Branch Institute for Human Infections & Immunity
The University of Texas Medical Branch Sealy Institute for Vaccine Sciences
NIH R01HD109095
NIH R01AI136031

Title: Periconceptional T. cruzi infection drives maternal ILC3 expansion, dysregulated risk assessment and social dysfunction in offspring

Authors: *L. RIOS[1,4], K. BUCHANAN[1], L. MATZ[4], I. J. BOLDING[4], N. J. GARG[2,3], S. A. BUFFINGTON[4,5];
[1]Biochem. & Mol. Biol. Grad. Program, [2]Dept. of Microbiology & Immunol., [3]Inst. for Human Infections & Immunity, UTMB, Galveston, TX; [4]Ctr. for Precision Envrn. Hlth., [5]Dept. of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Maternal immune activation (MIA) during pregnancy increases risk for neurodevelopmental disorders in offspring. The effects of maternal parasitic infection during pregnancy on offspring neurodevelopment, however, remain almost completely unexplored. Chagas disease is a prevalent, but neglected, tropical disease caused by the vector-borne parasite *Trypanosoma cruzi (Tc)*. *Tc* infection results in immune activation characterized by the induction of proinflammatory cytokines, including IL-6 and IL-17A, which play a complex role in host defense *versus* pathogenesis depending on the amounts, time, and site of production. Pathological activation of the IL-17A pathway is a well-established mediator of neurodevelopmental impairment in the MIA model for autism spectrum disorder (ASD). MIA disrupts cortical lamination, perturbs network connectivity, and increases neuroinflammation, which together can cause ASD-like behavioral deficits in offspring. Unfortunately, frontline anti-parasitic drugs are contraindicated during pregnancy, leaving pregnant women little recourse for protecting their developing child/ren from inflammatory sequalae resulting from *Tc* infection. Given that the proinflammatory response to *Tc* infection serves as a protective mechanism against neonatal infection but simultaneously leads to potentially pathogenic levels of circulating maternal IL-6 and IL-17A, we hypothesized that maternal periconceptional *Tc* infection would adversely affect offspring neurodevelopment and behavioral outcomes, irrespective of congenital transmission. Female mice were inoculated with *Tc* and mated seven days post-infection. *Tc*-infected dams demonstrated a notable rise in IL-17A cytokine production in type 3 innate lymphoid cells (ILC3s), as determined by post-weaning flow cytometry-based immune cell profiling. Neurobehavioral assays revealed dysregulated risk assessment, characterized by excessive risk tolerance, and anomalous social behavior in both male and female offspring born to *Tc*-infected dams, as compared to offspring of mock-infected dams. The results of our study indicate that implementing vaccination programs for women of reproductive age in regions where Chagas disease is prevalent could potentially reduce risk for adverse neurodevelopmental outcomes in children.

Disclosures: **L. Rios:** None. **K. Buchanan:** None. **L. Matz:** None. **I.J. Bolding:** None. **N.J. Garg:** None. **S.A. Buffington:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); S.A.B. is an inventor on a patent granted to Baylor College of Medicine related to the use of Limosilactobacillus reuteri for treating disorders characterized by social dysfunction, US Patent No. 1113.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.11

Topic: A.07. Developmental Disorders

Support: NIH Grant 5R01MH129732-02, A-CM

Title: Cell type specific contributions to anxiety deficits in a mouse model of complement 4 overexpression

Authors: *L. FOURNIER[1], R. PHADKE[2], M. SALGADO[2], A. BRACK[2], J. GRANT[2], S. BOLSHAKOVA[2], I. PICARD[2], N. PADRO[3], A. CRUZ-MARTIN[2];
[1]Neurobio., [2]Boston Univ., Boston, MA; [3]Univ. of Puerto Rico, Río Piedras, Puerto Rico

Abstract: Parvalbumin-positive interneurons (PV-INs) occupy the strongest role in regulating pyramidal neuron spiking and orchestrate brain oscillations subserving cognition. PV-INs form dense, non-specific inhibitory synapses with nearly all proximal excitatory neurons, thus serving an indispensable role in the cortical microcircuit. However, PV-INs are particularly susceptible to developmental stressors, and PV-IN dysfunction underlies many brain diseases, including schizophrenia (SCZ). Despite findings of reduced density of inhibitory markers and excitatory inputs to PV-INs in the prefrontal cortex (PFC) of SCZ patients, very few studies exist linking manipulations of SCZ-associated genes to PV-IN dysfunction in the PFC. In humans, specific structural variants of immune gene, C4A, increase C4A expression and confer greater risk for developing SCZ. Our group demonstrated that overexpression of C4A (C4-OE) in medial PFC (mPFC) pyramidal neurons led to alterations in synaptic developmental wiring and induced social behavioral deficits in mice. Our preliminary data using M-FISH in the mPFC suggest that PV-INs are a cellular source of C4. We hypothesize that specific C4-OE in PV-INs reduces the excitatory drive on this developmentally-vulnerable interneuron type, and this is sufficient to cause long-term deficits of mPFC-associated behavior. To test this hypothesis, we have developed a novel, conditional C4-OE mouse line and have crossed this to PV-Cre driver mice. Our preliminary data suggest that C4-OE exclusively in PV-INs is sufficient to cause a sexually dimorphic anxiety-like behavioral deficit. Our goal is to determine the mechanistic role of C4-OE in PV-INs in driving the pathology that underlies the observed anxiety-like deficits, at the synaptic level.

Disclosures: **L. Fournier:** None. **R. Phadke:** None. **M. Salgado:** None. **A. Brack:** None. **J. Grant:** None. **S. Bolshakova:** None. **I. Picard:** None. **N. Padro:** None. **A. Cruz-Martin:** A. Employment/Salary (full or part-time):; Center for Systems Neuroscience, Boston University, The Center for Network Systems Biology, Boston University, Department of Pharmacology and Experimental Therapeutics, Boston University.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.12

Topic: A.07. Developmental Disorders

Support: Jim and Betty Ann Rodgers Chair Fund

Title: Developmental nicotine exposure produces selective deficits in frontal cortical parvalbumin containing GABA neurons

Authors: *M. X. TRUPIANO[1], M. M. MARTIN[2], A. J. CANEKERATNE[1], D. M. MCCARTHY[1], P. G. BHIDE[1];
[1]Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL; [2]BASF, Research Triangle Park, NC

Abstract: Maternal smoking during pregnancy is a major public health concern due to the adverse effects on both the mother and offspring. Harmful effects of developmental nicotine exposure (DNE) include ADHD, learning disabilities, depression, and epilepsy. Preclinical models show DNE perturbs frontal cortical excitation-inhibition equilibrium by downregulation of GABA function. GABA neurons fall into multiple subtypes based on expression of specific molecular markers. Parvalbumin (PV) and somatostatin (SST) are two neuropeptides expressed by frontal cortical GABA neurons. Since PV- and SST-expressing GABA neurons make up 40% and 20% of frontal cortical GABA neurons respectively, and since both are associated with regulation of cognitive function, changes in the relative proportions of these GABA neurons expressing these neuropeptides, may have significant functional implications. However, the effects of DNE on GABA neuron subtypes are not known. Here we examined PV- and SST-GABA neurons in a mouse model of DNE. We used a Swiss-Webster GAD67-GFP knock-in reporter mouse to facilitate identification of GABA neurons based on GFP fluorescence in histological sections. Antibodies specific for PV and SST were used to identify GABA neuron subtypes. Female mice were exposed to plain drinking water or drinking water containing nicotine (200 µg/ml) beginning 3 weeks prior to mating with a nicotine-naïve GAD67-GFP male mouse. Oral nicotine exposure of the females continued throughout pregnancy and nursing. The effects of DNE on frontal cortical PV and SST neuron numbers were analyzed using 2-way ANOVA followed by Bonferroni's multiple comparisons in adult male and female mice at approximately 60-days of age. DNE produced a selective deficit in the density of PV-GABA

neurons in male but not female mice ($F_{(1,12)} = 12.78$; $p<0.005$). DNE spared SST-GABA neuron in both sexes ($F_{(1,12)} = 0.5582$; $p>0.05$). The total number of GABA neurons was not affected by DNE ($F_{(1,12)} = 0.05056$; $p>0.05$). Thus, our data show that DNE produces sex-specific and selective deficits in frontal cortical PV-GABA neuron numbers, which may be associated with the behavioral changes produced by DNE.

Disclosures: M.X. Trupiano: None. **M.M. Martin:** None. **A.J. Canekeratne:** None. **D.M. McCarthy:** None. **P.G. Bhide:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.13/B40

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NICHD NRSA F31HD101360
SFARI Bridge to Independence Award 381222

Title: Circuit consequences of Mef2c-mediated neurodevelopmental dysfunction in cortical parvalbumin interneurons

Authors: *C. WARD, E. SABRI, R. BATISTA-BRITO;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Cortical Parvalbumin-expressing inhibitory interneurons (PV-INs) sample input from large populations of pyramidal cells and synapse onto cell bodies, allowing them powerfully to regulate excitatory activity within the cortex. The dysregulation of parvalbumin has been implicated in several neurodevelopmental disorders (NDDs), including Autism Spectrum Disorder and Schizophrenia, however, the lack of an early marker for these cells that distinguishes them from other inhibitory neuron subtypes has precluded studies on the role of early PV-IN development on establishing a healthy cortical network. To address this, we used a transgenic mouse model to embryonically disrupt an NDD-linked gene in interneuron progenitors. Myocyte enhancer factor-2C (*Mef2c*), a critical regulator of neuronal development, is highly enriched within PV-INs. Differential expression of Mef2c among classes of interneurons allows for early genetic access to this cell population. Given that visual processing impairments are commonly reported in NDDs, we examined neuronal activity within in the mouse primary visual cortex (V1) as a readout of circuit function. We compared the response to visual stimuli between Mef2c conditional knockout animals and littermate controls by performing in vivo extracellular electrophysiology recordings within V1. Embryonic removal of Mef2c leads to weaker visually-evoked activity and increased noise correlations, with fewer cells strongly tuned to visual stimuli. Together these data show that the disruption of PV-IN development through embryonic removal of Mef2c leads to abnormal circuit function and

sensory processing, providing a foundation for future studies examining impaired PV-IN development and sensory perception.

Disclosures: C. Ward: None. **E. Sabri:** None. **R. Batista-Brito:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.14/B41

Topic: A.07. Developmental Disorders

Support: NIH Grant AA13023

Title: Effects of Δ^9-Tetrahydrocannabinol (THC) in the Primary Visual Cortex of Ferrets

Authors: *M. PRUITT[1], D. KEUM[2], A. E. MEDINA[3];
[1]Pediatrics, Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Pediatrics, Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Pediatrics, Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Cannabis use during pregnancy has dramatically increased over the last 15 years and has been exacerbated by dispensaries recommending use for expectant individuals to ease symptoms such as nausea, anxiety, and stress, despite the fetal risks. Δ^9-Tetrahydrocannabinol (THC), the main psychoactive component of marijuana, readily passes through the placenta, resulting in direct fetal exposure. Despite the importance of the topic, the consequences of prenatal cannabis exposure and underlying mechanisms remain elusive. Since most animal studies on this topic are in rodents, we decided to develop a ferret model of developmental THC exposure. Ferrets are gyrencephalic animals and have a visual cortex much closer to humans than rodents. We exposed ferrets daily to 4.0 – 6.0 mg/kg of either THC mixed in peanut butter (PB) or control PB orally between postnatal days (P) 10-32. This period in ferrets is equivalent to the third trimester of human gestation. At approximately P50 (ferret infancy), animals were euthanized, and the primary visual cortex (V1) extracted. One hemisphere was used for slice electrophysiology and the other for *in situ* hybridization (RNAscope). Whole cell patch clamp in V1 layer 2/3 showed that evoked inhibitory postsynaptic currents (IPSCs) were 44% smaller in THC (2.3 ± 0.58 nA) than control (4.1 ± 1.4 nA) animals. To evaluate cannabinoid type-1 receptor (CB1R) function we assessed the impact of the CB1R agonist WIN55212-2 (WIN) on IPSCs. We found that 10 minutes after WIN application IPSCs reduced by 32% in PB but were virtually unchanged (-3.2%) in THC neurons. To further evaluate the effects of developmental THC in CB1Rs we conducted RNAscope in parvalbumin-positive interneurons (PV) and pyramidal neurons (PYR). RNAscope revealed that density/μm^3 of CB1R mRNA did not differ in layer II/III PV neurons in THC-treated ($7.1 \times 10^{-3} \pm 2.5 \times 10^{-3}$) and control ($6.7 \times 10^{-3} \pm 2.5 \times 10^{-3}$) animals. Density/$\mu m^3$ of CB1R mRNA also did not differ in layers V-VI of PV neurons

in THC-treated ($1.3 \times 10^{-2} \pm 3.2 \times 10^{-3}$) and control ($2.3 \times 10^{-2} \pm 8.2 \times 10^{-3}$) animals. Density/$\mu m^3$ was also similar in layer II/III PYR neurons in THC-treated ($2.2 \times 10^{-2} \pm 2.4 \times 10^{-3}$) and control ($1.8 \times 10^{-2} \pm 2.7 \times 10^{-3}$) animals. However, CB1R mRNA density/μm^3 in layers V-VI of PYR neurons was higher in THC-treated ($2.2 \times 10^{-2} \pm 1.8 \times 10^{-3}$) than control ($1.2 \times 10^{-2} \pm 2.0 \times 10^{-3}$) animals ($p<0.005$). Future experiments will investigate changes in excitatory postsynaptic currents (EPSCs) and synaptic plasticity using patch clamp electrophysiology. Our findings suggest that THC exposure during the third trimester equivalent of human gestation affects the expression and function of CB1Rs in V1 of ferrets.

Disclosures: **M. Pruitt:** None. **D. Keum:** None. **A.E. Medina:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.15/B42

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA Grant AA027269

Title: Investigation of cell type-specific loss in the nucleus reuniens of the midline thalamus following PD9 single-day alcohol exposure in a rodent model of FASDs

Authors: *S. GUSTAFSON[1], I. SMITH[2], M. GROGIN[2], A. Y. KLINTSOVA[2];
[1]Psychological and Brain Sci., Univ. of Delaware Grad. Program In Behavioral Neurosci., Newark, DE; [2]Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Fetal Alcohol Spectrum Disorders (FASDs) is an umbrella term used to describe developmental disorders stemming from the prenatal alcohol exposure (AE) that are manifested by deficits in growth development, cognitive impairments, and physical abnormalities. FASDs result in lower brain volume, brain abnormalities, and behavioral deficits that include impaired executive function (EF). EF is a set of cognitive abilities that aid in the formation of goal-directed movements and regulating self-control; EF has been shown to rely on medial prefrontal cortex (mPFC) and hippocampus (HPC) functional synchrony. An intermediary structure, the nucleus reuniens (Re) of the midline thalamus, sends axonal projections to both the mPFC and HPC, coordinates communication between these two brain areas and is known to be damaged by prenatal alcohol exposure. Recently, we observed significant increases of apoptosis in Re 12 hours following a PD7 single day of binge-like AE. However, at this point, the cell phenotypes in Re that are susceptible to AE-induced damage are not known. This study employed a rodent model of third-trimester single-day binge alcohol exposure (AE) to evaluate neuroanatomical effects of neonatal alcohol exposure in a dose-dependent manner. Specifically, we assessed the numbers of distinct cell phenotypes in Re 12 hours after a moderate (3g/kg/day) or high (5.25g/kg/day) dose of ethanol compared to a sham intubated (SI) control group on postnatal day

9. The total population of neurons and oligodendrocytes in Re were estimated by utilizing immunohistochemical labeling coupled with unbiased stereological approaches. No significant effect of postnatal treatment was found on neuron or oligodendrocyte populations in Re, which indicates that cell loss might manifest itself later than 12 hours following AE. Additionally, no significant effect of alcohol exposure on reuniens volume was found, indicating that reuniens volume was not compromised within 12 hours of AE. Estimation of specific cell loss and volume changes in Re is being extended to 72 hours after AE. Our data demonstrate decreased vulnerability of Re to AE on PD9 when compared with PD7, at least in terms of timing of progressive cell loss paralleled by Re volume reduction. This study aids in our understanding of FASDs and adds to what we know of the immediate effects of ethanol on Re.

Disclosures: S. Gustafson: None. **I. Smith:** None. **M. Grogin:** None. **A.Y. Klintsova:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.16/B43

Topic: A.07. Developmental Disorders

Support: NIH grant AA025120
NIH grant 2R01AA015614-16
NIH grant P50-AA022534
NIH training grant 5T32AA014127

Title: Isolating neural circuits recruited during risky decision-making in mice with prenatal alcohol exposure

Authors: *A. MYRICK[1], D. FURLANO[2], N. M. MAPHIS[3], M. OROZCO[2], S. DAVID[2], Z. VILLASENOR[2], A. MOORE[2], D. N. LINSENBARDT[2];
[1]Neurosci., [2]The Univ. of New Mexico, Albuquerque, NM; [3]Neurosciences, Univ. of New Mexico, Albuquerque, NM

Abstract: Increased risk-taking is commonly observed in individuals with Fetal Alcohol Spectrum Disorders (FASDs) and contributes to a variety of detrimental outcomes such as increased rates of incarceration. Thus, it is critical that the neurobiological mechanisms leading to increased risk-taking in FASD be identified. The goal of the current study was to evaluate risk-taking and neural circuits involved in risk-taking using a preclinical mouse model of FASD and a Risky Decision-making Task (RDT). Prenatal alcohol exposed (PAE) or control transgenic 'TRAP2' mice were trained to distinguish between two images on a touch screen that dispensed either a large or small highly palatable reward. Once animals consistently chose the large reward with >80% frequency, risky decision-making was assessed by pairing the large reward with a 50% probability of a mild foot-shock. Mice were treated with tamoxifen following RDT daily for

five consecutive days to permanently label (i.e. TRAP) cFos-expressing neural circuits as they were recruited. Contrary to our previous studies in C57BL/6J (B6) mice, increased risk taking was not observed in PAE subjects versus control TRAP2 subjects, suggesting that genotype may interact with PAE to differentially regulate risk proclivity. However, we identified a robustly labeled neural circuit that included the pontine nuclei (PN), paraventricular nucleus (PVN), dorsal cortex of the inferior colliculus (IC), and several lateral cortex sub-regions. Furthermore, PAE subjects displayed significantly reduced IC labeling compared to control animals, suggesting a potential difference in auditory processing of sound cues associated with the task. These data support the use of RDT and TRAP2 mice for identifying and characterizing neural circuits involved in risky decision-making, and suggest the IC is vulnerable to PAE but may not be involved in regulating risk taking behavior in TRAP2 mice.

Disclosures: **A. Myrick:** None. **D. Furlano:** None. **N.M. Maphis:** None. **M. Orozco:** None. **S. David:** None. **Z. Villasenor:** None. **A. Moore:** None. **D.N. Linsenbardt:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.17/B44

Topic: A.07. Developmental Disorders

Support: NIH grant AA026421

Title: Reducing excitation of layer V pyramidal neurons in the prelimbic cortex alleviates attention deficits caused by prenatal ethanol exposure

Authors: *A.-L. WANG[1], R. WANG[1], S. BISWAS[2], K. A. HAUSKNECHT[1], V. B. MICOV[1], K. E. CZAJKA[1], M. A. SARTORI[1], K. ISHIWARI[1], R.-Y. SHEN[1];
[1]Dept. of Pharmacol. and Toxicology, Univ. at Buffalo, Buffalo, NY; [2]Dept. of Biol. Sci., Univ. at Buffalo, buffalo, NY

Abstract: Impaired executive functions are major deficits of fetal alcohol spectrum disorders (FASDs) caused by prenatal ethanol exposure (PE). Previous studies have shown that PE leads to impaired function of the medial prefrontal cortex – a brain area critical for executive functions. Our previous studies have found that PE increases excitatory synaptic neurotransmission in layer V pyramidal neurons in the prelimbic area of the medial prefrontal cortex and leads to increased impulsivity and impaired sustained attention. In this study, we tested the hypothesis that increased excitation in layer V pyramidal neurons in the prelimbic cortex contributed to attention deficits in PE rats. Pregnant Sprague-Dawley rats were treated with ethanol (0 or 3 g/kg, 15% w/v, twice daily with a 5-6 h interval) via intragastric gavage during gestational days 8-20. Female offspring underwent the training of the 2-choice reaction time task to examine action impulsivity and sustained attention when they were 12-13 weeks old. After training, rats were

bilaterally injected with an inhibitory channelrhodopsin (pAAV-CKIIa-stGtACR2) to inhibit layer V pyramidal neurons in the prelimbic region. The optic fibers were also implanted in the prelimbic cortex. Later, rats underwent the same task for further optogenetic experiments. The results show that optogenetic inhibition of pyramidal neurons significantly reduced augmented impulsivity in PE rats, reflected by decreased premature responses. In addition, incorrect responses were also reduced in PE rats. When optogenetic inhibition was applied in the second half of the trial, the reduction of premature responses was still observed in PE rats. Optogenetic inhibition also reduced premature responses in PE rats when task difficulty was increased in a non-salient condition. Furthermore, it reduced premature responses in control rats under the non-salient condition, reflecting the additional benefits of optogenetic inhibition. Lastly, optogenetic inhibition did not improve impaired sustained attention. These results show that the reducing excitation of pyramidal neurons could alleviate action impulsivity in PE rats. Taken together, these results support that PE-induced increases in the excitation of the prelimbic cortex contribute to attention deficit-like symptoms in PE rats.

Disclosures: **A. Wang:** None. **R. Wang:** None. **S. Biswas:** None. **K.A. Hausknecht:** None. **V.B. Micov:** None. **K.E. Czajka:** None. **M.A. Sartori:** None. **K. Ishiwari:** None. **R. Shen:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.18/B45

Topic: A.07. Developmental Disorders

Support: R00-045758
4T32ES007148-30
P20GM121334-06

Title: Sexually dimorphic neurobehavioral effects of gestational exposure to the organophosphate Insecticide Chlorpyrifos in the rat

Authors: *E. HAWKINS[1], M. BERRY[2], T. KRISHNA[3], D. KIM[3], S. KELSEN[2], G. S. ASTON-JONES[4], A. KOHTZ[5];
[1]Millsaps Col., Jackson, MS; [2]Univ. of Mississippi Med. Ctr., jackson, MS; [4]Brain Hlth. Inst., [3]Rutgers Univ., Piscataway, NJ; [5]Univ. of Missisippi Med. Ctr., Jackson, MS

Abstract: The organophosphate Chlorpyrifos (CPF) is classified as a moderately toxic agent. As a pesticide that took off en mass since 1965, CPF was most notably used to treat 50 different nut, fruit, vegetable, and cereal crops. Despite evidence suggesting significant health risks, a federal ban on CPF usage did not occur until 2021, and the long-term effects of extended exposure remain unknown. Although biochemical studies on the effects of CPF are widespread, behavioral

testing remains sparse and lacks depth. Here, we tested the effects of subthreshold (non-detectable in fetal tissue) exposure to CPF in development on neurobehavioral outcomes in adult rats. Pregnant Sprague-Dawley rats were given 6mg/kg/day CPF or safflower oil vehicle administered on cookies (readily eaten) daily during gestational day (GD) 6-20. Offspring were raised to adulthood (post-natal day 55), and CPF or vehicle rats were tested for addiction-like behavior, sucrose-seeking, anxiety-like, and depressive-like behavior, or behavioral flexibility tasks. Reinstatement behavior (context, cued, cocaine primed and stress) was tested following cocaine acquisition on an FR-1 schedule. CPF exposure resulted in shorter periods of cocaine-acquisition, greater cocaine-intake, and exacerbated extinction resistance compared to vehicle administered controls. Further, CPF-exposed females exhibited a decrease in sucrose-seeking and depressive-like behavior while males displayed an increase in anxiety and depressive-like behaviors but a decrease in sucrose seeking. We then performed immunohistochemistry to determine if CPF exposure affects locus-coeruleus norepinephrine (LC-NE), ventral tegmental area dopamine (VTA-DA), or dopamine beta hydroxylase fiber (DBH) densities in prefrontal cortex. CPF increased cortical DBH fiber densities by 4-fold in both sexes. LC-NE fiber density was diminished in both sexes, yet LC-NE neuron count remained insignificantly changed compared to controls. The number of VTA-DA neurons, but there was no significant change in cell density for either sex vs controls. This data indicates that enhanced DBH fiber densities in the cortex were likely not due to an increased number of forebrain projecting NE neurons. Stress responding was inversely predicted by DBH fiber density in females, whereas impulsivity measures were inversely predicted by fiber density in males. Together, these data indicate that increased NE innervation of the cortex by CPF may both promote stress-resilience, particularly in females, while also impairing behavioral flexibility in both sexes.

Disclosures: E. Hawkins: None. **M. Berry:** None. **T. Krishna:** None. **D. Kim:** None. **S. Kelsen:** None. **G.S. Aston-Jones:** None. **A. Kohtz:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.19/B46

Topic: A.07. Developmental Disorders

Title: Exposure to the organophosphorus pesticide chlorpyrifos in utero disrupts the critical period of neuroplasticity in the rat

Authors: *J. A. KOENIG, C. HAGA, A. KELLER;
Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Although there are ever increasing restrictions on their application, organophosphorus (OP) pesticides remain in widespread use throughout the world, in both agricultural and household settings. This is despite the known links between *in utero* exposure to these

compounds and an increased occurrence of neurodevelopmental disorders, such as autism spectrum disorder and ADHD. These outcomes occur even at environmentally relevant concentrations that are below the threshold for acetylcholinesterase inhibition or overt toxicity. An alternative causative mechanism for these early perturbations in brain development remains to be established. We used a rat model of gestational chlorpyrifos exposure, a widely used OP pesticide, and *ex vivo* electrophysiology to study lasting alterations in the primary somatosensory cortex of young rats. Dams were exposed to 5.0 mg/kg chlorpyrifos through subcutaneous injection on gestational days 18-21, with no outward signs of acute toxicity. Beginning on postnatal day (PND) 12, an age during the critical period of use-dependent plasticity, we performed whole-cell patch clamp recordings from layer 2/3 pyramidal neurons of the primary somatosensory cortex. A negative pairing (-25 ms) spike timing dependent plasticity protocol revealed significant alterations to both the progression of long-term depression (LTD) across ages (PND 12-20) and in the induction kinetics, with significantly enhanced LTD seen in the chlorpyrifos exposed group at younger ages (PND 12-14). Evaluating inhibitory synaptic inputs through paired pulse stimulation demonstrated a significant increase in the paired pulse ratio, suggesting a decrease in the presynaptic GABA release probability in chlorpyrifos exposed animals. Preliminary data suggest an increase in basal dendritic complexity induced by chlorpyrifos exposure. Overall, we provided evidence for novel functional alterations during brain development induced by *in utero* exposure to the OP pesticide chlorpyrifos that may account for the well-established behavioral outcomes.

Disclosures: **J.A. Koenig:** None. **C. Haga:** None. **A. Keller:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.20/B47

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 19K08078

Title: Juvenile social isolation affects synaptic and intrinsic excitability of prefrontal layer 5 pyramidal cells with subcortical axonal projection

Authors: *H. YOSHINO[1,2,4], **Y. NISHIHATA**[2], Y. OGAWA[3], T. SUGIMURA[3], K. OKAMURA[2], K. YAMAMURO[2], M. MAKINODAN[2], Y. SAITO[3], T. KISHIMOTO[2]; [2]Psychiatry, [3]Neurophysiol., [1]Nara Med. Univ., Kashihara / Nara, Japan; [4]Mie Prefectual Mental Ctr., Tsu / Mie, Japan

Abstract: Social experience during development is crucial for the functional maturation of the prefrontal cortex (PFC). Juvenile social isolation (JSI) causes severe dysfunction in PFC. In our previous studies, JSI reduced intrinsic and synaptic excitability of a subtype of layer-5 (L5)

pyramidal cells in the medial PFC, which cell has prominent h-current (PH), recognized as having axon projecting subcortically. However, it remains unknown which L5 pyramidal cells projecting axon to subcortical area are affected by JSI. With retrograde tracing and whole-cell patch clamp recording, we investigated how JSI affect the intrinsic and synaptic excitability of L5 pyramidal cells which project axons into mediodorsal thalamus, striatum and pons, followed by observing the fundamental properties of each pyramidal cells. We found that pyramidal cells projecting axon to pons have more excitatory synaptic inputs and more distinguishing intrinsic properties than pyramidal cells projecting to mediodorsal thalamus and striatum. JSI reduced excitatory synaptic inputs only on pyramidal cell projecting to pons. This suggests that JSI affect regional-dependently neuronal circuits from PFC to subcortical area.

Disclosures: H. Yoshino: None. **Y. Nishihata:** None. **Y. Ogawa:** None. **T. Sugimura:** None. **K. Okamura:** None. **K. Yamamuro:** None. **M. Makinodan:** None. **Y. Saito:** None. **T. Kishimoto:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.21/B48

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA Grant AA027269

Title: Running and environmental complexity as a means of ameliorating the impact of neonatal alcohol exposure on axonal representation in the prefrontal-reuniens-hippocampal circuit

Authors: *I. SMITH, S. GUSTAFSON, M. GROGIN, A. Y. KLINTSOVA;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Fetal Alcohol Spectrum Disorders (FASDs) are preventable developmental disorders that result from alcohol exposure (AE) *in utero*. Along with prominent behavioral and anatomical deficits, cognitive impairments are prevalent in FASDs. Cognitive deficits include impaired executive functions such as spatial working memory, which have been shown to rely on the coordination of activity between the medial prefrontal cortex (mPFC) and hippocampus (HPC) by the thalamic nucleus reuniens (Re). We have recently demonstrated that the total length of mPFC-originating axon projections within RE is reduced in females exposed to high-dose binge AE on postnatal days (PD) 4-9 in a rodent model of third trimester alcohol exposure, indicating reduced connectivity in the mPFC-Re-HPC circuit. While behavioral interventions to mitigate the adverse effects of AE are few, exercise in animal models of AE has been shown to increase proliferation of newly generated neurons and to increase white matter integrity. Additionally, exposure to environmental complexity following exercise (superintervention) is critical for the survival of newly generated cells. The goal of this study was to investigate the

effects of binge AE (5.25 g/kg/day, two doses 2 hours apart) on PD 4-9 on axonal projections from mPFC and HPC in Re while also exploring the efficacy of a superintervention consisting of wheel running followed by environmental complexity (WR/EC) to diminish AE-induced changes in axonal representation. AE and sham intubated (SI) male and female Long Evans rats were randomly assigned to either social housing (SH) or the WR/EC intervention, which consisted of 12 days of voluntary WR followed by 30 days of EC housing. Axon projections were labeled with anterograde viruses expressing the fluorophores tdTomato or GFP (AAV5-CAG-tdTomato or AAV5-CAG-GFP) stereotaxically injected into the left mPFC and HPC prior to housing in EC. Preliminary results indicate no difference between SI and AE animals in terms of cumulative mPFC- or HPC-originating axon length in Re as measured by the Spaceballs stereological probe (MBF Biosciences, Williston, VT) ($p > 0.05$). Collecting the complete set of data is underway to fully elucidate the impact of AE and superintervention on connectivity in the mPFC-Re-HPC circuit. This ongoing experiment will add to our understanding of intervention-related changes to axon representation in executive function-related brain circuitry in individuals affected by FASDs.

Disclosures: I. Smith: None. **S. Gustafson:** None. **M. Grogin:** None. **A.Y. Klintsova:** None.

Poster

PSTR450. Prenatal and Early Life Environment, Development, Inflammation: Effects on Behavior and Neural Function

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR450.22/B49

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIMH Grant R01MH110487

Title: Genetic and pharmacological rescue of myelination in neurodevelopmental disorders

Authors: *N. SADOWSKI[1], J. BOHLEN[2], C. CLEARY[3], G. SHIM[4], D. DAS[2], S. SRIPATHY RAO[5], I. BUCHLER[2], T. S. SCANLAN[6], D. K. MULKEY[7], A. J. KENNEDY[8], B. J. MAHER[5]; [1]Johns Hopkins Med. Institutions, Balitmore, MD; [2]Lieber Inst. of Brain Develop., Baltimore, MD; [3]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [4]Lieber Inst. for Brain Develop., Baltimore, MD; [5]Lieber Inst. For Brain Develop., Baltimore, MD; [6]Oregon Hlth. & Sci. Univ., Portland, OR; [7]Dept. Physiol. and Neurobio., Univ. of Connecticut Physiol. & Neurobio., Storrs Manfld, CT; [8]Bates Col., Lewiston, ME

Abstract: Autism spectrum disorder (ASD) manifests with severe symptomologies and presents an increasing health burden to patients and their families. Pitt-Hopkins Syndrome (PTHS), a neurodevelopmental disorder with features of ASD, is caused by autosomal dominant mutations in the transcription factor 4 gene (TCF4). Recently, we discovered that mutations in TCF4 result in hypomyelination caused by a reduction in the density of oligodendrocytes (OLs) in a mouse model of PTHS. Myelination is a dynamic neurodevelopmental process that continues

throughout the lifespan, and could be leveraged as a novel therapeutic target. Therefore, the PTHS mouse model is a useful model system to study the impact of genetic and pharmacological approaches targeting the OL lineage and the process of myelination. Here, we show that the promyelinating compounds, clemastine, sobetirome and Sob-AM2 are effective at restoring myelination deficits in the PTHS mouse model. Two week intraperitoneal administration of these promyelinating compounds in early adolescent mice (P28-P42) normalized the OPC/OL density in the cortex. Electron microscopy imaging showed a significant reduction in the g-ratio indicating an increased myelination of axons within the corpus callosum. Importantly, promyelinating drug treatment resulted in functional recovery by improving the physiology of compound action potentials and rescuing behavioral deficits. To confirm that rescue by these promyelinating compounds is specific to normalizing myelination and not due to off target effects, we performed OL lineage-specific genetic rescue experiments. We crossed the Tcf4 reinstatement mouse ($Tcf4^{+/LGSL}$) with several different OL lineage-specific Cre mouse lines (Pdgfra-Cre, Olig2-Cre, and Sox10-Cre) to restore Tcf4 expression in OPCs, and then examined functional recovery in early adolescent mice (P24 and P42). Immunohistochemistry experiments revealed that OPC-specific genetic reinstatement of Tcf4 normalized the OPC/OL density in the cortex. Additionally, Tcf4 reinstatement resulted in functional recovery by improving compound action potentials in the corpus callosum. Most importantly, genetic rescue of Tcf4 in OPCs improved hyperactivity, anxiety, and memory deficits in the PTHS mice. Altogether, our results provide preclinical evidence that promyelinating therapies may be an effective therapeutic approach to improve the health and wellbeing of individuals diagnosed with PTHS and potentially other forms of ASD.

Disclosures: **N. Sadowski:** None. **J. Bohlen:** None. **C. Cleary:** None. **G. Shim:** None. **D. Das:** None. **S. Sripathy Rao:** None. **I. Buchler:** None. **T.S. Scanlan:** None. **D.K. Mulkey:** None. **A.J. Kennedy:** None. **B.J. Maher:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.01/B50

Topic: A.09. Adolescent Development

Support: NIH Grant MH067924
Staunton Farm Foundation

Title: Dopamine modulation of prefrontal GABA/Glutamate balance suggests critical period plasticity during adolescence

Authors: *A. C. PARR[1], M. PERICA[2], F. CALABRO[3], B. TERVO-CLEMMENS[6], W. F. FORAN[1], V. YUSHMANOV[4], H. HETHERINGTON[4], B. LUNA[5];
[1]Psychiatry, [2]Psychology, [3]Psychiatry, Bioengineering, [4]Radiology, [5]Dept. of Psychiatry, Univ.

of Pittsburgh, Pittsburgh, PA; [6]Psychiatry and Behavioral Sci., Univ. of Minnesota, Masonic Inst. of the Developing Brain, Minneapolis, MN

Abstract: Changes in prefrontal cortex (PFC) excitatory (glutamatergic, Glu) and inhibitory (GABAergic) balance (E/I) have been identified in human adolescence, potentially reflecting critical period plasticity supporting maturation of PFC-dependent cognition. Adolescent increases in dopamine (DA) may be a trigger for critical period plasticity, and animal models implicate DA in regulating developmental changes in E/I. We assessed the role of striatal tissue iron indices of DA availability in the development of PFC GABA/Glu balance during adolescence.

Longitudinal 7T Magnetic Resonance Spectroscopic Imaging (MRSI) indices of GABA/Glu and T2*-based indices of tissue iron were obtained in 166 participants (86 female, ages 10-32 years-old, 1-3 visits, 267 visits total). PFC GABA/Glu was acquired via an oblique MRSI slice of 24x24 voxels (1.0x0.9x0.9mm) using a J-refocused spectroscopic imaging sequence. Striatal tissue iron was assessed by time-averaged and normalized T2*-weighted imaging (nT2*w) during an 8-min resting-state scan. General Additive Models (GAMMS) were used to characterize non-linear trajectories in GABA/Glu balance and nT2*w. To assess whether the relationship between nT2*w and DLPFC GABA/Glu changed with age, as we might expect should DA be differentially involved during distinct periods of adolescence and/or in GABA/Glu trajectories, we tested for interactions between nT2*w and age on GABA/Glu.

As in prior studies, striatal tissue iron (F=8.03, p=3.87e-05) and DLPFC GABA/Glu balance (F=10.76, p=9.88e-07) increased during adolescence. Critically, we observed a significant age by DA interaction on DLPFC GABA/Glu (F=3.01, p=.03). Post-hoc tests revealed that *higher* DA was associated with greater GABA/Glu *im*balance in early adolescence (age 10–15), and subsequently, steeper age-related increases in balance (i.e., decreases in *im*balance; F=12.44, p=2.49e-07) relative to *low* DA (F=.90, p=.44). Exploratory tests revealed that *higher* DA was associated with higher DLPFC Glu in early adolescence, followed by moderate age-related decreases (F=2.45, p=.06) relative to *low* DA (F=.44, p=.73).

Increased DA is associated with greater GABA/Glu *im*balance early in adolescence, potentially driven by DAergic enhancement of excitatory inputs to the PFC, creating a shift out of balance towards greater excitation. As DA stabilizes, Glu may be downregulated, potentially via synaptic pruning, facilitating developmental increases in GABA/Glu balance. These results provide novel, *in vivo*, support for critical period plasticity mechanisms whereby increases in DA are involved in fine-tuning GABA/Glu, and thus E/I balance, in adolescence.

Disclosures: A.C. Parr: None. **M. Perica:** None. **F. Calabro:** None. **B. Tervo-Clemmens:** None. **W.F. Foran:** None. **V. Yushmanov:** None. **H. Hetherington:** None. **B. Luna:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.02/B51

Topic: A.09. Adolescent Development

Support: Mallinckrodt Institute of Radiology Summer Research Program
Society for Neuroscience Trainee Professional Development Award
WUSTL McDonnell Center for Systems Neuroscience
NIH Grants U01DA041048, U01DA050989, U01DA051016, U01DA041022, U01DA051018, U01DA051037, U01DA050987, U01DA041174, U01DA041106, U01DA041117, U01DA041028, U01DA041134, U01DA050988, U01DA051039, U01DA041156, U01DA041025, U01DA041120, U01DA051038
NIH Grants U01DA041148, U01DA041093, U01DA041089, U24DA041123, and U24DA041147

Title: Bidirectional associations between waist circumference and basal ganglia microstructure in children over two years

Authors: *Z. A. LI[1], A. SANDERS[2], Y. CAI[4], M. RAY[2], Y. GU[1], T. HERSHEY[3]; [1]Psychiatry; Psychological & Brain Sci., [2]Psychiatry, [3]Psychiatry; Psychological & Brain Sciences; Neurology; Mallinckrodt Inst. of Radiology, Washington Univ. in St. Louis, Saint Louis, MO; [4]Neurol., Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Background
Basal ganglia gliosis has been noted as both a consequence and driver of diet-induced obesity in rodents. In children, obesity is cross-sectionally associated with greater diffusion-weighted magnetic resonance imaging-measured basal ganglia cellularity, consistent with neuroinflammation. Recent work has also suggested bidirectional associations between nucleus accumbens microstructure and weight gain over time, but it is unknown whether findings extend to other basal ganglia structures.
Methods
Baseline and two-year follow-up data were derived from the Adolescent Brain Cognitive Development (ABCD) Study. Tissue cellularity was assessed by scanner-harmonized restriction spectrum imaging (RSI) restricted normalized isotropic (RNI) diffusion. Bivariate latent change score models examined the longitudinal coupling between waist circumference and RSI-RNI in bilateral nucleus accumbens, caudate, putamen, and pallidum. Analyses were adjusted for sex, handedness, race/ethnicity, household income, parental education, and area deprivation index at baseline, and age, pubertal status, and mean head motion at both timepoints.
Results
A total of 3344 children were included (mean [SD] baseline age, 9.9 [0.6] years; 1696 [51%] boys; 119 [3%] with underweight, 2263 [68%] with normal-weight, 492 [15%] with overweight, and 470 [14%] with obesity). Greater baseline waist circumference was associated with larger two-year increase in RSI-RNI across the basal ganglia (eg, left caudate, $\beta = 0.89$, false discovery rate (FDR)-corrected $p < 0.001$; right putamen, $\beta = 0.67$, pFDR < 0.001). Further, greater baseline RSI-RNI across the basal ganglia was associated with larger two-year gain in waist circumference (eg, left caudate, $\beta = 0.58$, pFDR = 0.002; right putamen, $\beta = 0.60$, pFDR = 0.002). Gain in waist circumference was marginally correlated with increase in RSI-RNI in bilateral nucleus accumbens (eg, left, $\beta = 0.04$, pFDR = 0.06) and caudate (eg, left, $\beta = 0.05$, pFDR = 0.06). Similar findings were seen with body mass index (BMI) z-scores.
Conclusions
These results highlight bidirectional links between waist circumference and basal ganglia

cellularity in children over time. Research employing future ABCD Study data waves may extend these longitudinal findings and identify mediators.

Disclosures: Z.A. Li: None. **A. Sanders:** None. **Y. Cai:** None. **M. Ray:** None. **Y. Gu:** None. **T. Hershey:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.03/B52

Topic: A.09. Adolescent Development

Title: Gradients of myelination covariance during neurodevelopment

Authors: *Y. LI[1], J. BERO[1], C. HUMPHRIES[1], H. LEE[1], D. LEE[1,2];
[1]Neurogazer Inc., Baltimore, MD; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Structural covariance (SC) conceptualizes how morphological properties of different brain regions relates to each other at the group level and has been frequently utilized to study brain network and its development. In addition to the macrostructrual measures commonly used in constructing SC, such as cortical volume and thickness, the microstructure of the gray matter can be characterized by cortical myelin content estimated by T1w/T2w ratio. For example, to understand how the development of myeloarchitecture might be coordinated across brain regions, myelin covariance (MC) can be used to assess its topological organization. However, the macroscale cortical organization revealed by MC during neurodevelopment remains largely unexplored. In this study, we sought to situate MC in a low-dimensional space and assess age-related alterations in the principal modes of spatial variation in MC by comparing two age groups: adolescents (N=441, 10-15 years) from in-house dataset and young adults (N=1047, 22-35 years) from HCP dataset. MC matrix was constructed by correlating the T1w/T2w ratios between parcel pairs defined in Schaefer parcellation across individuals and its gradients were derived from the diffusion map embedding method. In adolescents, the principal gradient of MC (G1MC) accounted for 34% of the spatial variance and showed an anterior-posterior organizational axis from frontal cortex to occipital regions. The next three gradients showed a left-right, middle-pole, superior-inferior patterns, explaining 13%, 9%, and 8% variance, respectively. In young adults, The G1MC retained the anterior-posterior organization and accounted for 32% of the spatial variance. The next three MC gradients also exhibited patterns similar to those observed in adolescents. However, the order of these three gradients differed from that in adolescents, with superior-inferior, left-right, and somatosensory-occipital/frontal organizations accounting for decreasing variance. Nevertheless, close correspondence of the gradient modes between two age groups was confirmed by a Procrustes alignment. In sum, the observed organizational shifts in the major modes of MC suggest ongoing refinement of neural connections during neurodevelopment, with potential implications for cognitive development as well as the establishment of functional networks in the adult brain.

Disclosures: **Y. Li:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. **J. Bero:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **C. Humphries:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **H. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer Inc. **D. Lee:** A. Employment/Salary (full or part-time):; Neurogazer Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer Inc.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.04/B53

Topic: A.09. Adolescent Development

Support: NIH Grant R01 MH125479
NIH Grant R01 EB008374

Title: Development of Effective Connectome From Infancy to Adolescence

Authors: *G. LI, H. P. TAYLOR, K.-H. THUNG, Y. WU, Z. WU, G. LI, L. WANG, W. LIN, S. AHMAD, P.-T. YAP;
Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Delineating the normative developmental profile of functional connectome is important for both standardized assessment of individual growth and early detection of diseases. However, functional connectome has been mostly studied using functional connectivity (FC), where undirected connectivity strengths are estimated from statistical correlation of resting-state functional MRI (rs-fMRI) signals. To address this limitation, we applied regression dynamic causal modeling (rDCM) to delineate the developmental trajectories of effective connectivity (EC), the directed causal influence among neuronal populations, in whole-brain networks from infancy to adolescence (0-22 years old) based on high-quality rs-fMRI data from Baby Connectome Project (BCP) and Human Connectome Project - Development (HCPD). The rs-fMRI data included 288 subjects (158 females/160 males; 16 days-22 years) with a total of 428 scans. The BCP data was processed using an infant-dedicated pipeline and the HCPD data was processed by the HCP pipeline. Regional averaged blood-oxygen-level-dependent (BOLD) time series were extracted using the Desikan-Killiany atlas with 68 cortical regions of interest (ROIs) grouped into six functional networks (visual, somatomotor, salience, limbic, frontoparietal control, and default mode). We used linear mixed model to characterize the age-related continuous change in nodal EC computed as the sum of all incoming EC to a particular region. We found significant age effect on the mean nodal EC (averaged among 68 ROIs) which is best fit by a “U” shaped quadratic curve with minimal EC at around 2 years old. Further analysis indicates that five brain regions including the left and right cuneus, left precuneus, left supramarginal gyrus and right inferior temporal gyrus have the most significant age effect on

nodal EC ($p < 0.05$, FDR corrected). Moreover, the frontoparietal control network shows the fastest increase from early childhood (1-6 years) to late childhood and adolescence (6-21 years) followed by the visual and salience networks, indicating robust development of cognitive and sensory systems. Our study represents the first attempt to chart the developmental effective connectome from infancy to adolescence and suggests complex nonlinear developmental profile, which may reflect dynamic structural and functional maturation during this critical growth period.

Disclosures: G. Li: None. **H.P. Taylor:** None. **K. Thung:** None. **Y. Wu:** None. **Z. Wu:** None. **G. Li:** None. **L. Wang:** None. **W. Lin:** None. **S. Ahmad:** None. **P. Yap:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.05/B54

Topic: A.09. Adolescent Development

Support: This research was supported (in part) by the Intramural Research Program of the NIH (ZIAAA000550; National Institute on Alcohol Abuse and Alcoholism).

Title: Dissociable impact of perceived discrimination and structural racism on neural correlates of emotion regulation and inhibitory control: an ABCD study

Authors: *L. VINES[1], P. MANZA[1], D. TOMASI[1], G.-J. WANG[1], N. VOLKOW[2]; [1]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [2]NIH, Natl. Inst. On Drug Abuse, Gaithersburg, MD

Abstract: Perceived racial discrimination and structural racism affect neurocognitive function in children as early as 9 years old though few studies have disentangled these two factors. We examined how perceived discrimination (based on self-report) and structural racism (based on geographic location) each impact neural correlates of implicit emotion regulation and inhibitory control. To this end, we used the emotional-n-back (EN-Back) and Stop Signal (SST) fMRI tasks, respectively, in non-Hispanic Black (NHB; n=614) and non-Hispanic White (NHW; n=3968) children ages 9-10, and focused on pre-specified regions of interest based on a literature review. We employed mixed linear regression models with perceived discrimination or structural racism as predictors and EN-Back/SST brain responses as outcomes in pooled and race-stratified samples. In the pooled sample, for SST, lower perceived discrimination predicted greater activation of bilateral pars orbitalis (R B=-0.04, t=-2.68, p=7.50e-03; L B= -0.06, t=-3.96, p=8.29e-05) and right inferior frontal gyrus (B=-0.02, t=-2.02, p=0.04), and greater structural racism predicted greater activation of the left pars orbitalis (B=0.04, t=2.37, p=0.02). In NHB, for SST, we observed significant positive associations between perceived discrimination and all pre-defined ROIs; and for EN-Back task, greater perceived discrimination predicted greater

activation of bilateral nucleus accumbens (R B=0.10, t=2.48, p=0.02; L B=0.13, p= 2.54e-03) and lower activation of the left amygdala (B=-0.09, t=-2.34, p=0.02). In NHW, for SST, greater perceived discrimination was associated with lower activation of the bilateral inferior frontal gyrus (R B=-0.05, t=-2.84, p=4.73e-03; L B=-0.05, t=-2.99, p=2.93e-04) and pars orbitalis (R B=-0.05, t=-3.33, p=9.18e-04; L B=-0.07, t=-4.68, p=3.69e-06). In sum, we showed that perceived discrimination significantly impacts neural correlates of inhibitory control in the pooled, NHB, and NHW samples, with more global effects in NHB. Structural racism was only significantly associated with inhibitory control-related activation in the left pars orbitalis in the pooled sample. These results suggest that conscious perception of racism is more strongly associated with brain function during inhibitory control than structural racism, which may not play as pervasive a role in children at this young age. We also detected a significant effect of perceived discrimination on emotion regulation-related brain activation only in NHB. This race-specific result may demonstrate the pervasive effects of racism experienced by NHB based on their unique experiences in American Society.

Disclosures: L. Vines: None. **P. Manza:** None. **D. Tomasi:** None. **G. Wang:** None. **N. Volkow:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.06/B55

Topic: A.09. Adolescent Development

Support: R01HD081720

Title: The Corpus Callosum in Congenital Adrenal Hyperplasia

Authors: *E. LUEDERS[1], D. SPENCER[3], C. DALE[1], M. HINES[3], F. KURTH[2]; [2]Univ. of Auckland, [1]Univ. of Auckland, Auckland, New Zealand; [3]Cambridge Univ., Cambridge, United Kingdom

Abstract: Congenital adrenal hyperplasia (CAH) is a genetic variant that causes high levels of androgens during gestation in females, whereas levels in males are largely normal. Only little is known about the brain in CAH, and no study has specifically focused on the corpus callosum. Here we compared callosal area measures between 53 individuals with CAH and 53 control participants, who were pair-wise matched with respect to sex (33 women/20 men) and age (mean±SD: 30.2±7.8 years). The corpus callosum was manually outlined on T1-weighted brain images, obtained on a 3 Tesla scanner, and divided into seven sections according to the Witelson scheme (i.e., rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium). The midsagittal callosal areas were compared using a two-way ANOVA, while co-varying for age and total brain volume. Neither the main effect of biological sex (women vs. men) nor the group-by-sex interaction was significant. In contrast, there was a significant main

effect of group (CAH vs. controls) for some callosal areas. More specifically, women/men with CAH had significantly smaller callosal areas than control women/men within the isthmus (p=0.0024) and splenium (p=0.0048), both effects surviving Bonferroni corrections for multiple comparisons. In addition, there was a trend for a smaller posterior midbody in women/men with CAH compared to control women/men (p=0.0586). Given the lack of significant group-by-sex interactions (CAH-related effects were present in both sexes) it is likely that callosal abnormalities do not manifest as effects of prenatal androgens (otherwise effects would be restricted to women). Instead, they may reflect aspects of the disease and/or effects of treatment as both women and men with CAH receive supplements of glucocorticoids. The latter seems especially noteworthy because glucocorticoids are applied in a wide range of medical conditions, and possible adverse effects on the human brain may not be restricted to CAH.

Disclosures: **E. Lueders:** None. **D. Spencer:** None. **C. Dale:** None. **M. Hines:** None. **F. Kurth:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.07/B56

Topic: A.09. Adolescent Development

Support: Grant Nos. 31522028
Grant Nos. 31530031
Grant Nos. 81571056
Grant Nos. CNLZD1503
Grant Nos.CNLZD1703

Title: Positive coping shapes hippocampal-neocortical maturation: Behavioral evidence and neuroendocrinal mechanisms

Authors: T. TIAN[1], ***B. CHEN**[2], S. QIN[3];
[1]Beijing normal university, China, China; [2]Beijing Normal Univ., Beijing, China; [3]Beijing Normal Univ., Stanford Univ., Beijing, China

Abstract: A positive coping style is recognized as a stable disposition to foster emotional wellness and resilience, enabling an adaptive process of assessing and dealing with environmental challenges. Such an adaptive process is believed to rely on a nuanced interplay of the hippocampal system and the primary stress hormone cortisol activity. As a hallmark of diurnal cortisol rhythm, cortisol awakening response (CAR) is sensitive to upcoming stress and subserves the preparation of the hippocampal system for rapid behavioral adaption. Yet, little is known about how the hippocampal system and CAR contribute to the merit of positive coping on emotional wellness. By two studies, we investigate the effects of positive coping on children's emotional wellness and CAR, as well as longitudinal changes in hippocampal-neocortical

functional systems involved in emotional processing. Behaviorally, positive coping predicted better emotional regulation ability, but lower anxiety and lower response caution in emotional decision-making. At the endocrine and neurocognitive level, positive coping was associated with greater CAR, which further predicted higher connectivity of the hippocampus with ventrolateral prefrontal cortex (vlPFC) and stimulus-sensitive neocortex one year later. Furthermore, CAR mediated an indirect association between positive coping and longitudinal increases in hippocampal-neocortical connectivity. Positive coping and CAR together could account for the maturity of vlPFC through longitudinal changes in hippocampal-neocortical connectivity. Overall, our findings suggest a cognitive-neuroendocrinal framework in which positive coping shapes hippocampal-neocortical maturation via stress hormone response to support emotional wellness.

Disclosures: T. tian: None. **B. chen:** None. **S. Qin:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.08/B57

Topic: A.09. Adolescent Development

Title: Characterizing Developmental Patterns of Myelination as a Function of Pubertal Tempo in Typically Developing Children across Puberty

Authors: *I. M. WILDER[1], S.-M. WEI[2,3], J. KIPPENHAN[2], M. D. GREGORY[2], K. M. COLE[3], M. N. GOLDBERG[2], C. A. RECTO[2], D. S. WRIGHT[2], L. K. NIEMAN[4], J. A. YANOVSKI[5], P. J. SCHMIDT[3], K. F. BERMAN[2];
[1]NIH, Bethesda, MD; [2]Section on Integrative Neuroimaging, Clin. & Translational Neurosci. Branch,, [3]Behavioral Endocrinol. Br., Natl. Inst. of Mental Hlth., Bethesda, MD; [4]Diabetes, Endocrinology, and Obesity Br., Natl. Inst. of Diabetes and Digestive and Kidney Dis., Bethesda, MD; [5]Section on Growth and Obesity, Div. of Intramural Research, Eunice Kennedy Shriver, Natl. Inst. of Child Hlth. and Human Develop., Bethesda, MD

Abstract: BACKGROUND: The brain undergoes extensive structural changes, including myelination, during development, which greatly improves the speed and efficiency of neural network communications. Differences in pubertal tempo, the rate of progression through the pubertal stages, have been associated with structural and functional brain changes, but relevant literature is limited. Here, we characterized trajectories of myelin development associated with differential rates of pubertal tempo in a sample of typically developing children and adolescents between ages 8 and 18. **METHODS:** To measure myelination, quantitative maps of myelin water fraction (MWF) using mcDESPOT imaging were acquired longitudinally on a 3T-MRI scanner from 117 children over 460 visits (51 girls, mean age=12.1±2.8y, range 8.08-17.95; 66 boys, mean age=12.2±2.6y, range 8.08-17.98). Pubertal tempo was calculated at each visit by computing the rate of change of pubertal stage evaluated at 9-month intervals. A median split

generated a dichotomous, categorical variable of pubertal tempo (fast-vs.-slow). Average MWF values were extracted from a global white matter mask and from 20 canonical Johns Hopkins University-ICBM white matter tracts. Mixed-effects penalized-spline modeling of tempo and age were performed, separately for boys and girls, for both global and tract-based MWF across development using R's gamm4 package. **RESULT:** Pubertal tempo-related patterns were observed in the developmental trajectories of global MWF and specific tracts across puberty that differed notably between boys and girls. Specifically, in boys, fast tempo was associated with higher global MWF than was slow tempo (p=0.002), particularly in several major superior/posterior white matter tracts (p=0.0003). In contrast, girls showed an interaction of tempo with age (p=0.03) resulting in higher MWF for fast tempo at later ages compared with girls with slow tempo. **CONCLUSION:** We identified sex-specific tempo-related differences in developmental trajectories of global and tract-specific MWF across the pubertal transition. Future directions using this same cohort will probe pubertal timing, hormones such as estradiol and testosterone, and their impacts on developmental trajectories in brain structure.

Disclosures: I.M. Wilder: None. **S. Wei:** None. **J. Kippenhan:** None. **M.D. Gregory:** None. **K.M. Cole:** None. **M.N. Goldberg:** None. **C.A. Recto:** None. **D.S. Wright:** None. **L.K. Nieman:** None. **J.A. Yanovski:** None. **P.J. Schmidt:** None. **K.F. Berman:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.09/B58

Topic: A.09. Adolescent Development

Support: Adolescent Brains & Social Interactions Project, Bezos Family Foundation

Title: Covid-19 effects of adolescent brain structure suggest accelerated maturation

Authors: *N. M. CORRIGAN[1], A. ROKEM[2], P. K. KUHL[3];
[1]Inst. for Learning & Brain Sci., [2]Psychology, [3]Speech and Hearing Sci., Univ. of Washington, Seattle, WA

Abstract: Adolescence begets great changes in social and emotional development. The COVID-19 virus resulted in an extended period of social isolation, which had a particularly negative impact on adolescents. The hypothesis of this study was that disruptions to normal life caused by the COVID-19 pandemic altered the normal pattern of adolescent brain development, and that these alterations would be detectable with MRI. High-resolution MRI structural data were collected longitudinally at two time points: prior to the pandemic in 2018, and then after, in 2021 and 2022. The pre-COVID timepoint consisted of 158 subjects (79F) at 9, 11, 13, 15 and 17 years of age. The post-COVID timepoint consisted of 124 subjects (63F) at 12, 14, 16, 18 and 20 years of age. Cortical thickness for 68 brain regions were calculated from the MRI data using the

FreeSurfer software package. Bayesian linear regression was utilized to create a normative model of the relationship between cortical thickness and age for each brain region in a subset of the pre-COVID sample (N=108). This model was then used to calculate Z-scores for a different group of subjects in the post-COVID sample (N=50). Single-sample t-tests paired with FDR multiple comparison correction revealed that Z-score distributions for 29 brain regions, located in both hemispheres and all lobes of the cerebrum, had negative means that were significantly different from zero in the post-COVID measurements, indicating wide-spread acceleration in brain development typically associated with chronic stress or adversity. An exploratory analysis of gender differences in the post-COVID Z-score distributions suggested that the observed deviations in cortical thickness changes with development were primarily due to the female subjects in the sample. These findings suggest that lifestyle disruptions associated with the COVID-19 pandemic resulted in widespread changes in cortical thickness during adolescence that are typically associated with chronic stress.

Disclosures: **N.M. Corrigan:** None. **A. Rokem:** None. **P.K. Kuhl:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.10/B59

Topic: A.09. Adolescent Development

Support: NIH Grant 5R25NS114326

Title: The impact of early environmental adversity on autonomic nervous system: a longitudinal study

Authors: J. J. ESCATEL-FLORES[1], L. KOFLER[2], Y. GAO[1];
[1]Psychology, Brooklyn Col., Brooklyn, NY; [2]CUNY graduate center, New york, NY

Abstract: Respiratory sinus arrhythmia (RSA) is a heart rate variability measure indexing parasympathetic nervous system-linked cardiac activity and has been linked to emotion regulation and multiple psychopathologies. Environmental adversity (EA) is defined as risk factors such as neighborhood crime, parental marital conflict, low social economic status, stress, and abuse that occur in a person's lifetime. Researchers have found that EA has a significant impact on RSA changes in children and adolescents. Evidence has suggested that in children, girls who experience more EA have a lower resting RSA and are more likely to show poor emotional regulation (Feurer et al., 2019). Another study found evidence linking potential traumatic events to low resting RSA (Gray et al., 2017). In contrast, one study found that positive parenting style was associated with lower RSA in low-marital-stress settings and higher RSA in high-marital-stress settings (Lisitsa, 2021). In this study, we aim to examine the effects of different EA factors on RSA. Specifically, it was hypothesized that overall higher EA would be associated with lower RSA and that parental marital conflict would be the strongest EA factor

that impacts resting RSA. Data from a longitudinal study was used to test these hypotheses. Participants consisted of 8-11-year-old boys and girls and their caregivers who visited the laboratory for a battery of tests. Resting RSA was measured while children were relaxing for 2 minutes, and EA was assessed via parents' reports on social adversity index, prenatal maternal stress, neighborhood collectiveness, child abuse, domestic violence, and parenting styles. Results failed to support the proposition that exposure to higher amounts of EA is associated with a lower resting RSA. Limitations of the study included that participants were recruited from one neighborhood only and that they were from non-clinical populations with generally low rates of EA.

Disclosures: J.J. Escatel-Flores: None. **L. Kofler:** None. **Y. Gao:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.11/B60

Topic: A.09. Adolescent Development

Support: MH067924

Title: Maturation of prefrontal E/I balance during adolescence drives development of non-oscillatory neural activity

Authors: *F. J. CALABRO[1,2,3], M. PERICA[4], S. MCKEON[2], A. PARR[3], W. F. FORAN[3], V. YUSHMANOV[5], H. HETHERINGTON[5], B. LUNA[3];
[2]Bioengineering, [3]Psychiatry, [4]Psychology, [5]Radiology, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Introduction: Prefrontal cortex (PFC) undergoes protracted maturation through adolescence which may be supported by mechanisms of critical period plasticity, including increased inhibitory, GABAergic interneurons and decreased excitatory, glutamatergic processes. Recently, we reported *in vivo* Magnetic Resonance Spectroscopic Imaging (MRSI) evidence in humans for greater correlation, or balance, of glutamate and GABA emerging from adolescence into adulthood (Perica et al., 2022). It is not clear, however, how developmental changes in neurotransmitter measures of E/I balance may affect neural activity dynamics. Recent work has suggested the aperiodic component of EEG activity, represented by the slope and offset of the Power Spectral Density (PSD), may reflect underlying excitation/inhibition (E/I) balance (Donoghue et al., 2020) and neuronal population spiking (Manning et al., 2009) respectively. Here we test the hypothesis that developmental changes in E/I balance are associated with broadband background aperiodic neural activity. **Methods:** Longitudinal data (up to 3 visits at 18mo intervals) was collected from participants (n = 164; 87 AFAB, 10 - 32 years old, n = 286 total sessions) recruited from the community with no prior or current neurological or psychiatric diagnosis. 7 Tesla Magnetic Resonance Spectroscopic Imaging data acquired from DLPFC was used to estimate GABA/Cr and glutamate/Cr with LCModel (Provencher et al, 2001). On a

separate day, participants completed an EEG session using a high-impedance Biosemi ActiveTwo 64-channel EEG system, which was analyzed with the Fitting Oscillations and One Over f (FOOOF) toolbox (Donoghue et al., 2020). **Results:** In DLPFC, the correlation between glutamate/Cr and GABA/Cr, reflecting E/I balance, increased through adolescence ($F = 11.04$, $p = 0.0014$), while EEG-derived aperiodic slope ($F = 63.16$, $p < 0.0001$) and offset ($F = 240.07$, $p < 0.0001$) significantly decreased with age. Age-related decreases in aperiodic slope were associated with, and mediated by, changes in glu/GABA balance ($t = 2.01$, $p = 0.035$), indicating a link between MRSI-derived measures of E/I balance and developmental changes in aperiodic EEG activity. **Conclusion:** Maturation of glutamate and GABA through adolescence may underlie developmental changes in non-oscillatory neural activity, reflecting a neurobiological mechanism consistent with the framework of a prefrontal critical period for adolescent neurocognitive development. Understanding the development of E/I transmission during adolescence can inform psychopathologies, e.g., schizophrenia, that emerge during adolescence and may involve alterations in E/I.

Disclosures: **F.J. Calabro:** None. **M. Perica:** None. **S. McKeon:** None. **A. Parr:** None. **W.F. Foran:** None. **V. Yushmanov:** None. **H. Hetherington:** None. **B. Luna:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.12/B61

Topic: A.09. Adolescent Development

Title: Spatial and temporal autocorrelation of resting-state fMRI during adolescence

Authors: *J. BERO[1], Y. LI[1], A. KUMAR[1], C. J. HUMPHRIES[1], H. LEE[1], M. SHINN[2], J. D. MURRAY[3], T. J. VICKERY[4], D. LEE[1,5];
[1]Neurogazer USA Inc., Towson, MD; [2]UCL Queen Square Inst. of Neurol., Univ. Col. London, London, United Kingdom; [3]Psychiatry, Yale Univ., New Haven, CT; [4]Dept. of Psychology, Univ. of Delaware, Newark, DE; [5]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The spatial and temporal autocorrelations of functional magnetic resonance imaging (fMRI) data are highly robust and can provide a reliable description of the topological structure and temporal dynamics of the fMRI signal. For example, a recent study has shown that the spatial and temporal autocorrelation of resting-state fMRI can predict the individual variability in functional connectivity (FC) networks more parsimoniously than graph theoretic measures. In addition, although developmental studies have found systematic changes in the patterns of FC during adolescence, little is known about how the autocorrelation of resting-state fMRI changes during development. Therefore, in the present study, we quantified and compared temporal and spatial autocorrelations in resting-state fMRI data collected from an in-house adolescent dataset (N=443; age range = 10 to 15), the Human Connectome Project (HCP) 1200 subject dataset (N=1,084; age range = 22 to 37), and the HCP Developmental dataset (N=652; age range = 5 to

22). Comparison of temporal and spatial scales from the corresponding autocorrelation functions showed that the pattern of variation across the brain was highly consistent for adolescents and adults (r>0.9 for temporal and spatial autocorrelation). These patterns were robust and independent of the method used to measure the timescale, including the first-order autoregressive coefficient, parameters of double exponential functions fit to the autocorrelation functions, and power spectrum knee frequency. We also examined the effect of age on the autocorrelation, using both analysis of age-split subgroups as well as regression models, and found that the changes in temporal and spatial autocorrelation during adolescence followed the same trend observed from adolescents to adults. Patterns in regional variability and age-related change were further confirmed in the developmental dataset. These findings suggest that age-related changes in temporal and spatial autocorrelation in fMRI signals reflect brain development and might underlie changes in functional connectivity during adolescence.

Disclosures: **J. Bero:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. **Y. Li:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. **A. Kumar:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. **C.J. Humphries:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. **H. Lee:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc.. **M. Shinn:** None. **J.D. Murray:** None. **T.J. Vickery:** None. **D. Lee:** A. Employment/Salary (full or part-time):; Neurogazer USA Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer Inc.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.13/B62

Topic: A.09. Adolescent Development

Support: F32AA029930-01A1
K23AA025399-01
MUSC SCORE Pilot Project

Title: Multi-modal neuroimaging does not reveal neurometabolite level or alcohol-cue reactivity differences between adolescents who drink alcohol compared to controls

Authors: *A. E. KIRKLAND[1], R. GREEN[1], B. D. BROWNING[2], L. M. SQUEGLIA[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Neurosci., Med. Univ. of South Carolina (MU Neurosci. Inst. - Grad., Charleston, SC

Abstract: Heavy alcohol use has been related to brain changes in adults, including changes in metabolite levels and reactivity to alcohol cues. However, it is currently unknown if heavy alcohol use affects the brain in similar ways during adolescence, when alcohol use is typically initiated and escalates. The objective of this study was to use multi-modal neuroimaging to

assess the effects of heavy alcohol use during adolescence on (1) metabolite levels measured via proton magnetic resonance spectroscopy (MRS), and (2) alcohol-cue reactivity measured via fMRI blood oxygen level dependent (BOLD) signal. Non-treatment seeking adolescents (n= 46, average age= 18.9, 61% female) who drank alcohol heavily (≥4 drinking occasions/month, ≥3 standard drinks/occasion) and age-, gender-, and psychological diagnosis- matched controls (n=21, average age= 18.6, 52% female) were recruited. The neurometabolite levels were measured in the dorsal ACC (dACC), and metabolites of interest were glutamate, GABA, N-acetylaspartate, total choline, total creatine, *myo*-inositol, and glutathione. Regions-of-interest (8 mm) for alcohol-cue reactivity were the midline dACC and bilateral amygdala, caudate, putamen, insula, and nucleus accumbens. The fMRI contrast of interest was images of alcohol beverages compared to neutral, non-alcohol beverages (z-score). All models were covaried for biological sex and age, and metabolite models were corrected for brain tissue composition within the dACC. Pearson correlations were run between metabolite levels and alcohol-cue reactivity in the dACC within groups and across the full sample. We found no differences in metabolite levels or alcohol-cue activity between adolescents who drank heavily and controls. There was an effect of biological sex on alcohol-cue reactivity within the left putamen (β= -0.56, p= 0.03), where females showed stronger reactivity to alcohol cues than males. There was also an age effect in the right caudate (β= 0.36, p=0.05), where reactivity to alcohol cues was higher in older participants regardless of alcohol use. There were no significant correlations between metabolite levels and alcohol-cue reactivity in the dACC. The null findings may be due to the young age of participants, relatively low severity of alcohol use, and non-treatment seeking status of the sample. Future research should continue to investigate the progression of alcohol-related brain changes that have been noted in adults.

Disclosures: A.E. Kirkland: None. **R. Green:** None. **B.D. Browning:** None. **L.M. Squeglia:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.14/B63

Topic: A.09. Adolescent Development

Support: NSERC Discovery Grant
Canada Foundation for Innovation JELF
Ontario Research Fund
University of Toronto funds to MLS

Title: Prefrontal engagement during retrieval of overlapping memories at specific and general levels of detail in adolescents and adults

Authors: *M. WOODBURY[1], S. VIJAYARAJAH[2], M. L. SCHLICHTING[2];
[1]Psychology, Univ. of Toronoto, Toronto, ON, Canada; [2]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Remembering our lives accurately often requires telling apart similar, overlapping, experiences. However, prolonged prefrontal development suggests that adolescents may not have refined the top-down ventrolateral prefrontal cortex (vlPFC)-mediated processes used by adults to select between similar memories at recall. Here, we addressed this question by having adolescents (12-13 years) and adults retrieve overlapping information while collecting fMRI data. Participants first learned pairs composed of a natural object and a unique artifact. Natural objects came from four categories, which we selected to have high (apple, shell) or low (leaf, rock) intra-category similarity, yielding high or low overlap respectively among same-category pairs. At retrieval, participants were shown artifacts and asked to recall the paired natural item. To additionally test the impact of behavioural goals on retrieval selection, we cued participants before each trial as to whether the ensuing question would ask them to select the specific paired object (e.g., apple 1 or 2) or general category ("apple" or "rock"). We expected that higher overlap among learned pairs would place greater demands on selection processes, especially for specific retrieval. Adolescents were indeed less accurate and slower than adults on specific questions, especially for high overlap pairs, suggesting they were particularly impacted by high selection demands. In contrast to retrieving specific objects, where selecting between memories is necessary, adolescents showed very little impact of overlap when answering category-level questions. Adults even showed behavioural benefits of higher overlap for retrieving the category, consistent with abstraction across related pairs. When comparing activation within the vlPFC during retrieval we found greater activity for high compared to low overlap pairs across age groups. Activity in the vlPFC was also greater for specific compared to category questions. Together, these findings suggest that adolescents and adults may employ vlPFC-mediated processes when there is a high demand to select between related memories, either due to considerable memory overlap or need for high memory specificity. In future analyses, we will investigate whether the degree of vlPFC engagement during retrieval of high overlap pairs is related to accuracy and response time in either age group. Overall, this work will speak to the possibility that preliminary vlPFC retrieval selection processes are present in adolescence while their ongoing maturation may contribute to challenges in selecting among especially competitive memories.

Disclosures: M. Woodbury: None. **S. Vijayarajah:** None. **M.L. Schlichting:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.15/C1

Topic: A.09. Adolescent Development

Support: NSF Grant 2116707
NSF Grant 1649865

Title: The topological organization of developing brain circuits prior to the COVID-19 pandemic predicts adolescents' emotions and response to stress during the pandemic

Authors: L. HU[1], C. SMITH[3], *C. STAMOULIS[2];
[1]Biostatistics, [2]Pediatrics, Harvard Univ., Boston, MA; [3]Children's Hosp. of Boston, Boston, MA

Abstract: The COVID-19 pandemic has had extensive adverse impacts worldwide. The extent of its effects in youth, including on brain development and mental health, is unknown. However, the rapidly growing mental health crisis, particularly in adolescents, suggests that pandemic effects on the developing brain are likely profound, but may depend on the vulnerability and resilience of incompletely maturated brain circuits to stressors.This study investigated whether the topological organization of adolescent brain circuits prior to the pandemic predicted mental/emotional health outcomes during the pandemic. Survey data collected as part of the Adolescent Brain Cognitive Development (ABCD) study at 7 time points (2-3-month apart, starting in May 2020), and resting-state fMRI from subcohorts of youth who had been scanned ≤9 months prior to each survey were analyzed (median age = 144 months). Sample sizes varied from n = 671 at survey 1 (scan to survey was 0-8 months) to n = 218 at survey 7 (scan to survey from scan was 3-8 months). Outcomes included self-assessed emotional wellbeing, perceived stress, sadness, stress about uncertainty, and positive affect. In addition to demographics, and other individual characteristics, all analyses were also adjusted for parent engagement during the pandemic and prior youth mental health problems. Significant associations between brain topology and outcomes of interest were estimated primarily in surveys obtained October to December 2020. Higher stress was associated with lower median connectivity and topological robustness of the limbic network and amygdala ($p<0.04$, $\beta = -0.30$ to -0.16), higher network fragility and lower topological stability of basal ganglia and thalamus ($p<0.05$, $\beta = -0.25$ to -0.17), lower topological robustness and higher fragility of amygdala ($p<0.01$, $\beta = -1.64$ to -0.32), and lower median connectivity and global clustering of prefrontal cortices, and salience, limbic, temporoparietal, and reward networks ($p<0.05$, $\beta = -1.68$ to -0.67). In addition, increased sadness was associated with lower median connectivity between amygdala and thalamus ($p<0.04$, $\beta = -0.24$ to -0.22), higher fragility of the reward and prefrontal networks, as well as lower efficiency and global modularity (clustering) of these networks. Finally, higher stress and sadness ~15 months from the start of the pandemic was associated with lower connectivity between amygdala and thalamus ($p<0.03$, $\beta = -0.24$ to -0.22). These results suggest that the topological organization of adolescent brain circuits prior to the pandemic, may have played a significant role in youth emotional health and response to stress during the pandemic.

Disclosures: L. Hu: None. **C. Smith:** None. **C. Stamoulis:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.16/C2

Topic: A.09. Adolescent Development

Support: U01DA041117
U24DA041123, U24DA041147
U01DA041048, U01DA050989
U01DA041134, U01DA041156
U01DA041028, U01DA041025
U01DA051016, U01DA051039
U01DA041022, U01DA050988
U01DA051018, U01DA041089
U01DA051037, U01DA041093
U01DA050987, U01DA041148
U01DA041174, U01DA051038
U01DA041106, U01DA041120
https://abcdstudy.org/federal-partners.html

Title: Associations between adolescent sleep duration, impulsivity, and brain morphometry from the Adolescent Brain Cognitive Development (ABCD)® study

Authors: A. BEERAM[1], *C. CLOAK[2], A. ISAIAH[2], L. CHANG[2], T. M. ERNST[2];
[1]Penn State Univ. Park, State College, PA; [2]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Adolescence is marked by impulsive behavior, insufficient sleep, and ongoing brain maturation. Here we assess the relationship between sleep duration, impulsivity, and brain morphometry in youth. We hypothesize that youth with short sleep duration exhibit more impulsivity and have thinner cortices, smaller cortical volumes (CV), and smaller surface areas (SA) in the prefrontal cortex (PFC) but larger striatal and amygdalar volumes. Since the PFC is involved in mediating reward response via the striatum, we also hypothesize that smaller PFC measures reflect a weaker top-down control of impulsivity, and youths exhibiting more impulsivity have larger striatal and amygdalar volumes. Structural MRI measures, Urgency Premeditation Perseverance Sensation Seeking and Positive Urgency (UPPS-P) impulsive behavior scale scores, and wearable fitness tracker-measured sleep intervals were evaluated in 3769 youths (52% boys, 11.9±0.6 years old) from the ABCD® study 2nd follow-up visit. We used linear mixed models (covaried for site, age and sex for all measures, and intracranial volume for regional brain volumes) to assess associations between sleep duration, impulsivity traits, and regional morphometry. Shorter sleep duration was associated with more impulsivity (FDR-corrected $p<0.001$). Smaller SA and CV in the orbitofrontal cortex (OFC), cingulate cortex, and PFC were associated with shorter sleep duration and higher impulsivity scores (FDR $p<0.05$). Contrary to our hypothesis, smaller striatal and amygdalar volumes were associated with shorter sleep duration and more impulsivity (FDR $p<0.05$). Post hoc analyses of impulsivity sub-scores showed that shorter sleep duration was associated with less sensation-seeking but higher scores for positive and negative urgency, lack of perseverance, and lack of planning (FDR $p<0.001$). Additionally, less sensation-seeking was related to smaller SA and CV in multiple regions of the OFC and PFC, as well as smaller striatal volumes (FDR $p<0.05$). These findings demonstrate associations between adolescent sleep, impulsivity, and region-specific brain morphometry. This suggests less sleep may accelerate or amplify cortical and sub-cortical pruning during adolescent

development, altering typical developmental patterns of decreasing impulsivity and increasing sensation-seeking with age. These findings provide further evidence that adolescents need sufficient sleep for proper brain maturation. The ABCD study will follow these youths through their teen years; therefore, future longitudinal analyses may demonstrate causal relationships between sleep, brain maturation, and impulsivity.

Disclosures: **A. Beeram:** None. **C. Cloak:** None. **A. Isaiah:** None. **L. Chang:** Other; Drs Chang and Ernst are married. **T.M. Ernst:** Other; Drs Chang and Ernst are married.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.17/C3

Topic: A.09. Adolescent Development

Support: NIH Grant 5R01MH091864-10 (co-PIs: Tottenham, Milham).
NIH D-SPAN Award 1F99 NS134207-01 (PI: Vannucci)
Frank Putnam Trauma Research Scholar Award, International Society for Traumatic Stress Studies
Graduate Student Grant, Psy Chi International Honor Society

Title: Parental unpredictability is associated with broader medial prefrontal-subcortical circuitry recruitment during affective schema processing in human development

Authors: *A. VANNUCCI, C. VICIOSO, A. FIELDS, L. ABRAMSON, E. NIEMIEC, E. JOYCE, D. G. JUAREZ, L. GIBSON, N. TOTTENHAM;
Columbia Univ., New York, NY

Abstract: Background and Objective: Unpredictable parental care alters medial prefrontal cortex (mPFC)-subcortical circuitry development in rodents. Separately, cognitive neuroscience experiments show that mPFC-subcortical circuitry has a broader functional role in schema processes. This study asked: might the parental unpredictability-related neural patterns in this circuitry reflect (a) schemas of unpredictability (beliefs that people and the world are unpredictable) or (b) weak schemas with poor structural integrity (due to difficulty abstracting semantic information from the world reliably)?
Methods: The sample comprised 98 adolescents (10-17 years-old; 43F/55M), most of whom had a history of early caregiving adversity (72%). Parental unpredictability was assessed via parent reports, and adolescents self-reported on their unpredictability schemas. We measured adolescents' responses to abstract animated shape stimuli designed to evoke affective schemas (child 'needs-met' vs. 'needs-not-met' by others), including BOLD signal during an fMRI scan and verbal content during a post-scan recall of the task stimuli. The Linguistic Inquiry and Word Count software coded the affective semantic content and uncertainty of verbal responses. ROI analyses adjusted for age, sex, socioeconomic status, verbal IQ, head motion, and early adversity

exposure.
Results: Robust amygdala and ventral mPFC activation was found when processing the needs-not-met (vs. needs-met) schema in all adolescents. However, higher parental unpredictability was also associated with broader recruitment of hippocampal and striatal regions. Among adolescents exposed to high parental unpredictability, vmPFC-amygdala-hippocampal engagement was associated with greater negative affect inferred across both the needs-not-met and needs-met schemas, suggesting a lack of neuroaffective differentiation. Further, this aberrant amygdala engagement was associated with use of more uncertain language when recalling schemas for both conditions. By contrast, in those exposed to low parental unpredictability, vmPFC-amygdala engagement when processing the needs-not-met schema was linked to higher negative affect inferred solely from this schema, showing differentiation from the needs-met schema. Neither parental unpredictability nor schema-evoked neural activity was associated with adolescents' unpredictability schema content.
Conclusions: vmPFC-subcortical alterations linked to parental unpredictability may reflect overly general or imprecise affective schemas that may bias adolescents' meaning-making toward negative affect across contexts.

Disclosures: **A. Vannucci:** None. **C. Vicioso:** None. **A. Fields:** None. **L. Abramson:** None. **E. Niemiec:** None. **E. Joyce:** None. **D.G. Juarez:** None. **L. Gibson:** None. **N. Tottenham:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.18/C4

Topic: A.09. Adolescent Development

Support: OU Undergraduate Research Opportunities Program

Title: Impact of Childhood Parenting Style on Neural Correlates of Self-Perception in Young Adults

Authors: *J. HEIDARY[1], S. LINGAMALLU[1], R. GARG[1], S. PICO RODRIGUEZ[1], E. KELLY[1], J. NORRIS[1], L. ETHRIDGE[2];
[2]Psychology, [1]The Univ. of Oklahoma, Norman, OK

Abstract: Self-Perception is an image we hold about ourselves and our traits and the judgments we make about those traits. The type of parenting style experienced as a child (authoritative, authoritarian, or permissive) has been shown to impact future self-esteem in young adults, however little is known about the effects on more global self-concept of the potential impact on neural processing of self. Parenting style has been found to impact executive function measured via EEG resting alpha peak frequency in children, however whether these effects carry over into young adulthood is unknown. The current study surveyed 217 young adults regarding parenting styles, childhood experiences, attachment, and self-concept. Of these participants, 34 followed

up with dense-array EEG. EEG tasks included eyes open/eyes closed resting state and a behavioral task in which participants had to indicate yes/no whether adjectives characterized either themselves or a close friend. The purpose of this task is to evaluate the differences in processing concepts related to the self vs. a close other (self-referential processing). For the survey measures, significant differences were found between parenting styles in the self-perception domains of parent relationships $F(2,180)=5.2$, $p<.05$, athletic competence $F(2,180)=8.5$, $p<.001$), humor $F(2,180)=4.9$, $p<.05$, and global self-worth $F(2,180)=7.8$, $p<.001$. In these domains, participants with authoritative style parents generally had higher means than their peers with permissive or authoritarian styles, indicating that they had higher levels of competence in these domains. The EEG sample means followed a similar pattern to those in the larger survey sample. Across samples, students with authoritative style parents reported more secure styles of attachment than their peers. Power spectrum density was computed in the alpha frequency band (8-12 Hz) for eyes open and eyes closed resting EEG conditions. There were no significant differences between parenting style groups in relative alpha reactivity (eyes open minus eyes closed) or peak alpha frequency in the frontal and occipital lobes. This finding is important in that it indicates that baseline neural arousal and neural differences related to executive function and arousal found in children with different parenting styles do not carry over into adulthood. Anticipated outcomes for the behavioral EEG task include more differentiation in self-referential processing from other-referential processing in medial prefrontal cortex for individuals with an authoritative primary caregiver, consistent with survey results that indicate a stronger sense of self for these individuals.

Disclosures: J. Heidary: None. **S. Lingamallu:** None. **R. Garg:** None. **S. Pico Rodriguez:** None. **E. Kelly:** None. **J. Norris:** None. **L. Ethridge:** None.

Poster

PSTR451. Developmental Brain Changes: Human Imaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR451.19/C5

Topic: A.09. Adolescent Development

Title: Unveiling Brain Mechanisms in Early-Onset Anorexia Nervosa

Authors: *C. A. MOREAU[1], A. AYROLLES[3], R. BONICEL[3], N. TRAUT[4], C. STORDEUR[6], P. M. THOMPSON[2], T. BOURGERON[5], R. DELORME[7];
[1]USC Stevens Neuroimaging and Informatics Inst., USC, Venice, CA; [2]USC, Los Angeles, CA; [3]Child and Adolescent Psychiatry Dept., Robert Debré, APHP, Paris, France; [5]Inst. Pasteur, [4]Inst. Pasteur, Paris, France; [6]Child and Adolescent Psychiatry Dept., Robert Debré hospital, APHP, Paris, France; [7]Child and Adolescent Psychiatry Dept., APHP, Paris, France

Abstract: Anorexia nervosa (AN) is a serious psychiatric disorder associated with one of the highest mortality rates of any psychiatric disorder that typically starts during adolescence. Almost one-fifth of patients with AN have comorbidities with obsessive-compulsive disorder

(OCD). Neuroimaging studies conducted so far in AN have been constrained by small sample sizes and limited to females with a typical onset (TO). The largest study to date (conducted by ENIGMA) revealed lower cortical thickness (CT) in young women with TO-AN, with a gradient associated with weight restoration levels. We aimed to compare the impact of the early-onset (EO) form of AN and TO-AN on brain structure and to assess similarities at the brain level between AN and six psychiatric disorders.
We aggregated neuroimaging data from the Debré Hospital (France) on 198 children: 103 with EO-AN (83% female, mean age=10.5) and 95 with typical development (TD, 46% female, mean age=10.2). All 3D structural brain MRI scans were processed using the FreeSurfer pipeline and segmented using the Desikan-Killiany atlas into 68 cortical regions and 16 volumes. Linear regression with FDR adjustment was applied to assess group differences, taking into account age, sex, and scanner. CT, surface area (SA), and subcortical volumes were investigated. The EO-AN brain profile was correlated (Pearson, 5000 permutations) with six psychiatric disorders using the ENIGMA Toolbox, followed by a comparison between brain-based (r_B) and genetic-based (r_G) correlations.
Children with EO-AN exhibited a significant CSF volume increase and CT decrease compared to TD individuals (Cohen's d: +0.8 and -0.7 respectively). Global thickness reduction was mainly driven by the left precuneus and the left superior parietal cortex. We also observed significant thalamic volume reduction but did not detect an effect on SA. Results were concordant (76%) with prior findings on TO-AN, with regional effect sizes slightly larger in EO-AN than in TO-AN (absolute mean top-decile=0.96 and 0.87, respectively). Comparing the EO-AN effect size distribution with six psychiatric conditions revealed that EO-AN has one of the largest CT reductions ever seen in psychiatry. We finally observed a large concordance (CCC=0.84) between r_B and r_G across six pairs of psychiatric conditions (e.g., r_B[AN-OCD]=0.53 and r_G[AN-OCD]=0.48).
The similarities in brain structure abnormalities between EO- and TO-AN suggest general underlying mechanisms in AN, independent of age and sex. The brain and genetic overlaps between AN and other psychiatric diagnoses were in line with what has been observed clinically, supporting the need to conduct transdiagnostic studies.

Disclosures: **C.A. Moreau:** None. **A. Ayrolles:** None. **R. Bonicel:** None. **N. Traut:** None. **C. Stordeur:** None. **P.M. Thompson:** None. **T. Bourgeron:** None. **R. Delorme:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.01/C6

Topic: A.09. Adolescent Development

Support: Simons Foundation Autism Research Initiative Pilot Award 610850 (HM)
NIMH R01MH118297 (HM)
NIMH F31MH127805 (ML)

Title: Aberrant social experience after juvenile social isolation impairs recruitment of frontal corticothalamic neurons necessary for sociability

Authors: *M. LEVENTHAL, K. OKAMURA, A. LIDOSKI, M. JANIS, L. WALTRIP, H. MORISHITA;
Mount Sinai, New York, NY

Abstract: Juvenile social isolation (JSI) is known to disrupt social behavior in adulthood, but little is known about the neural mechanisms of social experience-dependent brain maturation that are disrupted by JSI. Previous studies suggest that, in male mice, there is a critical period between postnatal day (p) 21 and p35 when isolation will reduce adult sociability and induce prefrontal cortex (PFC) abnormalities, such as dampened excitability in medial PFC neurons projecting to the posterior paraventricular thalamus (mPFC-pPVT neurons). Interestingly, these circuit abnormalities were not present at the end of the isolation period (p35), raising the question of when and how JSI-induced social deficits emerge over the course of development. To investigate the developmental progression of JSI-induced social dysfunction, we assessed sociability at multiple timepoints using the three-chamber test and free reciprocal interaction. During the post-isolation developmental period, we conducted tests of affiliative behavior and aggression among cage mates and used patch clamp electrophysiology to examine the excitability of mPFC-pPVT neurons. We unexpectedly found that JSI-induced sociability deficits in the three-chamber test (where subjects interact with novel mice) and associated dysregulation of mPFC-pPVT neurons were not present at the end of isolation. Instead, deficits emerge during the first 2 weeks of the adolescent rehousing period (p36-p50). Detailed examination of the first week after rehousing revealed a dynamic transition of cagemate interaction from aggression at p35 to social withdrawal by p42. Of note, chronic social isolation (p21-p50) without rehousing did not induce sociability deficits or mPFC-PVT neuron deficits at p50, suggesting that developmental mismatch during the post-rehousing period plays a key role in driving the dysregulation induced by JSI. These results suggest that JSI may disrupt adult social behavior not only by impairing social development during the isolation period, but also by impairing subsequent development during the post-isolation developmental period. We propose that the prevailing “social deprivation model”, where adult social deficits are attributed to disruption of developmental processes occurring *during* the isolation period, should be supplemented by the “developmental mismatch model”, where social deficits are attributed to disruption of developmental processes occurring *after* the isolation period. An important implication of the developmental mismatch model is that social deficits induced by aberrant juvenile social experience may be treated by intervening during adolescence.

Disclosures: M. Leventhal: None. **K. Okamura:** None. **A. Lidoski:** None. **M. Janis:** None. **L. Waltrip:** None. **H. Morishita:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.02/C7

Topic: A.09. Adolescent Development

Support: Simons Foundation Autism Research Initiative Pilot Award 610850
NIMH R01MH118297
The Osaka Medical Research Foundation for Intractable Diseases 27-3-2

Title: Dysregulated maturation of frontal corticothalamic projections underlies autism-related social behavior deficits

Authors: *K. OKAMURA[1], A. LIDOSKI[1], M. LEVENTHAL[1], A. KAWATAKE-KUNO[1], Y. GARKUN[1], T. NISHIOKA[1], B. STEVENS[1], J. RICEBERG[1], K. YAMAMURO[2], H. MORISHITA[1];
[1]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New york, NY; [2]Psychiatry, Nara Med. Univ., Kashihara, Japan

Abstract: Impaired social processing is one of the hallmarks of autism spectrum disorder (ASD), yet little is known about the links between genetic risks and the circuit maturation underlying social processing. Genetic and transcriptomic studies have shown that ASD risk genes are enriched in maturing prefrontal cortical (PFC) deep layer projection neurons. Based on our recent finding that deep layer projections from medial prefrontal cortex (mPFC) to paraventricular nucleus of thalamus (PVT) (mPFC→pPVT) are necessary for proper sociability, but dysregulated by juvenile social isolation (Yamamuro et al, Nat. Neurosci 2020), we aimed to examine the impact of ASD risk genes on the maturation of mPFC→pPVT neurons and social processing. We employed whole-cell patch clamp recording to characterize mPFC→pPVT neuron function in Fmr1KO and Tsc2 Het mice. Fiber photometry imaging was used to interrogate the effects of the Fmr1KO genotype on mPFC→pPVT neurons. To explore potential therapeutic strategies, an optogenetic approach was combined with social behavior characterization of Fmr1KO mice. In two mouse lines harboring mutations in ASD risk genes (Fmr1-KO and Tsc2-Ht), we found that mPFC→pPVT neurons show reduced excitability and increased inhibitory drive in adulthood. Developmental characterization of mPFC→pPVT neurons in Fmr1KO mice revealed that deficits emerge during the juvenile period between p21 and p35, and persist until adulthood. Fiber photometry imaging of adult Fmr1KO mice during social behavior revealed blunted recruitment of mPFC→pPVT neurons during social contact, and optogenetic stimulation of mPFC→pPVT neurons was sufficient to acutely reverse social dysfunction in the adult Fmr1KO mice. These findings support that the frontal-thalamic projection to pPVT, essential for social processing, is not only a key converging circuit vulnerable to multiple ASD risk genes, but also a promising therapeutic target for circuit cure of social processing deficits in ASD.

Disclosures: **K. Okamura:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; The Osaka Medical Research Foundation for Intractable Diseases. **A. Lidoski:** None. **M. Leventhal:** None. **A. Kawatake-Kuno:** None. **Y. Garkun:** None. **T. Nishioka:** None. **B. Stevens:** None. **J. Riceberg:** None. **K. Yamamuro:** None. **H. Morishita:** 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.; Simons Foundation Autism Research Initiative Pilot Award 610850, NIMH R01MH118297.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.03/C8

Topic: A.09. Adolescent Development

Support: NIMH R01MH119523
SFARI pilot award #977966
FRAXA Research Foundation Fellowship

Title: Altered history-dependent recruitment of frontal-sensory cortical projections underlies visual attention deficits in a mouse model of fragile X syndrome

Authors: *S. ALLEN[1], T. NISHIOKA[2], Y. GARKUN[2], A. SERRATELLI[2], H. MORISHITA[3]; [1]Neurosci., Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [2]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York City, NY; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Attention deficits in Fragile X syndrome and in the relative mouse model (Fmr1KO) have been previously demonstrated, but the underlying cognitive circuit disruptions still remain unclear. We recently found that the maturation of frontal-sensory neurons projecting from anterior cingulate cortex to visual cortex, which normally plays an essential role in cognitive control by linking error monitoring and attention adjustment (Norman et al Neuron 2021), is dysregulated in the adult Fmr1KO mice due to failed pruning of glutamatergic input following adolescence (Falk et al Science Advances 2021). However, it is entirely unknown to what extent this dysregulation impacts the recruitment of frontal-sensory projection during attentional behavior in Fmr1KO mice. Here, we utilized fiber photometry recording of calcium signaling in GCaMP8 expressing-frontal-sensory projections of adult Fmr1KO mice during 5 Choice Serial Reaction Time Task (5CSRTT) to monitor the circuit activity associated with attention. We found that in WT mice frontal-sensory projection activity during the 5 second delay period—when anticipatory attention increases—was driven by error history in the early delay period (0-2.5 seconds) and by a consolidation of history and current trial performance during the later period (2.5-5 seconds). However, in Fmr1KO mice, activity during the delay period was found to be driven predominantly by correct performance history. Opposed to WT mice, circuit activity during the anticipatory delay period in Fmr1KO mice following error as well as the difference of post error and post correct circuit activity were significantly lower. These findings demonstrate that history-dependent recruitment of frontal-sensory projections is altered in adult Fmr1KO, contributing to cognitive control deficits. Given that frontal-sensory projection neurons receive excessive glutamatergic drive in adult Fmr1KO mice (Falk er al Science Advances 2021), our future study will utilize a glutamate sensor for fiber photometry recording in Fmr1KO mice to

evaluate how history and performance-related information is integrated in the context of cognitive deficits.
https://pubmed.ncbi.nlm.nih.gov/33609483/
https://pubmed.ncbi.nlm.nih.gov/33674307

Disclosures: S. Allen: None. **T. Nishioka:** None. **Y. Garkun:** None. **A. Serratelli:** None. **H. Morishita:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.04/C9

Topic: A.09. Adolescent Development

Support: NIH NIA T32 AG055378
NSF NCS #1926818
ARCS Scholar

Title: Environmental enrichment rescues brain and behavioral effects of early life sleep disruption during adolescent development in prairie voles (Microtus ochrogaster)

Authors: *N. E. P. MILMAN[1], J. LOEUNG[2], C. E. JONES[3], J. BABU[4], H. PANTAZOPOULOS[4], B. A. SORG[5], M. LIM[6];
[1]Behavioral and Systems Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Portland State Univ., Portland, OR; [3]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [4]Dept. of Psychiatry and Human Behavior, Univ. of Mississippi Med. Ctr., Jackson, MS; [5]Legacy Res. Inst., Portland, OR; [6]Veterans Affairs Portland Hlth. Care Syst., Portland, OR

Abstract: Background: Adequate sleep in early life is necessary for species-typical neurodevelopment, including the formation of functional sensorimotor networks and display of affiliative social behavior. Early-life sleep loss is implicated in the pathogenesis of the neurodevelopmental disorder Autism Spectrum Disorder (ASD). Prairie voles are a highly social, wild rodent species that display a variety of affiliative social behaviors toward familiar conspecifics, including prolonged huddling behavior. We have previously shown that early life sleep disruption (ELSD) during the 3rd post-natal week (P14-21) in prairie voles impairs typical affiliative social behavior in opposite-sex adults. However, it is unclear how ELSD impacts adolescent social behavior, or the mechanism by which rearing in an enriched environment (EE) may intervene during a critical period of neural development. Methods: Prairie vole pups were assigned to ELSD (gentle cage shaking) or Control conditions from P14-P21 and weaned into either standard housing (SH) or EE. At 28 days of age, same-sex sibling cagemates were placed in a novel-home cage to explore and interact. Affiliative social behavior was manually scored for huddling, allogrooming, nose-to-nose contact and play behavior. Parvalbumin-containing interneurons and perineuronal nets were quantified in brain tissue from EE and SH adolescent

voles. Results: ELSD siblings raised in standard housing (SH) had lowered levels of huddling compared to Control siblings, p = 0.0024, 2-way ANOVA, (n = 11 ELSD, SH 4M, 7F pairs vs n = 9 Control, SH 5M, 4F pairs). Rearing in an enriched environment (EE) for one-week rescues deficits in adolescent affiliative behavior but does not impact Control siblings: Control, EE (n = 12 6M, 6F pairs) show statistically equivalent levels of huddling as Control, SH (n = 9, 5M, 4F pairs) and ELSD, EE (n = 10, 4M, 6F pairs), while ELSD, SH had reduced huddling compared to the other three Group X Housing conditions ($p < 0.05$), 2-way ANOVA with post-hoc comparisons. Conclusions: Our data suggests that ELSD induces affiliative social deficits early in development in addition to previously observed differences in adulthood. Rearing voles in an EE for one-week is sufficient to rescue this affiliative social behavioral impairment. These results implicate that ELSD may be acting on cortical parvalbumin-interneurons and critical period plasticity dictated by perineuronal nets.

Disclosures: **N.E.P. Milman:** None. **J. Loeung:** None. **C.E. Jones:** None. **J. Babu:** None. **H. Pantazopoulos:** None. **B.A. Sorg:** None. **M. Lim:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.05/C10

Topic: A.09. Adolescent Development

Support: Inflammation Healing Foundation

Title: Early-life sleep disruption alters inhibitory interneurons and perineuronal nets in the hippocampus of adult prairie voles

Authors: *J. J. BABU[1], C. J. TINSLEY[3], N. E. MILMAN[4], L. REXRODE[1], J. HARTLEY[1], B. GISABELLA[2], M. M. LIM[5], H. PANTAZOPOULOS[2];
[1]Psychiatry and Human Behavior, [2]Univ. of Mississippi Med. Ctr., Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Oregon Hlth. and Sci. Univ., Oregon Hlth. and Sci. Univ., Portland, OR; [4]Oregon Hlth. and Sci. Univ., Oregon Hlth. & Sci. Univ. Behavioral Neurosci., Portland, OR; [5]Veterans Affairs Portland Hlth. Care Syst., Veterans Affairs Portland Hlth. Care Syst., Portland, OR

Abstract: Background: The pathogenesis of Autism Spectrum Disorder (ASD) remains poorly understood, limiting therapeutic and preventative strategies. Our recent gene expression profiling study on the hippocampus of children with ASD implicates gene expression pathways involved in extracellular matrix processing and synaptic signaling. REM sleep is critically involved in shaping brain circuitry early in life, and multiple lines of evidence indicate early life sleep disturbances are associated with ASD. Our recent studies demonstrated that early life sleep disruption (ELSD) results in impaired social behaviors in adult prairie voles (*Microtus ochrogaster*), including reduced affiliative behavior and partner preference. Perineuronal nets

(PNNs) are extracellular matrix structures involved in neuronal maturation and synaptic plasticity that have been implicated in neurodevelopmental disorders. We tested the hypothesis that adult voles with ELSD display alterations in PNNs and parvalbumin (PVB), a marker for the inhibitory neurons that PNNs surround. We also examined co-expression of PNNs and PVB neurons with MEF2C, a marker associated with ASD that is involved in PVB neuron maturation. **Methods:** Hippocampal sections from adult voles (P150-160) with ELSD (n=8) and controls (n=6) were used for histochemistry for PNNs and immunofluorescence for PNNs, PVB, and MEF2C. Stereology based microscopy was used to quantify PNNs and colocalization with PVB neurons and MEF2C neurons. We conducted QRT-PCR and Western blotting for MEF2C on hippocampal brain samples from children (ages 3-14) with ASD and age matched controls (n=8 per group). **Results:** Densities of PNNs were significantly decreased in CA1 stratum oriens of ELSD voles ($p<0.04$). Neurons triple-labeled for PNNs, PVB and MEF2C were significantly decreased in CA1 stratum oriens ($p<0.02$), CA1 stratum pyramidale ($p<0.005$), and CA4 ($p<0.01$). MEF2C mRNA ($p<0.05$) and protein expression ($p<0.03$) was decreased in the hippocampus of children with ASD. **Conclusion:** Our data suggest that ELSD may contribute to molecular changes observed in the hippocampus of children with ASD. Decreases of neurons co-labeled with PVB, MEF2C and PNNs indicate that ELSD contributes to impairment in inhibitory signaling and synaptic plasticity in the adult hippocampus of voles with behavioral deficits. ELSD may interfere with the critical role of REM sleep in brain development, contributing to changes in neural circuitry that persist throughout adulthood. Our data suggest that MEF2C signaling and sleep promoting strategies may be promising therapeutic targets for preventing or alleviating neurodevelopmental changes involved in ASD.

Disclosures: J.J. Babu: None. **C.J. Tinsley:** None. **N.E. Milman:** None. **L. Rexrode:** None. **J. Hartley:** None. **B. Gisabella:** None. **M.M. Lim:** None. **H. Pantazopoulos:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.06/C11

Topic: F.03. Stress and the Brain

Support: NIH Grant R01MH104603
NIH Grant R01AA030256

Title: Investigating resident-intruder aggression in hybrid B6-Tg(Htr2a-EGFP);129S6-Maoa^{tm1Shih} mice following early life stress

Authors: *N. M. RUSSELL[1], T. J. LIME[1], D. G. CARRIZALES[1], D. A. SAN MIGUEL[1], H. C. AZIZ[1], E. S. DE LEON[2], A. R. KARLA[2], M. BORTOLATO[3], R. A. MANGIERI[1];
[1]Div. of Pharmacol. and Toxicology, [2]Col. of Natural Sci., Univ. of Texas at Austin, Austin, TX; [3]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT

Abstract: Replicating observations from humans, mutant mice that express low activity monoamine oxidase A (MAOA), 129S6-*Maoa*^{tm1Shih} (MAOA-L), display heightened aggression following exposure to an early-life stress (ELS) paradigm. This phenotype requires early-life activation of the serotonin 2A receptor (5-HT$_{2A}$) and is particularly evident in males. In this work, we tested whether this gene by environment interaction could be replicated in a hybrid B6;129S6 strain of mice that express an enhanced green fluorescent protein (EGFP) reporter in 5-HT$_{2A}$-expressing cells, allowing for the targeted examination of these cells in MAOA-L mice. To produce ELS, mice experienced maternal separation for 1-3 hours/day during the first two weeks after birth, and a needle punch once/day on their flank. Aggression was measured in late adolescence (postnatal day 50-68), after a 7-day period of social isolation, using a 5-minute resident-intruder test during which a wildtype (WT) 129S intruder was placed in the resident's home cage. The test was video recorded and manually scored for total attack duration, latency to first attack, mounting, and tail rattling as indices of aggression. Recorders were blind to sex and genotype. Male resident-intruder pairs were categorized as being body weight-matched (ratio of resident to intruder body weight = 1.0 - 1.10), or not matched (ratio = 1.11 - 1.17). For body weight-matched male mice, 0/10 WT (0%) and 6/14 MAOA-L (43%) residents attacked the intruder mouse (χ^2= 5.7, p = 0.017), indicating that genotype was a significant factor in the probability of observing aggression. Total attack duration was significantly longer for MAOA-L (t_{13} = 2.54, p = 0.025) but there was no significant difference in number of tail rattles (t_{13} = 1.93, p = 0.076). However, when the resident outweighed the intruder by more than 10%, there was no significant effect of genotype; 4/13 WT (31%) and 2/9 MAOA-L (22%) residents attacked the intruder (χ^2 = 0.20, p = 0.66). Female data were not separated by body weight ratio as all, but one MAOA-L resident exceeded 1.10 (range = 1.07 - 1.24). 1/7 WT (14%) and 2/7 MAO-L (29%) females attacked the intruder; χ^2= 0.42, p = 0.51. In conclusion, the display of aggression by male MAO-L hybrid mice under conditions in which aggression was not displayed by WT is consistent with past results in the original 129S6-MAOA-L strain. Having established that the aggression phenotype is recapitulated in the hybrid strain, these mice can now be used to study the activity of 5-HT$_{2A}$-expressing cells, in order to gain more insight into the neural circuit mechanisms by which low MAOA activity in combination with early-life stress promotes aggression.

Disclosures: **N.M. Russell:** None. **T.J. Lime:** None. **D.G. Carrizales:** None. **D.A. San Miguel:** None. **H.C. Aziz:** None. **E.S. De Leon:** None. **A.R. Karla:** None. **M. Bortolato:** None. **R.A. Mangieri:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.07/C12

Topic: F.03. Stress and the Brain

Support: NIH Grant R01MH104603
NIH Grant R01AA030256

Title: Examining basic membrane properties and excitability of Layer V mPFC pyramidal neurons in a mouse model of a critical gene-by-environment interaction in pathological aggression

Authors: *T. J. LIME[1], H. C. AZIZ[1], N. M. RUSSELL[1], D. A. SAN MIGUEL[1], D. G. CARRIZALES[1], E. S. DE LEON[2], A. R. KARLA[2], M. BORTOLATO[3], R. A. MANGIERI[1]; [1]Div. of Pharmacol. and Toxicology, [2]Col. of Natural Sci., The Univ. of Texas at Austin, Austin, TX; [3]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT

Abstract: The gene-by-environment (GxE) interaction between low functioning of the gene encoding monoamine oxidase A (MAOA) and early-life stress (ELS) is important in the development of pathological aggression (PA). Previous work has described an animal model of this GxE interaction in mice which recapitulates the aggressive phenotype seen in PA (ELS-$MAOA^{Neo}$). Adult aggression in ELS-$MAOA^{Neo}$ mice can be prevented by early-life serotonin 2A receptor ($5HT_{2A}$) blockade in the prefrontal cortex (PFC). Thus, $5HT_{2A}$-expressing Layer V medial PFC pyramidal neurons (5HT-PN) are implicated in the development of PA in ELS-$MAOA^{Neo}$ mice. The purpose of this study was to examine the basic membrane properties and excitability of 5HT-PN in male ELS-$MAOA^{Neo}$ mice in adulthood. To do this, we replicated the model using a hybrid strain of B6;129S6 mice with MAOA knockdown and a $5HT_{2A}$-green-fluorescent-protein (GFP) reporter. Child maltreatment was modeled via an ELS paradigm (3 hours of maternal separation and one needle poke in the flank daily from postnatal day (P) 2 – 14). On ~P90, 300 µm coronal mPFC slices were prepared from male ELS (wildtype (WT)=4, Neo=4) mice for ex vivo patch-clamp slice electrophysiology. Despite utilizing a hybrid line with a GFP reporter, fluorescence was not always detectable, potentially due to loss of $5HT_{2A}$ expression in our cells of interest in adulthood. Therefore, we collected and are reporting data regardless of GFP expression (GFP+: 83%, GFP-:17%). All recordings were taken in Layer V mPFC (n_{neo}=12, n_{wt}=12). Cells were patched with whole cell access and current was injected in steps from -180 pA to 200 pA (+20 pA/step). Membrane voltage was recorded, and basic membrane and excitability properties were extracted using python and R. A repeated measures ANOVA was performed to compare the effect of genotype on action potential firing, but found no significant effect ($F(1,11) = 0.62$, $p = 0.44$). Two-sample t-tests were performed to compare the resting membrane potential ($p = 0.83$), input resistance ($p = 0.41$), and action potential threshold potential ($p = 0.48$) between ELS-$MAOA^{Neo}$ and ELS-WT mice but found no significant differences. These results indicate that there are no gross alterations in the excitability of 5HT-PN of adult ELS-$MAOA^{Neo}$ compared to ELS-WT, which suggests that, during adulthood, these neurons may not play a role in the enhanced aggressiveness of ELS-$MAOA^{Neo}$. This project will next perform electrophysiological recordings at earlier developmental time points, and retrograde tracing will be used to classify cell types by projection target to study specific mPFC-containing circuits. Females will also be included to examine sex differences.

Disclosures: T.J. Lime: None. **H.C. Aziz:** None. **N.M. Russell:** None. **D.A. San Miguel:** None. **D.G. Carrizales:** None. **E.S. De Leon:** None. **A.R. Karla:** None. **M. Bortolato:** None. **R.A. Mangieri:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.08/C13

Topic: A.09. Adolescent Development

Support: NIH Grant DK124727
NIH Grant GM060507
NIH Grant MD006988
Loma Linda University School of Medicine GRASP Seed Fund

Title: Prefrontal cortical protease TACE/ADAM17 regulates neuroinflammation and stress-related eating alterations.

Authors: *F. SHARAFEDDIN, M. GHALY, T. SIMON, P. ONTIVEROS-ANGEL, J. FIGUEROA;
Loma Linda Univ., Loma Linda, CA

Abstract: Traumatic stress during childhood is associated with obesity and eating disorders later in life. However, the mechanisms contributing to obesity and disordered eating behaviors in trauma victims remain largely unknown. Early traumatic stress profoundly influences brain immune responses, which may, in turn, disrupt the maturation of prefrontal cortical networks regulating top-down control of eating. The tumor necrosis factor alpha-converting enzyme / a disintegrin and metalloproteinase 17 (TACE/ADAM17) is a sheddase with essential functions in brain maturation, behavior, and neuroinflammation and is upregulated in rats exposed to traumatic stress. This study aimed to determine the role of prefrontal cortical TACE/ADAM17 in a rat model of early trauma. Fifty-two (52) adolescent Lewis rats (postnatal day, PND, 15) were exposed to either predator stress and chronic social isolation (trauma) or typical housing conditions (controls). Rats were subsequently injected intracerebrally either with a novel Accell™ SMARTpool TACE/ADAM17 siRNA or the corresponding siRNA vehicle. The RNAscope Multiplex Fluorescent v2 Assay was used to determine spatial mRNA expression. Observation cages were used to monitor ethological behaviors in a more naturalistic environment over long periods. Early traumatic stress blunted startle reactivity and increased food intake while disrupting eating behavior structure. We also found that the rats that received prefrontal cortical TACE/ADAM17 siRNA exhibited decreased eating and increased grooming behaviors compared to controls. These changes were associated with decreased AIF-1 expression (a typical marker of microglia and neuroinflammation). This study suggests prefrontal cortical TACE/ADAM17 regulates neuroinflammation and eating behaviors after early trauma. TACE/ADAM17 represents a promising target to ameliorate trauma-induced brain and behavior alterations.

Disclosures: F. Sharafeddin: None. **M. Ghaly:** None. **T. Simon:** None. **P. Ontiveros-Angel:** None. **J. Figueroa:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.09/C14

Topic: A.09. Adolescent Development

Support: T32 DA007261

Title: A Phenome-Wide Association Study (PheWAS) Identifies Elevated Genetic Risk for Inflammation is Associated with Body Composition, Screen Time, and Mental Rotation in Children of European Ancestry

Authors: *S. NORTON, A. GORELIK, S. PAUL, E. JOHNSON, A. HATOUM, N. KARCHER, A. MILLER, A. AGRAWAL, R. BOGDAN;
Psychology & Brain Sci., Washington Univ. in St. Louis, ST. LOUIS, MO

Abstract: Background: Recent research has highlighted chronic low-grade inflammation as a risk factor through which long-term physical and mental health outcomes may manifest. One inflammatory biomarker, C reactive protein (CRP), has been previously associated with increased risk of health-related outcomes. However, it remains unclear if this and other correlates are present during childhood.
Methods: We conducted a PheWAS of psychosocial, behavioral, and neural phenotypes (total n>1,300) in the context of genetic risk for elevated levels of CRP using baseline data of 5,560 children of PCA-defined European ancestry from the Adolescent Brain Cognitive Development (ABCD) Study. Polygenic risk score (PRS) derived from summary statistics from the largest GWAS(s) of CRP were used. Mixed models were used to nest data by family and site and the following fixed effect covariates were included: sex, age, and 10 ancestrally-informative principal components with false discovery rate (FDR) and Bonferroni correction used to adjust for multiple testing.
Results: Results from 5,560 children at baseline revealed positive associations between genetic risk for elevated CRP and several body composition measures (e.g., waist circumference, weight; Bs = 0.071 - 0.079, Ps_{fdr} = 6.25E-06 - 1.61e-05) and screen time measures (e.g. total weekday screentime; Bs = 0.061 - 0.048, Ps_{fdr} = .001 - .04). In addition, there was a negative association between genetic risk for CRP and total score on a mental rotation task (B = -0.068, P_{fdr} = 0.0001).
Discussion: These preliminary results indicate that genetic risk for inflammation is associated with psychosocial and behavioral phenotypes during middle childhood. Analyses with associations between genetic risk for CRP and neuroimaging variables are ongoing.

Disclosures: S. Norton: None. **A. Gorelik:** None. **S. Paul:** None. **E. Johnson:** None. **A. Hatoum:** None. **N. Karcher:** None. **A. Miller:** None. **A. Agrawal:** None. **R. Bogdan:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.10/C15

Topic: A.09. Adolescent Development

Support: NIH R21 DA055105

Title: Exposure to methamphetamine influences the volume and the number of neurons and glia in the female rat medial prefrontal cortex

Authors: *A. S. BRINKS[1], L. K. CARRICA[2], J. M. GULLEY[3], J. M. JURASKA[4];
[1]Univ. of Ilinois At Urbana-Champaign, Champaign, IL; [2]Univ. of Illinois, Urbana-Campaign, Champaign, IL; [3]Psychology Dept., Univ. of Illinois At Urbana-Champaign, Champaign, IL; [4]Psychology, Univ. of Illinois, Champaign, IL

Abstract: Onset of drug use in adolescence has been associated with a greater risk of developing a substance use disorder later in life. This may be especially true with methamphetamine (METH), as those with a METH use disorder who began drug taking during adolescence have greater rates of relapse compared to those who use other addictive drugs. In previous work, we found that daily exposure to METH (3.0 mg/kg, i.p.) during early [postnatal day (P) 30-38] and late adolescence (P40-48) had opposite effects on the number of neurons expressing parvalbumin (PV) in the medial prefrontal cortex (mPFC) of female Sprague-Dawley rats but no effects in male rats. At present, we are examining the specificity of the changes in PV neurons in tissue from the early and late adolescent METH exposed females. Both the volume and the number of neurons and glia in the mPFC are being quantified in brains collected 24-hours following drug exposure and stained with methylene blue/azure II. Results show that females exposed to METH during early adolescence had an increased volume of the mPFC, while females exposed in late adolescence had no significant change. Preliminary cell counts suggest that METH exposure decreases the number of neurons and glia during the late adolescent period within the female mPFC. Ongoing data analysis will further elucidate the extent of this effect and include tissue from females exposed to METH in adulthood.

Disclosures: A.S. Brinks: None. **L.K. Carrica:** None. **J.M. Gulley:** None. **J.M. Juraska:** None.

Poster

PSTR452. Mechanisms of Vulnerability and Early Life Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR452.11/C16

Topic: A.09. Adolescent Development

Support: NIH GrantEY033950
NIH Grant MH128176

Title: Schizophrenia-relevant mutation produces region-dependent dendritic remodeling

Authors: *A. RADER[1,2], T. J. SUTTON[1], J. M. ROSS[1,3], R. A. SWEET[4,5], M. J. GRUBISHA[4], J. P. HAMM[1,2,3];
[1]Neurosci. Inst., [2]Ctr. for Neuroinflam. and Cardiometabolic Dis., [3]Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA; [4]Dept. of Psychiatry, [5]Dept. of Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Altered dendritic architecture in cortical pyramidal neurons is commonly reported in schizophrenia (SZ) and may underlie cortical sensory-perceptual aberrations characteristic of the disorder. Despite a heavy focus on morphological deficits in prefrontal regions, aberrant architecture has been reported in primary sensory cortices such as primary auditory (A1) and visual cortex (V1) as well. While these abnormalities likely arise from several different etiological factors, identifying specific genetic models of dendritic alterations may provide better insight into the relationship between effectors and effects of this phenotype. A rare mutation in the cytoskeletal regulator *Kalrn* was identified from a population of individuals with SZ. Characterization of this mutation revealed a gain-of-function effect on RhoA signaling downstream of longer kalirin isoforms. We recently developed a genetically altered mouse model with this *Kalrn* mutation, which exhibits decreased dendritic length and complexity in layer III pyramidal cells in A1, as well as auditory processing aberrations relevant to SZ. However, the uniformity and extent of dendritic alterations and functional changes across other cortical regions downstream of this *Kalrn* mutation remain unknown. Here we explore how altered *Kalrn* function may induce dendritic alterations in other sensory cortices.To determine how altered *Kalrn* function influences dendritic morphology in V1, we Golgi-stained and imaged layer II/III neurons in V1 from male and female animals homozygous for the *Kalrn* mutation (n=24 neurons) or wild-type controls (WT; n=24 neurons). Using the Imaris Filament Tracer, we traced and skeletonized dendritic arbors and used Imaris's Python Sholl Extension to analyze the dendritic complexity (i.e., number of Sholl intersections) and length. Unlike the dendritic reductions observed in A1, the *Kalrn* mutant mice showed increased dendritic length and complexity in V1. This effect was found in both apical (length: $p=0.003$, complexity: $p<0.001$) and basal (length: $p=0.02$, complexity: $p=0.01$) arbors. Local field potential recordings in these mice revealed decreased long-range synchrony between frontal cortex and V1 and reduced visual mismatch responses, further linking the mouse model to SZ-related phenotypes. Overall, these data illuminate the importance of investigating effects across multiple regions. The results invite further studies of how *Kalrn* differentially regulates dendritic morphology across higher order cortical regions and how disruptions in this function may contribute to disease-relevant pathology.

Disclosures: A. Rader: None. **T.J. Sutton:** None. **J.M. Ross:** None. **R.A. Sweet:** None. **M.J. Grubisha:** None. **J.P. Hamm:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.01/C17

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: European Union's Horizon 2020 research and innovation program, Marie Skłodowska-Curie grant agreement No: 860954

Title: Modulation of dopamine transporter function by zinc in functionally impaired neuropsychiatric disease mutants

Authors: *A. CAMPANA[1], C. KLEIN HERENBRINK[1], J. F. STØIER[1], A. H. NEWMAN[2], T. WERGE[3], F. HERBORG[1], U. GETHER[1];
[1]Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; [2]Natl. Inst. on Drug Abuse - Intramural Res. Program, Baltimore, MD; [3]The Lundbeck Fndn. Initiative for Integrative Psychiatric Res., Copenhagen, Denmark

Abstract: The dopamine transporter (DAT) plays a pivotal role in the regulation of extracellular dopamine (DA) levels in the brain, as it exerts high-selectivity, Na^+-dependent reuptake of dopamine. Missense mutations in the DAT gene (*SLC6A3*) have been suggested to be associated with neuropsychiatric diseases including ADHD, autism and bipolar disorder. From an exome-sequenced Danish cohort of 19,005 individuals, including 6,162 controls (iPSYCH2012) and 12,843 patients, we characterized 53 DAT missense mutants predominantly identified in patients with ADHD, ASD, schizophrenia or bipolar disorder to identify and classify mutational phenotypes. We assessed DAT-mediated DA uptake through a classical, cell-based radioligand uptake assay and found a large fraction of mutants with impaired uptake capacity. We next assessed surface expression/binding of DAT mutants using a fluorescently tagged cocaine analogue (DG3-80)-based assay, which also revealed variants with lower surface binding and $KD_{DG3\text{-}80}$ values significantly higher than wild-type. A screen based on use of T-Rex 293 cells "sniffer cells" expressing a genetically encoded DA sensors did not reveal any mutants displaying constitutive DA efflux, a phenotype earlier reported for disease-associated DAT mutants. Finally, selected variants were tested for zinc (Zn^{2+}) sensitivity as DAT is known to possess an endogenous high-affinity Zn^{2+} binding site. Binding of Zn^{2+} to this site stabilizes the transporter in an outward-facing conformation and biphasically inhibits DA uptake in wild-type DAT. However, in mutants shifted towards the inward facing conformation, it was demonstrated that Zn^{2+} can rescue uptake capacity. Interestingly, our analysis identified three new disease mutants (Arg60Trp, Glu121Ser and Cys135Tyr) in which uptake capacity was partially restored by micromolar concentrations of Zn^{2+}, consistent with an inward conformational bias in these mutants. Summarized, our results exhaustively classify the phenotypic repertoire of disease-associated DAT mutations and thereby provide a new framework for a better understanding of how dysregulation of dopamine signaling might be coupled to neuropsychiatric disease.

Disclosures: A. Campana: None. **C. Klein Herenbrink:** None. **J.F. Støier:** None. **A.H. Newman:** None. **T. Werge:** None. **F. Herborg:** None. **U. Gether:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.02/C18

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Lundbeck Foundation R359-2020-2301
Lundbeck Foundation R266-2017-4331
Lundbeck Foundation R276-2018-792
Lundbeck Foundation R303-2018-3540
Independent Research Fund Denmark – Medical Sciences 7016-00325B)

Title: Three-dimensional simulations of striatal dopamine dynamics posits dopamine transporter uptake plasticity as a key regulator

Authors: A. L. EJDRUP[1], J. K. DREYER[2], M. D. LYCAS[1], S. H. JØRGENSEN[1], T. W. ROBBINS[3], J. W. DALLEY[3], F. HERBORG[1], *U. GETHER[1];
[1]Univ. of Copenhagen, Copenhagen N, Denmark; [2]H Lundbeck A/S, H Lundbeck A/S, Valby, Denmark; [3]Univ. Cambridge, Cambridge, United Kingdom

Abstract: Dopamine (DA) release in the striatum is important for reward, learning, motivation, and motor function, but the extracellular dynamics that make up the underlying information processing are still up for debate. To better understand the nature of the signalling, we developed a 3D computational model of the dorsal and ventral striatal DA systems based on a wealth of reports from the literature. Our model accurately predicted results from *in vivo* voltammetry recordings as well as biosensor-based amphetamine experiments. Importantly, we found that DA did not accumulate to a uniform tonic level in the dorsal striatum due to the high uptake capacity of the dopamine transporter (DAT). Rather, hotspots of elevated extracellular DA were found to be segregated, suggesting a high spatial specificity in DA signaling in the dorsal striatum. In contrast, a much lower DAT uptake in the ventral striatum allowed for a build-up of a more homogenous tonic concentration of DA in this region. Further, our simulations showed that the ventral-specific tonic concentration was highly sensitive to alterations in DAT capacity. Using new kinetic estimates from biosensors and recent pharmacological studies, we found that dopamine D1 receptor occupancy tracked the extracellular dynamics with only millisecond delays, but dopamine D2 receptors required seconds-long reductions in DA to significantly affect occupancy. Our model also predicted that nanoclustering of DAT decreased uptake capacity, and using super-resolved fluorescence microscopy that nanoclusters were twice as frequent in the ventral striatum compared to the dorsal striatum. These findings implicate uptake plasticity as a central regulator of DA activity in the striatum. Nanoclustering may be a form of neuroplasticity orthogonal to internalization that may change DA uptake rates on the same time scale as D2 receptor occupancy.

Disclosures: A.L. Ejdrup: None. **J.K. Dreyer:** None. **M.D. Lycas:** None. **S.H. Jørgensen:** None. **T.W. Robbins:** None. **J.W. Dalley:** None. **F. Herborg:** None. **U. Gether:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.03/C19

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Lundbeck Foundation R359-2020-2301
Lundbeck Foundation R276-2018-792
Lundbeck Foundation R303-2018-2896

Title: Novel insights into the dopamine transporter interactome in health and disease

Authors: *J. SCHMIDT[1], B. GYÖRFFY[1], B. SINTES[1], T. RAVNSBORG[2], A. EJDRUP[1], O. JENSEN[2], F. HERBORG[1], U. GETHER[1];
[1]Dept. of Neurosci., Univ. of Copenhagen, Copenhagen, Denmark; [2]Dept. of Biochem. and Mol. Biol., Univ. of Southern Denmark, Odense, Denmark

Abstract: Dopamine (DA) neurotransmission differs from classical fast synaptic transmission by operating perhaps primarily via "volume transmission"; that is, DA is predominantly released from non-synaptic release sites to act on target cells often located micrometres away. The availability of dopamine in the extracellular space is strictly controlled by the dopamine transporter (DAT). Distortion in the reuptake of released DA by DAT, and thereby the precise termination of dopaminergic signaling is known to have serious consequences ranging from deterioration of cognitive functions and learning capabilities to severe movement and psychiatric disorders and addiction. To better our understanding of the *in vivo* protein interactions of DAT, their dynamics upon exposure to drugs of addiction and in the context of disease associated variants, we coupled acute amphetamine exposure and a clinically relevant DAT variant, associated with ADHD-like symptoms and parkinsonism, to proteomic profiling in mouse striatum via liquid chromatography-mass spectrometry. Subsequent analysis of the identified interaction partners was performed using STED, SMLM and SIM² super resolution microscopy as well as [^{3}H]DA uptake assays in primary midbrain neuronal cells and heterologous cell lines. Global profiling of DAT interaction partners upon acute amphetamine exposure and the DAT variant identified 22 and 26 altered interaction partners, respectively, including proteins involved in signal transduction, scaffolding and membrane trafficking. Functional analysis of select interaction partners indicates high co-localization with DAT and shows altered uptake capacity of [^{3}H]DA in HEK293 cells. Overall, based on high-throughput investigation of the DAT interactome, novel interaction partners are proposed. Imaging and functional studies have provided further clues towards the role of selected proteins in DAT's localization and DA uptake function. These findings add valuable information for the improved treatment of DA linked addiction and disease.

Disclosures: J. Schmidt: None. **B. Györffy:** None. **B. Sintes:** None. **T. Ravnsborg:** None. **A. Ejdrup:** None. **O. Jensen:** None. **F. Herborg:** None. **U. Gether:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.04/C20

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant R01DA038616 (MER)
NIH Grant DP2NS105553 (NXT)
Attilio and Olympia Ricciardi Research Fund (MER and JCP)
Marlene and Paolo Fresco Postdoctoral Fellowship (RM)

Title: Autoregulation of striatal dopamine release by co-released GABA dampens phasic-to-tonic dopamine signaling

Authors: *J. C. PATEL[1], A. D. SHERPA[1], R. MELANI[2], B. O'NEILL[1], N. X. TRITSCH[2], M. E. RICE[1,2];
[1]Neurosurg., [2]Neurosci. and Physiol., NYU Grossman Sch. of Med., New York, NY

Abstract: Striatal dopamine (DA) axons co-release GABA that is acquired from the extracellular compartment via GAT1 transporters on DA axons and packaged in vesicles using the vesicular monoamine transporter, VMAT2. However, the consequences of co-released GABA on DA signaling are unknown. Given that DA axonal D2 receptors can autoinhibit DA release and that DA and GABA most likely share the same vesicle, we tested whether co-released GABA also autoregulates DA release via $GABA_A$ receptors ($GABA_AR$s) on DA axons. To test for the presence of functional $GABA_AR$s on DA axons, we first examined the effect of a $GABA_AR$ agonist on optically-evoked increases in extracellular DA concentration ($[DA]_o$), monitored with fast-scan cyclic voltammetry in *ex vivo* slices from male and female Ai32:DAT-Cre mice. Activation of $GABA_AR$s with muscimol decreased single-pulse (1 p) evoked $[DA]_o$ by ~25% in both the dorsal striatum (dStr) and nucleus accumbens core (NAc), confirming functionality. Conversely, the $GABA_AR$ channel blocker, picrotoxin (PTX), increased 1 p optically evoked $[DA]_o$ in both regions consistent with an endogenous $GABA_AR$ tone in striatal slices, as shown previously. Using optical pulse-train stimulation (10 p, 10 Hz) to mimic phasic DA axonal activity we found that PTX increased pulse-train evoked $[DA]_o$ throughout the striatum in both sexes, but to a greater extent than seen with 1 p. This is consistent with inhibition of DA release by co-released GABA during subsequent pulses in the pulse-train. Consequently, PTX amplified the ratio of 10 p-to-1 p-evoked $[DA]_o$ which provides an index of phasic-to-tonic DA signaling. Moreover, in both the dStr and NAc the greater amplification of 10 p *versus* 1 p by PTX persisted in the presence of glutamatergic and nAChR antagonists. This eliminated a possible indirect contribution by co-released glutamate from DA axons activating striatal cholinergic interneurons and subsequent nAChR facilitation of DA release. Importantly, however, the differential effect of PTX on optically-evoked phasic-to-tonic DA signaling was lost in mice devoid of GAT1 in DA neurons and lack GABA co-release. Together, these data provide evidence for direct autoinhibition of axonal DA release by co-released GABA that is likely faster than G-protein coupled D2 autoreceptors, thereby introducing a novel autoregulatory mechanism by which co-released GABA acts as a first responder to dampen phasic-to-tonic DA signaling.

Disclosures: **J.C. Patel:** None. **A.D. Sherpa:** None. **R. Melani:** None. **B. O'Neill:** A. Employment/Salary (full or part-time):; Addgene. **N.X. Tritsch:** None. **M.E. Rice:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.05/C21

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Cannabidiol modulates dopamine neurotransmission in a striatal compartment-specific manner

Authors: *M. R. KOLANOWSKI[1], S. A. ZIMMERMAN[2], E. S. RAMSSON[2];
[1]Grand Valley State Univ., Goodrich, MI; [2]Dept. of Biomed. Sci., Grand Valley State Univ., Allendale, MI

Abstract: Cannabidiol (CBD) is a non-psychotropic compound present in large amounts in cannabis sativa. Recently, CBD was identified as a modulator for GABA, glutamate, and dopamine (DA). This modulation is attributed to CBD's interaction with the endocannabinoid system. Previous work in our lab showed changes in DA neurotransmission after CBD exposure, however, these changes were inconsistent. CBD strongly influences habit-forming behaviors related to striosomes, small μ-opioid receptor-positive regions within the striatum. Also, striosomes are differentially affected by compounds such as substance P. Therefore, we hypothesized that CBD influences DA signaling differently in striosomes than in the surrounding striatum (matrix). Measurements of DA neurotransmission in the dorsal striatum of mice demonstrates exposure to CBD can be categorized by striatal region (striosome or matrix). This work identifies a compartment-specific change in DA in response to CBD exposure.

Disclosures: **M.R. Kolanowski:** None. **S.A. Zimmerman:** None. **E.S. Ramsson:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.06/C22

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: CIHR Grant #PJT-183931

Title: Characterization of the mechanisms underlying the exuberant axonal development of dopamine neurons.

Authors: *R. DENIS[1], S. BURKE[1], A. TCHUNG[2], N. GIGUÈRE[2], M.-J. BOURQUE[2], L.-É. TRUDEAU[3];
[1]Neurosci., [2]Pharmacol. and Physiol., [3]Pharmacol. and Physiology, Neurosci., Univ. of Montréal, Montréal, QC, Canada

Abstract: Dopamine (DA) neurons in the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) are known to have highly developed axonal arborization endowed with a much larger number of axon terminals compared to most other types of neurons. This characteristic has been suggested to underlie their vulnerability in Parkinson's disease. We aim to identify the cellular and molecular mechanisms underlying the development of such morphological features. We hypothesize that DA neurons develop an unusually large axonal arbor either because their growth kinetics are faster or because they continue growing for a longer period compared to other neurons. To tackle this question, we used time-lapse confocal microscopy and primary postnatal neurons obtained from transgenic mice expressing the red fluorescent protein tdTomato selectively in DA neurons or glutamatergic neurons of the thalamus. Our results reveal no significant difference in the rate of initial axonal growth within the first 24h in vitro when comparing SNc DA neurons to VTA DA neurons or thalamic glutamate neurons. However, at 3 days in vitro (DIV), SNc DA neurons have a larger axonal arbor compared to the other groups. This suggests that a key stage of axonal development occurs between 1 and 3 DIV. We are presently finalizing a more complete comparison of axon growth at 3 DIV, examining growth cone dynamics at 7 DIV as well as investigating the role of activity-dependent mechanisms. This project will provide a better understanding of the development of DA neurons, which could ultimately help to identify new strategies to reduce their vulnerability in Parkinson's disease.

Disclosures: R. Denis: None. **S. Burke:** None. **A. Tchung:** None. **N. Giguère:** None. **M. Bourque:** None. **L. Trudeau:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.07/C23

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: R01NS101104
F32MH123088

Title: Histamine receptor-mediated mechanisms regulate dopamine levels in the striatum in a sexually dimorphic manner

Authors: *M. A. VAN ZANDT, C. J. PITTENGER;
Psychiatry, Yale Univ., New Haven, CT

Abstract: Many neuropsychiatric disorders are sexually dimorphic in prevalence and presentation. Tourette's Syndrome (TS), for example, is diagnosed approximately 3-5 times more often in males than females. TS ranges affects 4-8 per 1000 school age children, with severity varying between cases. Dysregulation of the cortico-basal ganglia circuitry is implicated in tic-disorders. Imaging studies and other evidence implicate elevated dopamine in the striatum of TS patients, indicating a role for dopamine in the pathology of TS. Similarly, several genetic studies in humans have identified deficits in histaminergic signaling as a potential mechanism underlying TS; while knocking out the *histidine decarboxylase* gene, encoding an enzyme essential for histamine biosynthesis, potentiates stereotyped repetitive behaviors in mice. Previous work in our lab revealed that, in male mice, ICV infusion of histamine reduced levels of dopamine in the striatum, revealing a regulatory role of histamine in striatal dopaminergic circuitry. However, the mechanism behind histaminergic regulation of dopamine was still unknown. To address this, we infused agonists for the three brain-expressed receptors of histamine - H1, H2, and H3 - into either the striatum or Substantia Nigra (SN) of adult male and female C57BL/6 mice while recording striatal dopamine levels using microdialysis. Microdialysis samples, which were collected every 10 minutes, were analyzed using a HPLC system. Changes in dopamine were calculated from baseline levels prior to infusion of agonist. We found that the H2 receptor agonist dimaprit infused into the SN produced significant reduction in the levels of dopamine in the striatum over the 60 minutes post infusion, but only in males. Infusion of agonists for H1 and H3 in males did not alter dopamine levels. In females, in contrast, striatal infusion of the H3 agonist RAMH produced significant elevation in dopamine levels, but only when infused during the proestrus or estrus phase of the menstrual cycle. Females showed a trend toward decreased striatal dopamine with SN H2 infusion, during the estrus phase of the cycle, but this did not reach statistical significance. These results suggest that histamine regulates dopamine dynamics in the striatum in a distinct manner in male and female mice. These sexually dimorphic mechanistic differences may be relevant to the sexual dimorphism seen in TS and other disorders of basal ganglia function. Future work will clarify the neuronal substrates of these disparate effects in males and females to further understand these sex-specific mechanisms.

Disclosures: M.A. Van Zandt: None. **C.J. Pittenger:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.08/C24

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Intramural program at NIAAA and NIMH
Center on compulsive behaviors

Title: Enhanced inhibitory tone over striatal dopamine in macaque compared to mice is mediated by GABA receptors

Authors: *J. SHIN[1], H. GOLDBACH[1], E. SWANSON[1], J. KWON[1], J. MEHR[1], S. O. VASU[1], M. AUTHEMENT[1], R. BOCK[1], D. BURKE[2], S. HERNANDEZ[2], M. BOCARSLY[2], A. PLOTNIKOVA[1], A. CUMMINS[1], M. ELDRIDGE[1], B. AVERBECK[1], V. ALVAREZ[1]; [1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: We studied and compared dopamine (DA) transmission in the striatum using *ex vivo* brain slices of macaques and mice. Electrically evoked DA transients measured with fast-scan cyclic voltammetry were significantly larger in mice compared to macaques, as reported previously. Using photometry measurements of fluorescent dopamine sensors dLight and GRAB-DA, we confirmed the interspecies differences observed with voltammetry. Our study also revealed two main interspecies differences in the modulation of DA signals in the striatum. First, the nicotinic receptor blocker DHβE (1 μM) depressed the evoked DA signals by 68% in mice vs. 40% in macaque (n=44-58), indicating a stronger regulation by cholinergic interneurons of the DA signals in mice than macaques. Second, blockers of the GABA-A and GABA-B receptors (5 μM gabazine and 2 μM CGP55845) potentiated the amplitude of evoked DA signals to a larger extent in macaques compared to mice (54% vs. 16%, n=9-12). This finding suggests a stronger inhibitory tone by GABA over striatal DA signals in the striatum of macaques than rodents. In macaques, we also found regional differences in the magnitude of the evoked DA signals, with the largest signals being in the putamen, followed by the caudate and the nucleus accumbens. We also observed differences along the anterior-posterior axis, with signals being the smallest in the anterior caudate and largest in the posterior sections. Quantification of density of cholinergic interneurons along the anterior-posterior axis will be performed. Taken together, we describe interspecies differences in the magnitude of the evoked DA signals and in the modulation of these signals by acetylcholine and GABA receptors within the striatum of macaques and rodents. We hope these findings will be helpful when implementing translational studies.

Disclosures: J. Shin: None. **H. Goldbach:** None. **E. Swanson:** None. **J. Kwon:** None. **J. Mehr:** None. **S.O. Vasu:** None. **M. Authement:** None. **R. Bock:** None. **D. Burke:** None. **S. Hernandez:** None. **M. Bocarsly:** None. **A. Plotnikova:** None. **A. Cummins:** None. **M. Eldridge:** None. **B. Averbeck:** None. **V. Alvarez:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.09/C25

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: BYU Mentored Research Award

Title: P39 as an ZMP Prodrug Activator of AMPK Enhances Accumbal Dopamine Function

Authors: *R. J. CAMPBELL[1], S. LEE[1], J. MCFARLANE[1], H. A. WADSWORTH[1], O. SAUNDERS[2], D. M. THOMSON[1], J. T. YORGASON[1];
[1]Brigham Young Univ., Provo, UT; [2]Skylark Biosci., Clovis, CA

Abstract: AMP-activated protein kinase (AMPK) is a vital regulator of metabolic processes in mammalian cells and has applications as a potentially drug-able target for muscle growth, diabetes, and neuronal longevity. 5-Aminoimidazole-4-carboxamide-1-B-D-ribofuranoside (AICAR) is a well-characterized AMPK activativator. However, some studies indicate that AICAR-stimulated AMPK activation yields relatively small effects and is highly variable. In addition, the high concentrations of AICAR needed to elicit AMPK activation can lead to off-target effects and make it unviable as a pharmacological agent. Prodrug-39 (P39) is an exogenous activator of the AMPK pathway and is a prodrug for composed of phosphorylated AICA-ribotide called ZMP (5-aminoimidazole-4-carboxamide-ribotide), a known endogenous AMPK activator and analog of AMP. ZMP works to increase cell metabolism. The present work examined P39 effects on mesolimbic dopamine terminal function. P39 was detected in striatal cells using fluorescent microscopy. P39 enhanced evoked dopamine (DA) in the accumbens of adolescent and aged mice. P39 effects on dopamine terminals were dose-dependent. Cholinergic interneurons are well-known regulators of dopamine release, and the effects of P39 on cholinergic firing were determined. The present studies reinforce ZMP as a key modulator of AMPK-activated dopamine release which has implications for delayed cognitive deficits observed in aging. The present studies were performed using methods to ensure scientific rigor, including experimenter blinding when possible, statistical analysis and appropriate sample sizing for replication and hypothesis testing.

Disclosures: R.J. Campbell: None. **S. Lee:** None. **J. Mcfarlane:** None. **H.A. Wadsworth:** None. **O. Saunders:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Skylark Bioscience. **D.M. Thomson:** None. **J.T. Yorgason:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.10/C26

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: CNPq
CAPES
FAPERJ
INNT

Title: Cyclic AMP regulation by dopamine D1 and adenosine A1 receptors controls CREB phosphorylation during retinal development

Authors: *R. PAES-DE-CARVALHO, M. GUIMARÃES-SOUZA, L. G. R. XIMENES; Neurobio., Fluminense Federal Univ., Niterói, Brazil

Abstract: Distinct signaling pathways regulate specific functions during Central Nervous System (CNS) development such as neuron survival and differentiation. The chicken retina is a useful model to study the role of neurotransmitters or neuromodulators on CNS neurochemistry and development. Our previous work showed that the levels of the ubiquitous second messenger cyclic AMP are regulated by dopamine and adenosine since early stages of chicken retina development. We have now studied the effects of activation or inhibition of adenylyl cyclase, respectively by dopamine D1 and adenosine A1 receptors, on CREB phosphorylation during retinal development. Retinas from 10-day-old chicken embryos (E10), an early developmental stage, and E16, a more developed stage, were dissected and stimulated in Hanks saline with the direct adenylyl cyclase stimulator forskolin, dopamine or the D1 agonist SKF 38393, and the adenosine A1 receptor agonist cyclohexyladenosine (CHA) to respectively activate or inhibit adenylyl cyclase. After the incubation period, retinas were lysed and analysed by western blotting using antibodies against phosho-CREB or the respective total protein. Dopamine or the D1 agonist SKF 38393 stimulates CREB phosphorylation in E10 but not in E16, effects blocked by the D1 antagonist SCH 23390 or the adenylyl cyclase inhibitor SQ 22536. In agreement with these data, forskolin stimulates CREB in E10 in a concentration and time-dependent manner but a much smaller effect is observed in E16. Surprisingly, although adenosine A1 receptors are negatively coupled to adenylyl cyclase, CHA stimulates CREB phosphorylation in a Src kinase, PKC and MEK/ERK-dependent way in E10 retinas, but this effect is no longer observed in E16, when an inhibition of CREB phosphorylation by CHA takes place. The effect of CHA on CREB activity in E10 is blocked by the A1 antagonist DPCPX or by Thyrphostin AG 1478, an inhibitor of EGF receptor activity, suggesting a transactivation of EGF receptor mediated by adenosine A1 receptor in this stage of development. Our results indicate that cyclic AMP and its regulation by dopamine D1 or adenosine A1 receptors differentially controls different signaling pathways leading to CREB phosphorylation during retinal development.

Disclosures: R. Paes-de-Carvalho: None. **M. Guimarães-Souza:** None. **L.G.R. Ximenes:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.11/C27

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant NS129675
NYS DOH Grant C37715GG

Title: Uptake$_2$-mediated dopamine internalization and intracellular D_5-receptor-dependent CDP-diacylglycerol signaling in neural cells

Authors: C. C. OLUIGBO, A. D. SIEWE, M. O. ARIJESUDADE, W. KANG, *A. S. UNDIEH;
Biomed. Sci., CUNY Sch. of Med., New York, NY

Abstract: Dopamine induces phosphoinositide metabolism leading to the release of inositol phosphate and diacylglycerol messengers. Concomitantly, dopamine receptor stimulation facilitates phosphatidylinositol resynthesis, thus amplifying subsequent responses to activation of phospholipase C-coupled receptors. Phosphatidylinositol synthesis critically depends on the nucleolipid CDP-diacylglycerol. Dopamine robustly increases microsomal CDP-diacylglycerol biosynthesis through D_1-like receptors, particularly the D_5 subtype the majority of which are intracellularly localized. We explored the mechanism by which extracellular dopamine acts to modulate intracellular CDP-diacylglycerol biosynthesis. Dopamine stimulated CDP-diacylglycerol synthesis in organotypic and primary neuronal cultures devoid of the dopamine transporter. Dopamine was saturably transported into cortical primary neurons or B35 neuroblastoma cells expressing wild-type Uptake$_2$ molecular transporters, particularly PMAT/ENT4. Cellular dopamine uptake and CDP-diacylglycerol biosynthesis were inhibited by microtubule disrupters which block cytoskeletal transport, and by decynium-22 which blocks Uptake$_2$-like transporters. Dopamine effects were selectively mimicked by D_1-like agonists SKF38393 and SKF83959, competitively inhibited by D_1-like antagonist SCH23390, and unaffected by D_2-like agonist or antagonist. These observations indicate that dopamine is actively internalized by Uptake$_2$ into postsynaptic-type cells where the monoamine can stimulate its intracellular D_5-type receptors to increase CDP-diacylglycerol production. This finding counters the conventional notion that postsynaptic-type cells internalize supra-threshold levels of synaptic dopamine only to inactivate the transmitter. Given the critical involvement of CDP-diacylglycerol in phospholipase C and phosphatidylinositol-3-kinase signaling systems, our findings imply that intracellular dopamine could play an important role in cellular responses and adaptation to high levels of extracellular dopamine.

Disclosures: C.C. Oluigbo: None. **A.D. Siewe:** None. **M.O. Arijesudade:** None. **W. Kang:** None. **A.S. Undieh:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.12/C28

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant 1R01MH125903-TU-1
Program in Pharmacology and Drug Development, Tufts Univ. Grad Sch Biomed Sciences

Title: The role of the astrocytic vesicular nucleotide transporter in modulating mesolimbic dopamine release and behavior

Authors: Y. R. ABUHASAN[1], L. LI[1], J. S. YANG[1], S. AGRAWAL[1], W. LI[1], J. EGHOLM[1], Y. LIU[1], H. LEE[2], Q. HUANG[2], B. VOLPE[2], *W. CAI[2], **E. N. POTHOS**[1];
[1]Program in Pharmacol. and Exptl. Therapeut. and Pharmacol. and Drug Development, Grad. Sch. of Biomed. Sciences; Program in Biomed. Sciences, Tufts Univ. and Dept. of Immunology, Tufts Univ. Sch. of Med., Boston, MA; [2]New York Inst. of Technol., Old Westbury, NY

Abstract: ATP sequestration by the astrocytic vesicular nucleotide transporter (VNUT) and ATP exocytosis in astrocytes is potentially regulated by central insulin receptors and signaling. According to our prior work, this mechanism of action may be linked to the depressive-like and stress-induced behavior and dopamine deficits exhibited by neuronal insulin receptor and astrocytic insulin receptor null mice. In the present study, we have crossed VNUT-flox mice with astrocyte-specific Aldh1l1-CreERT2 mice to delete VNUT in astrocytes in a tamoxifen-dependent manner (iA-VNUTKO), thus inhibiting ATP exocytosis by about 60%. Given the important role of purinergic signaling on dopamine synaptic plasticity as suggested by previous studies, we employed carbon fiber amperometry to measure dopamine exocytosis in real time in acute coronal slices from the nucleus accumbens (Nacc) and dorsal striatum (DS) of iA-VNUTKO and VNUT-floxed male and female mice with half the groups of animals being exposed to a battery of chronic mild stressors (CMS) for a few weeks. Amperometric electrodes with 5 μm carbon fibers (Amoco) and a positive 700 mV voltage (vs Ag-AgCl ground) were employed to measure electrically evoked dopamine release. Electrodes were placed in the DS or Nacc in acute 300 μm coronal slices. A bipolar stimulating electrode was placed 100-200 μm away from the carbon fiber electrode and a current stimulus of +500 μA was applied 3 times per site every 5 min for 2 msec. The output was digitized at 50 kHz to record dopamine exocytosis in real time and low-pass filtered at 1 kHz. The number of dopamine molecules oxidized was determined by Faraday's equation, N=Q/nF where Q is the charge of the spike, n is the number of electrons transferred (two for catecholamines), N is the number of moles, and F is Faraday's constant (96485 coulombs per unit charge). The potential sex differences in dopamine exocytosis may drive behavioral aberrations observed specifically in female mice. The VNUT deletion alone reduced motivation in iA-VNUTKO female mice to a degree similar to female mice subjected to CMS, as characterized by breakpoint differences in total active pokes for palatable pellets under a progressive ratio of reinforcement. We did not observe a VNUTKO additive effect in mice subjected to CMS, indicating astrocytic VNUT is possibly in one key node within the CMS-induced neuromodulation. Together, our study considers whether exocytosis of ATP from astrocytes regulates multiple metabolic and neuronal pathways that could contribute to the modulation of dopamine signaling and related behavior.

Disclosures: Y.R. Abuhasan: None. **L. Li:** None. **J.S. Yang:** None. **S. Agrawal:** None. **W. Li:** None. **J. Egholm:** None. **Y. Liu:** None. **H. Lee:** None. **Q. Huang:** None. **B. Volpe:** None. **W. Cai:** None. **E.N. Pothos:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.13/C29

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: DFG (LI-1745/11-3, GRK1789, SFB1506)
FWF (F44-12)
Wellcome Trust Collaborative Award

Title: Deep learning-based image-analysis identifies a novel DAT-negative subpopulation of dopaminergic neurons in the lateral Substantia nigra

Authors: *S. ROY[1], N. BURKERT[1], M. HÄUSLER[1], M. OLDRATI[1], D. WUTTKE[2], S. MÜLLER[1], C. PÖTSCHKE[1], J. DUDA[1], M. MÜNCHMEYER[2,3], R. PARLATO[4], B. LISS[1,5]; [1]Univ. of Ulm, Ulm, Germany; [2]Wolution GmbH & Co.KG, Munich, Germany; [3]Dept. of Physics, Univ. of Wisconsin-Madison, Madison, WI; [4]Med. Fac. Mannheim Heidelberg Univ., Mannheim, Germany; [5]Linacre and New College, Univ. of Oxford, Oxford, United Kingdom

Abstract: Dopaminergic (DA) midbrain neurons are mainly located in the Substantia nigra (SN) and the ventral tegmental area (VTA). They are important for a variety of brain functions, and their dysfunction can cause diseases like Parkinson's (PD) or Schizophrenia. Hence, a better understanding of their functional activity and its modulation is desired. However, manual analysis of DA neuron-subtypes at the single cell level is very time consuming and prone to error. Thus, we combined tailored deep learning based image analysis approaches with immunohistochemistry, to quantify the expression of the plasmalemma dopamine transporter (DAT) in SN DA neurons, as their preferential degeneration is the hallmark of PD. DAT is important for axonal and somatodendritic dopamine-reuptake and -transmission, and thus a major determinant of DA neuron activity and excitability. In addition, it is commonly used as marker for SN DA neurons, and for their specific targeting. We also analysed the expression of the dopamine autoreceptor (D2-AR), the calcium binding protein calbindin-d28k (CB), and the aldehyde dehydrogenase Aldh1A1, all markers for subpopulations of DA neurons, differentially affected in disease.
We analyzed about 40.000 tyrosine hydroxylase (TH, the key-enzyme for dopamine-synthesis) immuno-positive SN neurons in fixed midbrain sections from adult mice. By co-expression analysis, we identified a novel subpopulation of neurons (~5% of all TH-positive SN neurons) that was immunofluorescence-negative for DAT. These neurons were ~35% smaller in size, compared to DAT-pos. SN neurons, and localized mainly in the very lateral SN (~18% of lateral TH-pos. neurons). Further anatomical mapping of their 3D-coordinates identified a ~7-fold enrichment in the caudo-lateral SN, compared to its rostral parts. In addition, we identified a lower co-expression rate of D2-ARs in TH-pos. DAT-neg. SN neurons (only ~70% compared to almost all DAT-pos. neurons). Contrastingly, the co-expression rate of CB, a marker for less vulnerable SN DA neurons, was ~4-fold higher in the DAT-neg. SN neurons, with an abundance of ~40%. Aldh1A1, a marker for more vulnerable, ventral tier SN DA neurons, was not expressed in the lateral DAT-neg. SN neurons, compared to ~60% co-expression in the DAT-pos. SN DA neurons, with a higher co-expression rate in medial SN DA neurons. These findings suggest a novel subpopulation of non-classical DAT-negative DA neurons in the caudo-lateral SN that might be less vulnerable in PD, compared to the classical, CB negative ventral tier SN

DA neurons. We are currently further characterizing these neurons, in view of their functional anatomy in health and disease.

Disclosures: **S. Roy:** None. **N. Burkert:** None. **M. Häusler:** None. **M. Oldrati:** None. **D. Wuttke:** None. **S. Müller:** None. **C. Pötschke:** None. **J. Duda:** None. **M. Münchmeyer:** None. **R. Parlato:** None. **B. Liss:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.14/C30

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Stiftelsen Professor Bror Gadelius minnesfond
Systembolagets alkoholforskningsråd
Swedish Medical Research Council

Title: The pharmacological effect of acamprosate originates from different drug moieties

Authors: *K. ADEMAR, Jr[1], A. LOFTÉN, Jr[2], M. NILSSON, Jr[2], A. DOMI, Jr[2], L. ADERMARK, Sr[2], B. SÖDERPALM, Sr[2,3], M. ERICSON, Sr[2];
[1]Psychiatry and Neurochemistry, [2]Neurosci. and Physiol., Univ. of Gothenburg, Göteborg, Sweden; [3]Beroendekliniken, Sahlgrenska Univ. Hosp., Göteborg, Sweden

Abstract: Alcohol misuse significantly contributes to the global disease burden and mortality. Alcohol use disorder (AUD) is characterized by neuronal changes, particularly within the mesolimbic dopamine system, a major part of the brain reward pathway. However, available pharmacological treatment options are limited, with Campral® (acamprosate; calcium-bis(N-acetylhomotaurinate)) being used as a relapse prevention therapy. Previous studies suggest that acamprosate reduces alcohol intake by increasing dopamine levels in the nucleus accumbens (nAc), thus partially substituting the dopamine-releasing effect of ethanol. Furthermore, it has been suggested that the relapse-preventing effect of acamprosate is primarily mediated by its calcium moiety rather than by *N*-acetylhomotaurinate.The present study aimed to investigate the effects of acute local and systemic administration of regular acamprosate (CaAcamp, 0.5 mM; 200 mg/kg i.p.), calcium chloride ($CaCl_2$, 0.5 mM; 73.5 mg/kg i.p.), the sodium salt of acamprosate (NaAcamp, 1 mM; 200 mg/kg i.p.), and the combination of $CaCl_2$ and NaAcamp on extracellular dopamine and taurine levels in the nAc using *in vivo* microdialysis. Additionally, the impacts of these drugs on ethanol intake using a voluntary ethanol consumption model with a two-bottle choice paradigm were examined. All experiments were conducted in male Wistar rats.Local administration of CaAcamp and $CaCl_2$, as well as the combination of $CaCl_2$ and NaAcamp, increased accumbal dopamine levels in a glycine receptor-sensitive manner. Systemic administration resulted in a significant dopamine elevation only with the combination treatment. Accumbal taurine levels increased following local administration of CaAcamp and the

combination of $CaCl_2$ and NaAcamp, as well as with systemic administration of NaAcamp alone and in combination with $CaCl_2$. Ethanol intake was significantly reduced by all treatment regimens, but the addition of NaAcamp prolonged the $CaCl_2$-induced reduction of ethanol intake.In conclusion, these findings suggest that the combination of $CaCl_2$ and NaAcamp has beneficial effects in terms of elevating dopamine and taurine levels within the nAc and reducing ethanol consumption. Enhancing our understanding of the mechanisms of action of acamprosate could facilitate the development of novel drugs to combat AUD.

Disclosures: K. Ademar: None. **A. Loftén:** None. **M. Nilsson:** None. **A. Domi:** None. **L. Adermark:** None. **B. Söderpalm:** None. **M. Ericson:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.15/C31

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: LGFG, State of Baden-Württemberg
DFG CIBSS EXC-2189—Project ID: 390939984
DFG—322977937/GRK2344

Title: A genetic model for development, physiology and behavior of catecholamine-devoid zebrafish larvae

Authors: S. PAREDES-ZÚÑIGA, R. PETERS, K. ØSTEVOLD, G. ARREY, D. FRANK, J. OSWALD, *W. DRIEVER;
Albert-Ludwigs-University Freiburg, Freiburg, Germany

Abstract: The catecholamines dopamine, noradrenaline and adrenaline have conserved roles in control of physiology and behaviors of vertebrates. Adrenergic dysfunctions may cause heart failure, while dopaminergic impairments are linked to neuropathologies including Parkinson's disease, schizophrenia and drug abuse. So far, most of our knowledge about catecholamine systems derives from pharmacological approaches or neuronal ablation. This has limited analysis of complex functions of catecholaminergic neurons, which have dual transmitter GABAergic or glutamatergic, and in some cases also peptidergic phenotypes. However, a genetic model to study vertebrate catecholamine systems function by selective elimination of catecholamines has not been available so far. We generated a genetic zebrafish model completely devoid of catecholamines by combining mutations in all genes involved in L-DOPA synthesis: the two *tyrosine hydroxylase* genes (*th* and *th2*) in neurons, and *tyrosinase* (*sdy*) in melanocytes. We verified by ELISA that *th, th2, sdy* triple mutant larvae are completely devoid of dopamine. Catecholamine-deficient zebrafish larvae are viable and develop an anatomically normal nervous system including neuron somata that express a catecholaminergic marker transgene, albeit with reduced cell numbers in some clusters. In *th, th2, sdy* triple mutants, the global organization of

catecholaminergic tracts and projection targets appear normal. In contrast, physiological functions that depend on catecholamines are impaired, including larval hatching and cardiac function. Behavioral assays reveal that specific visually guided locomotor outputs are impaired in triple mutants. Comparison of single and multiple *sdy*, *th* and/or *th2* mutant larvae reveals that inactivation of the *th* locus has the strongest contribution to the mutant phenotypes. Overall, our results show that catecholamine depletion impairs the fine tuning of physiological and behavioral responses in a way that resembles mammalian dopamine-derived dysfunctions, making the catecholamine-free zebrafish a suitable model for the translational study of dopaminergic systems. However, it is surprising that larvae develop and have a largely normal behavioral repertoire, indicating that our model may be also useful to investigate how at the circuit level, or through compensation by other neuromodulatory systems, physiology and function are regulated in catecholamine deficient larvae.

Disclosures: S. Paredes-Zúñiga: None. **R. Peters:** None. **K. Østevold:** None. **G. Arrey:** None. **D. Frank:** None. **J. Oswald:** None. **W. Driever:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.16/C32

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Horizon 2020 grant 848002

Title: Dopamine and serotonin interactions within the Dorsal Raphe Nucleus - Central Amygdala circuit in the regulation of anxiety-related behavioral responses

Authors: *C. RAIS[1], S. BARILE[1], Y. PELLOUX[1], Y. LI[2], R. TONINI[1];
[1]Isituto Italiano di Tecnologia, Genova, Italy; [2]Peking Univ., Peking Univ., Beijing, China

Abstract: The Dorsal Raphe Nucleus (DRN) not only contains serotoninergic (DRN_{5HT}) neurons, but it also comprises a wide diversity of cell subpopulations, including dopaminergic neurons (DRN_{DA}), which represent a potential source of local DRN modulation. The DRN plays a role in a wide range of behaviors, such as Pavlovian learning processes that underpin anxiety responses. Accordingly, the DRN forms a connectional hub with the Central Amygdala (CeA), a region implicated in the processing of aversive stimuli; the DRN-CeA circuit involves reciprocal connections between these two brain regions. While the contribution of $DRN_{5\text{-}HT}$ to CeA projections in fear learning and anxiety has been previously studied, the role of DRN_{DA} cells in the formation of anxiety-related behaviors is still poorly understood. Moreover, recent data from the lab suggest that DRN_{DA} neurons modulate the firing activity of DRN_{5HT} cells that project to the CeA. This raises the possibility that DRN_{DA}-$DRN5_{HT}$ cell interaction is involved in the regulation of the DRN-CeA circuit, and thereby in anxiety-related behavioral responses. In this study, we have been investigating the role of the DRN_{DA}-DRN_{5HT} functional relationship in

regulating anxiety through the modulation of the CeA circuit. By using intersectional viral strategies and in-vivo imaging tools, we monitored the activity of $DRN_{5\text{-}HT}$ to CeA and DRN_{DA} to CeA projections as well as the release of dopamine and serotonin during a behavioral task in mice based on the presentation of compound stimuli following Pavlovian learning. A similar behavioral conceptualization has been shown to reliably assess anxiety in multiple species, including non-human and human primates. Thus, our investigation has the potential to relate dysfunctional DRN microcircuit and encompassing circuit network to the development of comorbid affective symptoms in neurological disorders characterized by dopaminergic cell loss, such as Parkinson's disease.

Disclosures: C. Rais: None. **S. Barile:** None. **Y. Pelloux:** None. **Y. Li:** None. **R. Tonini:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.17/C33

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: CIHR project grant to Ali Salahpour

Title: Characterization of the pharmacological chaperone and inhibitory effects of ibogaine to the human serotonin transporter

Authors: *E. Q. WILLIAMS[1], H. GIANG[2], Y. YIN[2], W. HORSFALL[2], M. SCHAPIRA[3], A. SALAHPOUR[2];
[1]Pharmacol. & Toxicology, [2]Univ. of Toronto, Toronto, ON, Canada; [3]Structural Genomics Consortium, Toronto, ON, Canada

Abstract: Pharmacological chaperones have been shown to selectively bind SLC6 transporters, such as the dopamine (DAT) and serotonin (SERT) transporters, and in doing so, increase mature protein levels at plasma membranes. This drug action could prove critical for conditions in which mature transporter proteins are absent or greatly decreased at the plasma membrane. One such pharmacological chaperone is ibogaine, an atypical reuptake inhibitor that is structurally similar to the endogenous substrate serotonin (5-HT). Our preliminary results indicate ibogaine's pharmacological chaperone effect may not be mediated through the primary, orthosteric binding site (S1) on SERT. As a result, we hypothesize ibogaine elicits its two drug effects through different sites of action: 1) the inhibitory effect mediated through S1, and 2) the chaperone effect mediated through an allosteric (S2) site. In this study, we utilized several available reference structures to determine potential binding sites for ibogaine on SERT. Following our structural studies, we generated several S1 and S2 mutants that are predicted to decrease ibogaine binding at these two sites. We will utilize these mutants in several *in vitro* assays to directly assess changes in inhibition and chaperone activity of ibogaine on SERT. By identifying the site of

action of ibogaine's chaperone effect, we hope to identify more efficacious and potent pharmacological chaperones for SERT and other SLC6 transporters.

Disclosures: E.Q. Williams: None. **H. Giang:** None. **Y. Yin:** None. **W. Horsfall:** None. **M. Schapira:** None. **A. Salahpour:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.18/C34

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Fondecyt 1221030
Fondecyt 1161375
ANID Doctorado Nacional

Title: Conformational changes in the Human Serotonin Transporter (SERT): Insights from Molecular modeling

Authors: *L. DINAMARCA[1], K. BRUNEL[2], G. TORRES[4], G. ZAPATA[5], A. FIERRO[3]; [1]Organic Chem., Pontificia Univ. Catolica de Chile, Santiago, Chile; [3]Organic Chem., [2]Pontificia Univ. Católica de Chile, Santiago, Chile; [4]Mol. Pharmacol. and Neurosci., Loyola Univ. of Chicago, Chicago, IL; [5]Inorganic Chem., Univ. de Chile, Santiago, Chile

Abstract: Monoamine transporters (MATs) are 12 transmembrane domain proteins involved in the reuptake of monoamines including dopamine (DA), norepinephrine (NE) and serotonin (5HT). By utilizing ion gradients, the MATs transport monoamines from the extracellular to the intracellular space, thereby regulating their concentration outside the cells. Due to its highly relevant function in regulating monoamine concentrations, MATs have been the target of different recreational drugs, antidepressants, and antipsychotics, among others. In this work, we evaluated how the 5HT transporter (SERT) interacts with different amphetamine derivatives using molecular modeling. Using different computational methods, four amphetamine derivatives and 5HT were evaluated to describe specific interactions of ligands with SERT. We employed Molecular Docking in AutoDock 4.0.2, Molecular Dynamics Simulations (1.5 μs), and Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) calculations in the Amber18 program to determine the stabilization and free energy values for each system. We observed distinct interaction patterns with lower energies for 5HT, Amphetamine (AMPH), and Methamphetamine (METH) in comparison to Methylenedioxymethamphetamine (MDMA) and Methylenedioxyamphetamine (MDA). The latter two compounds exhibited interacting patterns similar to 5HT, but with improved energies and alterations in the electrostatic potential, which may be associated with the efflux process. In summary, our results offer novel structural insights into the diverse patterns of substrate interactions within the binding cavity of the transporter.

These findings include changes in macromolecule flexibility, which could potentially contribute to the translocation mechanism in SERT.

Disclosures: L. Dinamarca: None. **K. Brunel:** None. **G. Torres:** None. **G. Zapata:** None. **A. Fierro:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.19/C35

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Some kinetic features of Na,K-ATPase

Authors: *G. CHKADUA;
Membranology, Iv. Beritashvili Ctr. of experimental biomedicine, Tbilisi, Georgia

Abstract: A comparative kinetic analysis of Na,K-ATPase from rat brain synaptic and kidney plasma membrane fractions revealed different sensitivity to the neurotransmitter noradrenaline. Noradrenaline inhibits Na,K-ATPase in rat brain synaptic membranes, while the Na,K-ATPase in kidney plasma membranes is not sensitive to noradrenaline. However, the detailed intracellular mechanism of noradrenaline's action remains unknown. Since there is a different tissue distribution of catalytic alpha subunits of Na,K-ATPase, it is hypothesized that they would exhibit different kinetic features. To investigate this, we studied the kinetic characteristics of the alpha1 and alpha2/3 subunits separately using varying concentrations of the specific Na,K-ATPase inhibitor ouabain. The study demonstrated that alpha2/3 subunits possess an Mg2+-dependent cycle, while this characteristic is not observed for the alpha1 subunit. Noradrenaline shifts the enzyme system from an MgATP-dependent cycle to an Mg^{2+}-dependent cycle, resulting in the inhibition of brain Na,K-ATPase, where both alpha1 and alpha2/3 subunits are present. However, noradrenaline does not exert a modulatory effect on kidney Na,K-ATPase, which contains the alpha1 subunit and lacks the Mg^{2+}-dependent cycle.

Disclosures: G. Chkadua: None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.20/C36

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Intramural funds of the National Institute on Drug Abuse

Title: Systemic administration of dipyridamole increases the striatal extracellular concentration of adenosine. Supporting the adenosine hypothesis of Restless Legs Syndrome

Authors: *M. VALLE LEON[1], C. QUIROZ-MOLINA[1], C. ROMERO-LEGUIZAMÓN[1], Y. LI[2], D. GARCÍA-BORREGUERO[3], F. CIRUELA[4], C. EARLEY[5], S. FERRE[1];
[1]NIH, NIDA IRP, Baltimore, MD; [2]Sch. of Life Sci., Peking Univ., Beijing, China; [3]Sleep Res. Inst., Madrid, Spain; [4]Dept. of Pharmacol. and Exptl. Therapeut., Univ. de Barcelona, L' Hospitalet de Llobregat, Spain; [5]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Restless Legs Syndrome is a very prevalent sensorimotor disorder characterized by an urge to move the legs. Brain iron deficiency (BID) remains the key initial pathobiological factor and relates to alterations of iron acquisition by the brain. Rodent models based on diet-induced BID provide RLS animal models with face and construct validity, which recapitulate pathogenetic mechanisms of RLS. The BID rodent model has also showed predictive value with translational therapeutic implications. We previously found that rodents with BID show a hypoadenosinergic state with a downregulation of adenosine receptors of the A_1 subtype in the cortex and striatum, which determines an increased striatal presynaptic glutamatergic neurotransmission. It was then found that the intracranial or systemic administration of dipyridamole, an inhibitor of equilibrative nucleoside transporters ENT1 and ENT2, reestablishes this dysfunction of striatal glutamatergic neurotransmission. As predicted, we could demonstrate that dipyridamole provides a significant amelioration of RLS symptoms in two clinical trials, including a randomized, placebo-controlled crossover study. However, a valid critic to these clinical results was that dipyridamole crosses poorly the blood brain barrier, and its therapeutic efficacy could then be related to some peripheral effects. This was contested with experiments in reserpinized mice, were doses of 30 and 100 mg/kg were shown to counteract the locomotor activity induced by dopamine agonists, and this effect was counteracted by the non-selective adenosine receptor antagonist caffeine. With the present study we wanted to provide a more direct demonstration, that systemic administration of dipyridamole significantly increases the striatal extracellular levels of adenosine *in vivo* in the freely moving mouse. We used the fiber-photometry technique with the recently introduced adenosine biosensor GRABAdo1.0, which corresponds to a modified adenosine A_{2A} receptor. After obtaining a significant signal with the systemic administration of an exogenous A_{2A} receptor agonist (CGS21680, 0.5 mg/kg i.p.), we analyzed the effect of dipyridamole (i.p.) and the respective vehicle. As predicted, the doses of 30 and 100 mg/kg significantly increased the extracellular levels of adenosine, by about 100% and 200% versus the effect of the vehicle, respectively. The therapeutic effect of dipyridamole in RLS is therefore most probably related to its ability to increase adenosine in the brain. Work supported with the intramural funds of the National Institute on Drug Abuse.

Disclosures: M. Valle leon: None. **C. Quiroz-Molina:** None. **C. Romero-Leguizamón:** None. **Y. Li:** None. **D. García-Borreguero:** None. **F. Ciruela:** None. **C. Earley:** None. **S. Ferre:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.21/C37

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant DA045284
SUNY Research SEED Grant

Title: Distinct methamphetamine-enhanced norepinephrine transmission in the ventral bed nucleus of the stria terminalis dependent on estrous cycle stage

Authors: *R. BHIMANI, R. PAULY, J. PARK;
Biotechnical and Clin. Lab. Sci., Univ. At Buffalo, Buffalo, NY

Abstract: Norepinephrine in the ventral bed nucleus of the stria terminalis (*v*BNST) has recently been highlighted for its roles in encoding the valence of sensory stimuli as well as driving drug seeking behaviors. The *v*BNST receives the densest norepinephrine innervation in the brain primarily from A2 noradrenergic neurons of the nucleus of the solitary tract (NST). Increasing evidence has highlighted distinct anatomical and neurochemical sexual dimorphisms in this pathway, however, their contributions to norepinephrine transmission remain to be elucidated. Furthermore, estradiol has been hypothesized to play a key role in modulating norepinephrine regulatory mechanisms. In this study, we used fast-scan cyclic voltammetry, a neurochemical sensing technique, coupled with pharmacological manipulations to characterize the role of different stages of the estrous cycle on norepinephrine regulation (release and clearance). In addition, we investigated how different stages (diestrus/proestrus and estrus) of the cycle impact methamphetamine-induced modulation of norepinephrine regulation. All experiments were conducted in intact females and data was compared with male rats. Our results highlight that female rats in estrus show a blunted response to methamphetamine. Moreover, female rats exhibit less regulation by α2-adrenergic autoreceptors. These results provide the neurochemical framework necessary to establish the heightened sensitivity and greater drug seeking behavior in females.

Disclosures: R. Bhimani: None. **R. Pauly:** None. **J. Park:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.22/C38

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: Roy J. Carver Charitable Trust

Title: Inhibition of noradrenaline-dependent synaptic transmission in the dorsal raphe nucleus by alpha2-adrenergic receptors

Authors: *A. GUGEL[1], H. S. HAKE[3], S. C. GANTZ[2];
[1]Mol. Physiol. and Biophysics, [2]Mol. Physiol. & Biophysics, Univ. of Iowa, Iowa City, IA;
[3]Neurosci. Grad. Program, Univ. of Washington, Kirkland, WA

Abstract: The dorsal raphe nucleus (DR) is the largest serotonergic nucleus in the brain and the predominant source of central serotonin. Previously we have shown that in the DR, synaptic release of noradrenaline and the subsequent activation of metabotropic alpha1-adrenergic receptors (alpha1-A_R) on serotonin neurons produces a slow alpha1-A_R-mediated excitatory postsynaptic current (alpha1-A_R-EPSC) that drives action potential firing. But the source of noradrenaline to the DR and the presynaptic mechanisms that regulate noradrenaline release are not established. First, to determine the source of noradrenaline to the DR, the light-gated ion channel, Channelrhodopsin-2 (ChR2) was virally expressed in locus coeruleus (LC) neurons. Using whole-cell patch-clamp recordings from DR serotonin neuron in acute mouse brain slices, we found that activation of ChR2 in LC axon terminals was sufficient to produce an alpha1-A_R-EPSC in DR serotonin neurons. Thus, LC noradrenergic axons project to the DR and release noradrenaline to produce alpha1-A_R-dependent synaptic transmission. Inhibitory alpha2-adrenergic receptors (alpha2-A_R) are expressed in LC neurons and function as autoreceptors - receptors that when activated inhibit release of their cognate ligand. Here we show that application of an alpha2-A_R agonist, UK-14,304 nearly abolished the alpha1-A_R-EPSC. To determine whether activation of alpha2-A_R was inhibiting noradrenaline release, we bypassed the presynaptic element and activated alpha1-A_R by focal application of exogenous noradrenaline (I-NA). UK-14,304 had no effect on I-NA indicating that alpha2-A_R activation inhibited the alpha1-A_R-EPSC via a presynaptic mechanism. Increasing extracellular potassium from 2.5 mM to 10.5 mM had no effect on the alpha2-A_R-mediated inhibition of the alpha1-A_R-EPSC, indicating that the inhibitory effect of alpha2-A_R activation did not depend on the membrane potential of the innervating axon terminals. Application of UK-14,304 also produced an outward current in ~60% of the recorded neurons, likely due to activation of GIRK channels. Taken together, these results demonstrate that alpha2-A_R are presynaptic receptors located on noradrenergic projections from the LC to the DR. Activation of alpha2-A_R in the DR is expected to reduce the excitability of serotonin neurons by 1) limiting noradrenaline release thereby decreasing alpha1-A_R-mediated excitation and 2) direct hyperpolarization via activation of GIRK channels.

Disclosures: A. Gugel: None. **H.S. Hake:** None. **S.C. Gantz:** None.

Poster

PSTR453. Catecholamines and Purines

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR453.23/C39

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Val/met polymorphism in the comt gene and personality trait - openness to experience in peruvian students

Authors: *E. APARICIO[1], D. HUERTA[2], R. FUJITA[3], M. GUEVARA[4], O. ACOSTA[1]; [2]Facultad de Medicina- Ciencias Dinámicas., [1]UNIVERSIDAD NACIONAL MAYOR DE SAN MARCOS, Lima, Peru; [4]Lima, [3]Ctr. de Genética y Biología Molecular, Facultad de Medicina Humana, Univ. de San Martin de Porres, Lima, Peru

Abstract: Aim: To evaluate the impact of the Val/Met polymorphism in the COMT gene on the personality trait - openness to experience in a sample of Peruvian university students.**Design:** Observational, comparative.**Institutions:** Faculty of Pharmacy and Biochemistry¬-UNMSM, Faculty of Medicine¬-UNMSM, Centre of Genetics and Molecular Biology USMP.**Subjects:** Apparently healthy first-year university students, sampled by convenience, over 18 years of age and with signed informed consent.**Methods:** Assessment by NEO PI-R personality trait test, DNA extraction (blood/oral epithelium), analysis of the Val/Met polymorphism in the COMT gene by RFLP-PCR and automated Sanger sequencing. Statistical analysis by ANOVA test.**Results:** Genotypic frequencies are in Hardy-Weinberg equilibrium. With the ANOVA test, considering the genotypes Val/Val, Val/Met and Met/Met, differences were found at the limit of significance (F=3.187, p=0.053), and in the post-hoc - Tukey analysis a significant difference was found in the pair Val/Val vs Met/Met (p=0.045).**Conclusions**: Preliminary evidence is provided of a possible relationship between the Val/Met polymorphism in the COMT gene and the personality factor - openness to experience in a sample of Peruvian university students, for which further confirmation is needed. **Keywords:** COMT, personality traits, openness to experience.

Disclosures: E. Aparicio: None. **D. Huerta:** None. **R. Fujita:** None. **M. Guevara:** None. **O. Acosta:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.01/C40

Topic: B.03. Ion Channels

Support: University of Arizona/Anesthesiology

Title: Sensory neurons' activity is modified by silent VGKCs subunits

Authors: L. MOREIRA-JUNIOR[1], ***J. CARVALHO-DE-SOUZA**[2]; [1]Anesthesiol., Univ. of Arizona, Tucson, AZ; [2]Anesthesiol., Univ. of Arizona, TUCSON, AZ

Abstract: Voltage-gated potassium channels (VGKCs) play the fundamental role of repolarizing the membrane potential of virtually every cell in the body. In neurons, VGKCs are responsible for the repolarization phase of action potentials. Extraordinarily, VGKC are These channels

normally heteromeric complexes formed by Kv2 subunits together with other subunits from families Kv5-6 and Kv8-9 (aka silent subunits). Kv2-containing channels are the major cause of the delayed rectifying K^+ currents (IKdr) that tune neuronal firing frequency but not its threshold. Interestingly, heteromeric VGKCs possess distinct biophysical properties, yielding diversification and tissue specificity to IKdr. In addition, the expression of Kv5-6 and Kv8-9 subunits enables inhibition of Kv2 expression, providing another layer of modulation for IKdr. Here, we present data pertaining to the expression of these channels in sensory neurons from dorsal root ganglia (DRG), and heterogously in HEK cells. We used Kv6.4 as archetypal silent subunit expressed together with Kv2.1 in HEK cells. In DRG neurons we observed cell size dependence in Kv6.4 expression and strong modulation of IKdr (therefore of firing frequency) through Kv2-specific inhibitors. In HEK cells, isolated heteromeric VGKCs currents were inhibited by Kv6.4, in addition to a crucial change in the inactivation pattern of the currents. We found a significant hyperpolarizing shift in inactivation V50 (-79.6 mV vs -53.5 mV, t-test, p=0.0001), significantly larger activation slope (20.7 vs 14.9, t-test, p=0.0014) and faster activation kinetics (2-way ANOVA, p=0.0354) in heteromeric VGKCs currents compared to Kv2.1-only currents. Overall, these results suggest silent subunits may be a strong modulator of neuronal activity by tweaking voltage and time dependence of IKdr inactivation.

Disclosures: L. Moreira-Junior: None. **J. Carvalho-de-Souza:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.02/C41

Topic: B.03. Ion Channels

Support: NIDA Grant R01 DA053743
NIMH Grant R01 MH114990
NIDA Grant R01 DA054714
NIDA Grant F32 DA058453

Title: Pharmacological manipulation of KCNQ channels alters striatal neuron activity and excitability

Authors: *E. T. JORGENSEN[1], J. J. DAY[2];
[1]Neurobio., [2]Univ. of Alabama At Birmingham, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: KCNQ “m-type” K+ currents are important for controlling neuronal excitability, serving as a brake against hyperexcitable states in the nervous system. As such, changes in KCNQ channel expression and activity have been implicated in several neuropsychiatric disorders including epilepsy, mood disorders, and substance abuse. KCNQ channels in the nucleus accumbens (NAc) are altered by chronic ethanol consumption and several studies

suggest that pharmacologically manipulating these channels can block cocaine reinstatement after self-administration. To better understand the function of KCNQ channels in the striatum, we used a high-density multielectrode array (MEA) recording system to measure single-neuron electrophysiological activity from rat primary striatal neuron cultures. We complemented MEA recordings with whole-cell patch clamp recordings from the adult rat NAc. For MEA experiments, neurons were harvested from embryonic day 18 striatal tissue, and MEA recordings were performed at DIV 12 with pharmacological manipulation of KCNQ channels. Following a 20 min baseline, we administered the KCNQ agonist, flupirtine maleate (3 µM and 30 µM), and KCNQ antagonist, XE-991 (1 µM and 10 µM), in addition to vehicle controls. We found a dose-dependent effect with both pharmacological reagents. KCNQ antagonism produced a heterogeneous effect on neuronal action potential frequency, with 10 µM XE-991 significantly decreasing spike frequency in one cell population while increasing activity in a separate population. In contrast, KCNQ stimulation with flupirtine maleate increased activity in a small population of cells at 3 µM, but severely decreased activity in ~50% of cells at the higher concentration of 30 µM. In slice physiology experiments, we patched medium spiny neurons (MSNs) and injected 15 current steps starting at -100pA, increasing in increments of 25pA. Following successful baseline sweeps, 10 µM XE-991 was added to the perfusing aCSF and a second set of recordings were taken to determine the effects of KCNQ2/3 channel blockade from the same cell. XE-991 makes the resting membrane potential significantly less negative in addition to significantly decreasing the latency to fire, which is a hallmark of MSN excitability. These results suggest that KCNQ channel manipulations produce divergent responses in distinct cell populations, and have potential relevance for understanding drug-mediated changes in KCNQ expression and function. Future projects will use gene editing and RNAscope techniques to further identify how KCNQ channels influence striatal neuron activity and excitability.

Disclosures: E.T. Jorgensen: None. **J.J. Day:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.03/C42

Topic: B.03. Ion Channels

Support: NIH grant NS102239

Title: Cytoplasmic C-terminus of Slack (KCNT1) channel mediates positive cooperativity in gain-of-function mutations

Authors: *H. WANG[1], I. H. QURAISHI[2], T. MALONE[1], H. MCCLURE[1], J. KRONENGOLD[1], L. K. KACZMAREK[3];
[1]Yale Univ., New Haven, CT; [2]Yale Sch. of Med., Yale Univ., NEW HAVEN, CT; [3]Yale Univ. Sch. Med., Yale Univ. Sch. Med., New Haven, CT

Abstract: Gain-of-function mutations in the Slack (KCNT1, KNa1.1) Na^+-activated K^+ channel are associated with early-onset epilepsy and severe intellectual disability. Electrophysiological recordings in cRNA-injected oocytes have shown increases in evoked current ranging from two-fold to over 20-fold for different mutations. One of the highest gain-of-function mutations, and one most highly represented in the patient population is KCNT1-R474H. While many mutations alter sensitivity to internal Na^+ concentrations or voltage-dependence, recordings of single channels in isolated patches do not typically show the same degree potentiation as whole oocyte macroscopic currents. We hypothesize that the gain-of-function in mutations results from positive cooperativity between individual channels, which is mediated by the large cytoplasmic C-terminal domains of Slack. We therefore generated and tested two expression constructs related to this region. The first, Slack-CT, contains this C-terminal domain alone. Co-expression of Slack-CT with full-length Slack-R474H reduced K^+ current by 80%, suggesting such isolated cytoplasmic domains compete with intact channels for interacting sites on neighboring channels, reducing the likelihood of cooperative interaction. In contrast, co-expression of Slack-CT with wild-type Slack did not significantly reduce total current. This is consistent with our previous findings that recordings of single channels in isolated patches containing four or more mutated channels showed greatly positive cooperativity between individual channels. The second construct we evaluated was a truncation mutation Δ804 Slack in which the distal C-terminal region was deleted. Currents from a patch containing multiple truncated channels could be explained by a linear combination of single channel activity from patches with a small number of channels, indicating that cooperativity was lost by deletion of the C-terminus. Furthermore, we have demonstrated that positive cooperativity provides a good fit to multi-channel Slack recordings. Markov models simulating single-channel recordings show that cooperativity is sufficient to explain observed recordings, while alternative mechanisms cannot readily explain the patterns of channel openings. To further confirm specificity, we are testing the effect of other C-terminal constructs. Our findings support the hypothesis that Slack channels gate cooperatively, an effect mediated by the cytoplasmic C-terminus. Moreover, human disease-causing mutations are associated with a higher degree of cooperativity than wild type Slack channels.

Disclosures: H. Wang: None. **I.H. Quraishi:** None. **T. Malone:** None. **H. McClure:** None. **J. Kronengold:** None. **L.K. Kaczmarek:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.04/C43

Topic: B.03. Ion Channels

Support: NIH NINDS Grant R21NS125503
NIH NINDS Grant U54NS108874
New York Stem Cell Foundation

Falk Medical Research Trust
Davee Foundation

Title: Patient-specific iPSC-based models of KCNQ2-related developmental epilepsy capture disease severity endophenotypes

Authors: *D. SIMKIN[1], S. M. WAFA[2], M. GHARIB[1], K. A. MARSHALL[1], A. L. GEORGE, Jr.[2], E. KISKINIS[1];
[1]Neurol., [2]Pharmacol., Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: Heterozygous loss-of-function mutations in KCNQ2 are associated with developmental and epileptic encephalopathy (KCNQ2-DEE) and self-limiting familial neonatal epilepsy (KCNQ2-SLFNE). While the effects of KCNQ2 mutations have been studied in heterologous systems, their impact on human neurons remains unclear. Using induced pluripotent stem cells (iPSCs) and CRISPR/Cas9 gene editing we established a KCNQ2-DEE disease model system and previously demonstrated that neurons derived from a patient with KCNQ2-DEE (carrying a R581Q pathogenic variant) exhibit enhanced burst-suppression-like firing, as neurons mature on multi-electrode arrays (MEAs), in contrast to isogenic mutation-corrected controls (PMID 33544076). This maladaptive maturation time course is associated with upregulation of Ca^{2+}-activated K^+ channels leading to faster action potential repolarization and larger post-burst afterhyperpolarizations over several weeks in culture.
Here, we examined the broader relevance of our findings by establishing four additional pairs of KCNQ2-DEE and one KCNQ2-SLFNE patient-specific and respective, isogenic mutation-corrected control iPSC lines (*KCNQ2* pathogenic variants: R207W, H228R, T274M, P335L, and E257GfsX6), and investigated whether maladaptive homeostatic responses are characteristic of KCNQ2-DEE and SLFNE in iPSC-derived excitatory neurons. Despite varying levels of channel loss-of-function caused by patient variants, all four DEE patient-derived neuronal lines exhibited enhanced bursting propensity compared to their respective isogenic controls on MEAs. Application of multivariable machine learning algorithms on MEA-based datasets identified common and distinct firing features across patients and gene expression studies revealed upregulation of Ca^{2+}-activated (SK) K^+ channel genes across DEE lines. Furthermore, we find that this aberrant bursting phenotype is specific to chronic M-current blockade rather than hyperactivity through inhibition of other voltage-gated K^+ channels. We hypothesize that upregulation of SK channels, associated with this bursting phenotype, is likely a specific response to M-channel dysfunction. In contrast, SLFNE patient neurons did not differ in bursting propensity from their isogenic controls. This difference in cellular phenotypes between DEE and SLFNE neurons suggests that upregulation of SK channels may be contributing to neurodevelopmental deficits that are exclusively seen in DEE cases. Our studies provide crucial insights into the functional consequences of KCNQ2 mutations in human neurons that may enable discovery of alternative therapeutic strategies.

Disclosures: D. Simkin: None. **S.M. Wafa:** None. **M. Gharib:** None. **K.A. Marshall:** None. **A.L. George:** None. **E. Kiskinis:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.05/C44

Topic: B.03. Ion Channels

Support: NIH Grant R03 AA026997
NIH Grant U01 AA013519
NIH Grant P60 AA010760
NIH Grant AA020889
NIH Grant TL4 GM118965
VA Grant IK2 BX002488
VA Grant BX004699
John R. Andrews Family

Title: Major role of BK potassium channel in behavioral responses to alcohol in mouse revealed by ethanol-insensitive mutation

Authors: K. G. TOWNSLEY[1,2], ***I. ANDERSON**[1,2], L. TZAB[1,2], Z. USMANI[1,2], E. J. FIRSICK[1,2], A. TRAN[1,2], B. E. JENSEN[1,2], G. HOMANICS[3], W. SHAWLOT[4], L. SCOTT[5], J. PIERCE[5], A. R. OZBURN[1,2];
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Res. & Develop., VA Portland Hlth. Care Syst., Portland, OR; [3]Anesthesiol. & Perioperative Med., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [4]Mouse Genet. Engin. Facility, [5]Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: Alcohol acts through many targets to elicit various behavioral responses. A direct way to determine if a molecular target of ethanol mediates these responses is by testing an animal with an ethanol-insensitive version of the molecule. While ethanol is known to act on GABA (γ-Aminobutyric acid sub-type A) and NMDA (N-methyl-D-aspartate) receptors, the BK potassium channel (voltage-gated potassium channels that conduct large amounts of potassium) has been implicated in behavioral responses to ethanol in invertebrate and mammalian models. At pharmacologically relevant concentrations, ethanol activates BK which typically hyperpolarizes excitable cells and may account for many behavioral responses to alcohol. To test the contribution of the BK channel to these responses, we generated a knock-in (KI) mouse on the BALB/cj background that carries the ethanol-insensitive mutation T352I (threonine at position 352 changed to an isoleucine in the alpha subunit of BK). We carried out a battery of behavioral tests in homozygous KI, heterozygous (HET), or wild-type (WT) male and female mice. We found that the mutation markedly reduced behavioral sensitivity to ethanol in mice in the loss of righting reflex assay. Specifically, compared to wild type mice, the T352I mice 1) took longer than WT mice to lose their righting reflex, and 2) recovered their righting reflex from ethanol sedation (3.6g/kg) twice as fast as WT mice (9-13/sex/genotype; p's<0.05). No effects of genotype on righting reflex were observed for the GABA agonist pentobarbital (50mg/kg) or the NMDA antagonist ketamine (100mg/kg) (n's 8-20/genotype). We next determined whether BK KI mice were less sensitive to ethanol by identifying the ethanol dose needed to produce a loss of righting reflex [using the up and down method to ascertain the ED50 (effective dose for 50% of

population)]. We found that BK KI mice required a significantly higher dose of ethanol to lose their righting reflex (9-13/sex/genotype; $p<0.05$). Together, these data show that BK KI mice exhibit lower sensitivity to the inhibitory effects of ethanol. The T352I mouse also displayed more severe withdrawal at a low but not a high dose of ethanol (13-16/sex/genotype/dose; $p<0.05$). Importantly, the T352I mouse showed normal weight, locomotor activity, and ethanol metabolism (n's >8/sex/genotype/experiment), suggesting that this mutation does not interfere with overall development or physiology. Our results suggest that the threonine at position 352 of BK channel alpha subunit could represent a novel molecular target of ethanol; however, it is also possible that the mutation alters channel configuration to prevent or blunt activation by ethanol.

Disclosures: K.G. Townsley: None. **I. Anderson:** None. **L. Tzab:** None. **Z. Usmani:** None. **E.J. Firsick:** None. **A. Tran:** None. **B.E. Jensen:** None. **G. Homanics:** None. **W. Shawlot:** None. **L. Scott:** A. Employment/Salary (full or part-time):; Mahana Therapeutics. **J. Pierce:** None. **A.R. Ozburn:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.06/C45

Topic: B.03. Ion Channels

Support: the National Natural Science Foundation of China (grants 31925022, 91949206)
the National Key R&D Program of China (2018YFC2000400)
Innovative research team of high-level local universities in Shanghai (SHSMU-ZDCX20212501)
the Strategic Priority Research Program of the Chinese Academy of Science (XDB32020100)
the Shanghai Municipal Science and Technology Major Project (2018SHZDZX05)

Title: Dna topoisomerase 2-associated proteins patl1 and patl2 regulate the biogenesis of herg k+ channels

Authors: *Y. LI[1], R. MEI-YU[2], Y. SHI-WEI[1], C. SHI-QING[1];
[1]Inst. of Neurosci. and State Key Lab. of Neuroscience, CAS Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai, China; [2]Sch. of Life Sci. and Technology, ShanghaiTech Univ., Shanghai, China

Abstract: The human ether-a-go-go related gene (hERG) K^+ channel conducts a rapidly activating delayed rectifier K^+ current (I_{Kr}), which is essential for normal electrical activity of the heart. Precise regulation of hERG channel biogenesis is critical for serving its physiological functions and deviations from the regulation result in human diseases. However, the mechanism

underlying precise regulation of hERG channel biogenesis remains elusive. Here, by using forward genetic screen, we found that PATR-1, the *C. elegans* homologue of the yeast DNA topoisomerase 2-associated protein PAT1, is a critical regulator for the biogenesis of UNC-103, the ERG K^+ channel in *C. elegans*. A loss-of-function mutation in *patr-1* down-regulates the expression level of UNC-103 proteins, and suppresses the phenotypic defects resulted from a gain-of-function mutation in the *unc-103* gene. Furthermore, down-regulation of PATL1 and PATL2, the human homologues of PAT1, decreases protein levels and the current density of native hERG channels in SH-SY5Y cells and human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Knockdown of PATL1 and PATL2 elongates the duration of action potentials in hiPSC-CMs, suggesting that PATL1 and PATL2 affect the function of hERG channels and hence electrophysiological characteristics of the human heart. Further studies found that PATL1 and PATL2 interact with TFIIE, a general transcription factor required for forming the RNA polymerase II preinitiation complex, and dual-luciferase reporter assays indicated that PATL1 and PATL2 facilitate the transcription of hERG mRNAs. Together, our study discovers that evolutionarily conserved DNA topoisomerase 2-associated proteins regulate the biogenesis of hERG channels via a transcriptional mechanism.

Disclosures: **Y. Li:** None. **R. Mei-Yu:** None. **Y. Shi-Wei:** None. **C. Shi-Qing:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.07/C46

Topic: B.03. Ion Channels

Support: AHA Predoctoral Fellowship ID 899111
NIH Grant NS105616
NIH Grant NS127146

Title: Investigating the role of glial KCNQ K^+ channels in neuronal function in C. elegans

Authors: *B. GRAZIANO, L. WANG, O. R. WHITE, D. H. KAPLAN, J. FERNANDEZ-ABASCAL, L. BIANCHI;
Dept. of Physiol. and Biophysics, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: KCNQ channels are voltage-gated K^+ channels that are expressed in the nervous system where their function is to reduce cellular excitability. Genetic mutations in KCNQ channels are associated with epilepsy and autism spectrum disorder (ASD) in children. KCNQ channels are expressed both in neurons and in glia, but their role in glia is still unknown. We used the model organism *C. elegans* to elucidate the function of KCNQ channels in glia and their influence on neuronal function. First, we tested neuronal function by performing octanol avoidance assays, which test the sensitivity of the nematode to the repulsive odor octanol. We found that both global knockout of the KCNQ nematode homolog *kqt-2* and its glial specific

knockdown delayed the response to octanol [KO: *t*=4.3 +/- 0.23; n=142; RNAi: *t*=4.4 +/- 0.55; n=40] as compared to the wild type [*t*=1.8 +/- 1.05; n=144] [Anova; *p*<0.0001]. Expression of *kqt-2* in glia rescued the defective avoidance response [*t*=1.5 +/- 0.12; n=42] [Anova; *p*<0.0001]. Taken together, these data support a role for glial KCNQ channels in regulating neuronal function. Rescue of *kqt-2* knockout phenotype was also achieved by expression of the human KCNQ2 and KCNQ3 channels in glia [KCNQ2: *t*=2.09 +/- 0.19; n=100; KCNQ3: *t*=2.1 +/- 0.12; n=175]. This result supports the homology of function of KCNQ channels across species. To investigate the role of *kqt-2* in glial and neuronal activity, we performed Ca^{2+} imaging experiments *in vivo* using the genetically encoded calcium sensor GCaMP6s. We found that *kqt-2* knockout decreases Ca^{2+} transients in glia in response to octanol [mean=31.2 +/- 7.65; n=11], while it increases Ca^{2+} transients in the neurons [mean=251.1 =/- 29.83; n=10] as compared to the wild type [glia: mean=181.6 +/- 36.12, n=13; neurons: mean=97.3 +/- 11.78, n=10]. Both glial and neuronal Ca^{2+} changes were rescued by expression of the nematode and human KCNQ channels in glia. The increase in neuronal Ca^{2+} transients in *kqt-2* knockout is a sign of neuronal hyperexcitability, suggesting reduction of inhibition or increase in excitation in this mutant. Thus, we tested whether glial KCNQs might be involved in glial GABA release. To do this, we performed imaging and behavioral assays in which we enhanced GABA signaling in *kqt-2* knockout, or reduced GABA signaling in wild type using genetic, optogenetic, and pharmacological approaches. Results that we will present support the novel idea that glial KCNQs regulate GABA release from glia thereby reducing neuronal activity. To conclude, our data point to the contribution of glial KCNQs to the expression of epilepsy and ASD phenotypes via reduction of GABA release from glia.

Disclosures: **B. Graziano:** None. **L. Wang:** None. **O.R. White:** None. **D.H. Kaplan:** None. **J. Fernandez-Abascal:** None. **L. Bianchi:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.08/C47

Topic: B.03. Ion Channels

Support: NIH Grant GM127184
NS101596
HL137094

Title: KCNQ3 gain-of-function variants: does it affect KCNQ protein levels and cortical layering?

Authors: *R. PAZ ZAVALA, N. VARGHESE, K. SPRINGER, A. TZINGOUNIS; Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: The members of the voltage-gated potassium channel family, KCNQ, are associated with multiple neurodevelopmental disorders, including developmental and epileptic encephalopathy and autism spectrum disorders. Although the KCNQ family includes five members known as KCNQ1-5 (Kv7.1-5), most neurodevelopmental disorders associated with KCNQ channels are due to dysfunction of KCNQ2 and KCNQ3 channels. Over the last few years, KCNQ3 gain-of-function (GOF) mutations have been identified in patients with autism spectrum disorders with or without epilepsy. Considering that KCNQ3 channels express early in development, it's possible that KCNQ3 GOF variants might alter neuronal development. To start addressing this question we used a recently generated knockin mouse line that expresses the most commonly identified KCNQ3 GOF variant, R231C (R230C in humans) constitutively. We found that these mice survive to adulthood even though they exhibit an increase in L2/3 pyramidal neuron excitability. Despite this, we have found that KCNQ3 protein levels, as well as the levels of KCNQ2 and KCNQ5, did not have a substantial change in either the neocortex or the hippocampus of *Kcnq3*$^{R231C/+}$ mice. Similarly, we have not found any changes to the neocortical size of *Kcnq3*$^{R231C/+}$ mice, in contrast to mice expressing a KCNQ2 GOF function variant in forebrain neurons. We are currently investigating whether expression of KCNQ3 GOF mutants alters cortical layering. Hence, our work will provide insight into the effects of autism spectrum disorders associated with KCNQ3 GOF variants in the brain.

Disclosures: R. Paz Zavala: None. **N. Varghese:** None. **K. Springer:** None. **A. Tzingounis:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.09/C48

Topic: B.03. Ion Channels

Support: NIH grant DC01919 to LKK

Title: Kv3.3 channels may regulate the aggregation of the guanine nucleotide exchange factor Plekhg4 in Purkinje neurons

Authors: *Y. ZHANG, A. S. ANDRAWIS, L. K. KACZMAREK;
Yale Univ. Sch. Med., New Haven, CT

Abstract: Kv3.3 potassium channels are highly expressed in cerebellar Purkinje neurons and contribute to the ability of these neurons for fire at high rates. In addition to their role in regulating excitability, Kv3.3 channels form a complex with several cytoplasmic proteins, including Hax-1, Arp2/3, Rac1 and TBK1. This stimulates the nucleation of actin filaments under the plasma membrane. Mutations in *KCNC3*, the gene encoding Kv3.3, lead to spinocerebellar ataxia type 13 (SCA13), and an SCA13-causing mutation, Kv3.3-G592R, differs from wild type channels in that it fails to trigger actin nucleation. Another gene linked to

spinocerebellar ataxias is that for Pelkhg4, a guanine nucleotide exchange factor (GEF) responsible for regulating Rac1 activity. Purkinje cell degeneration in humans has been found to be associated with the formation of cytoplasmic aggregates of Pelkhg4. We have now found that activation of Kv3.3 channels can trigger the formation of such insoluble Pelkhg4 aggregates. Using biochemical, electrophysiological and confocal imaging techniques, we found that Pelkhg4 is distributed uniformly in the cytoplasm of untransfected CHO cells or those expressing wild type Kv3.3. In contrast, Plekhg4 aggregates form spontaneously in Kv3.3-G592R expressing cells, and the formation of these aggregates can be further enhanced by depolarization of these cells. Aggregates can also be induced by depolarization of cells expressing wild-type Kv3.3, but not of untransfected cells. Moreover, in the nervous system, increased levels of Plekhg4 and its interacting protein β-spectrin III are found in the triton-insoluble fraction prepared from Kv3.3-G592R knock-in mouse cerebellum compared to those in wild type mice. Plekhg4 coimmunoprecipitates with Kv3.3, and colocalizes with the channel at the plasma membrane and neurites of Purkinje neurons. The interaction between Kv3.3 channel and Plekhg4 modifies channel gating, as overexpression of Plekhg4 slows the inactivation rate of Kv3.3 channels, a process that is known to be regulated by the actin cytoskeleton. Because the interaction between Kv3.3 and the Hax-1/actin cytoskeleton is regulated by TBK1 (Tank Binding Kinase 1) and because Kv3.3-G5923 channels significantly increase basal TBK1 activity, we tested the effect of TBK1 inhibitor on Plekhg4 aggregation. Our preliminary data indicate that inhibition of TBK1 reduces the number of Plekhg4 aggregates in G592R Kv3.3 expressing cells. These results suggest that Purkinje cell activity, mediated by Kv3.3 channels, may regulate Pelkhg4 aggregation and provide a potential new therapeutic approach to the treatment of spinocerebellar ataxias.

Disclosures: Y. Zhang: None. **A.S. Andrawis:** None. **L.K. Kaczmarek:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.10/C49

Topic: B.03. Ion Channels

Title: 14-3-3 proteins promote eag1 potassium channel degradation via a cul7-dependent mechanism

Authors: *C. HSIEH[1], C.-Y. TANG[2], C.-J. JENG[1];
[1]Natl. Yang Ming Chiao Tung Univ. Inst. Of Anat. And Cell Biol., Taipei, Taiwan; [2]Natl. Taiwan Univ. Dept. of Physiol., Taipei, Taiwan

Abstract: Mutations in the neuron-specific Eag1 potassium (Kv10.1; KCNH1) channel are associated with congenital neurodevelopmental disorders. Disease-causing mutations may in part cause aberrant Eag1 protein homeostasis (proteostasis), manifesting as enhanced protein degradation and defective membrane trafficking. The E3 ubiquitin ligase cullin 7 (Cul7) is

known to promote degradation of wild-type and mutant Eag1 proteins through proteasomal and lysosomal pathways. To further understand the molecular regulation of Eag1 channel protein by Cul7, we focused on the role of an Eag1-binding partner, the small acidic protein 14-3-3. Disruption of endogenous 14-3-3 function with the peptide inhibitor difopein led to an increase in Eag1 protein level in a transcription-independent manner. The difopein effect was abolished by Cul7 knock-down, suggesting that 14-3-3 may hinder Cul7-mediated degradation of Eag1. Among the seven 14-3-3 isoforms, isoforms β, η and θ displayed most prominent binding efficiency with Eag1. Upon shRNA knock-down of 14-3-3 isoform β or θ, but not η, we observed a sizeable up-regulation of total Eag1 protein level. Although Eag1-14-3-3 interaction did not appear to require prior phosphorylation of the potassium channel, treating cell lysates with phosphatases notably enhanced the interaction between 14-3-3 proteins and Eag1/Cul7, implying a modulating role of phosphorylation of 14-3-3 proteins (e.g., at serine 232). Consistent with this notion, serine-to-alanine mutation at residue 232 in either 14-3-3β or 14-3-3θ, designed to mimic a dephosphorylated state, effectively promoted the interaction of 14-3-3 proteins with Eag1/Cul7, as well as significantly reducing Eag1 protein level. Our findings suggest that phosphorylation of 14-3-3 proteins may serve as a potential molecular mechanism for regulating Cul7-mediated Eag1 degradation.

Disclosures: C. Hsieh: None. **C. Tang:** None. **C. Jeng:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.11/C50

Topic: B.03. Ion Channels

Support: NICHD intramural grant

Title: Activity-dependent degradation of Kv4.2 by Angelman Syndrome protein Ube3A contributes to synaptic plasticity and behavioral phenotypes

Authors: *J.-H. HU, C. MALLOY, Y. LIU, A. PRATT, M. A. WELCH, D. HOFFMAN; NICHD, Bethesda, MD

Abstract: Angelman syndrome (AS) is a severe neurological disorder characterized by intellectual disability, absence of speech, spontaneous seizure, and motor dysfunction. The absence of functional maternally derived Ube3A protein is considered the primary cause of AS, yet the downstream signaling pathways remain elusive. Here we identify the voltage-gated K^+ channel Kv4.2 as a seizure/activity-dependent substrate for Ube3A. Kv4.2-Ube3A association is induced along the apical dendrites in hippocampal pyramidal neurons by seizure. We show that Ube3A binding of Kv4.2 at its N-terminus, ubiquitinating residue K103, is required for activity-induced Kv4.2 protein loss. Further, Ube3A is associated with internalized Kv4.2 that complexes with the Kv4 auxiliary subunit DPP6. A p38 kinase-Pin1 isomerase cascade that regulates

Kv4.2-DPP6 dynamics facilitates activity-dependent Ube3A-mediated degradation of Kv4.2. In a mouse model of AS, we observed elevated Kv4.2 protein level, abolished seizure-induced Kv4.2 protein loss, impaired dendritic gradient of Kv4.2 and reduced Kv4.2-DPP6 association. Moreover, deficits in mEPSC frequency and spike-timing-dependent LTP, as well as certain behaviors including cognitive inflexibility found in AS mice, were partially rescued when bred with Kv4.2 conditional knockout mice. These findings reveal a novel Ube3A downstream pathway regulating plasticity and cognitive behaviors, and provide potential targets for the treatment of AS.

Disclosures: J. Hu: None. **C. Malloy:** None. **Y. Liu:** None. **A. Pratt:** None. **M.A. Welch:** None. **D. Hoffman:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.12/C51

Topic: B.03. Ion Channels

Support: NIH Grant NS102239

Title: Slack channels regulate neuronal RNA translation through complexing with FMRP/CYFIP

Authors: J. WU, T. MALONE, Y. ZHANG, R. CHEN, P. LICZNERSKI, M. PEDRAM, N. SHEIKH, E. JONAS, ***L. KACZMAREK**;
Yale Univ. Sch. Med., New Haven, CT

Abstract: Local translation of mRNAs in neurons is known to be regulated by the RNA-binding protein Fragile X Mental Retardation Protein (FMRP) and Cytoplasmic FMRP-Interacting Protein 1 (CYFIP1). These two proteins form a complex that represses the initiation of mRNA translation by binding to the initiation factor eukaryotic initiation factor 4E (eIF4E). The conditions under which the FMRP-CYFIP1 complex dissociates from eIF4E to trigger the synthesis of new proteins in neurons are, however, not understood. We have found that FMRP and CYFIP1 each binds to the cytoplasmic C-terminus of sodium-activated potassium Slack (KCNT1, $K_{Na}1.1$) channel. Our new findings suggest that activation of Slack channels triggers the translocation of these regulatory proteins to the channel complex, relieving the inhibition of translation. A mutation in the C-terminus of the Slack (*Slack-R455H*) that constitutively activates the channel results in higher levels of FMRP and CYFIP1 bound to the channel, but lower levels bound to eIF4E. Direct stimulation of cortical slices from wild type mice with a Slack activator also triggers translocation of FMRP to the channel. Both the constitutively active Slack mutation and pharmacological activation of wild-type channels stimulate the translation of an mRNA reporter construct with the 5' and 3' UTRs of β-actin mRNA for β-actin, an mRNA target of FMRP. To test the effects of Slack activation on translation of native proteins, a proximity ligation assay with β-actin and puromycin was used to detect sites of β-actin translation in

cortical neurons in culture. The number of positive puncta was very markedly enhanced by the *Slack-R455H* mutation in the dendrites but not the somata of the neurons. Our findings suggest that Slack channel activity in the dendrites of cortical neurons regulates local protein synthesis by removing the inhibitory effects of FMRP and CYFIP1 on translation initiation. The effects of Slack mutations on activity-dependent translation may explain the very severe intellectual disability produced by these mutations in humans.

Disclosures: J. Wu: None. **T. Malone:** None. **Y. Zhang:** None. **R. Chen:** None. **P. Licznerski:** None. **M. Pedram:** None. **N. Sheikh:** None. **E. Jonas:** None. **L. Kaczmarek:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.13/C52

Topic: B.03. Ion Channels

Title: The molecular mechanism of frequency-dependent spike broadening and peripheral pain sensitization in DRG neurons

Authors: *T. ALEXANDER[1], A. ALICEA-PAUNETO[1], S. TYMANSKYJ[1], C. BENSON[2], A. TAN[2], Y. ZHANG[2], L. K. KACZMAREK[2], M. COVARRUBIAS[1];
[1]Neurosci., Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA; [2]Yale Univ. Sch. of Med., New Haven, CT

Abstract: Kv3.4 channels can tune the repolarization rate of the DRG neuron action potential (AP) in a manner that depends on the phosphorylation status of their cytoplasmic N-terminal inactivation domain (NTID) at four non-equivalent serines. However, the mechanism underlying this link is unknown. Based on computational modeling, we hypothesize that use-dependent AP broadening depends on Kv3.4 recovery from inactivation, which, in turn, depends on the phosphorylation status of the NTID. To test this hypothesis, we used an AAV6-based approach in rat DRG neurons to overexpress wild-type Kv3.4 (susceptible to basal phosphorylation) and mutant Kv3.4 channels that were either dephosphorylated (Ala mutations = phospho-null) or fully phosphorylated (Asp mutations = phosphomimic) at four serines and characterized the expressed Kv3.4 currents and APs under repetitive stimulation conditions that induce cumulative inactivation and use-dependent AP broadening. We found that that robust use-dependent AP broadening is associated with profound cumulative inactivation and slow recovery from inactivation of the phosphonull Kv3.4. In contrast, modest AP broadening is associated with less severe cumulative inactivation and faster recovery from inactivation of the wild-type Kv3.4. Strikingly, when overexpressing the phosphomimic Kv3.4, use-dependent AP broadening and cumulative inactivation are nearly eliminated - which is associated with faster recovery from inactivation. Furthermore, as expected, phosphonull, wild-type and phosphomimic exhibit dramatically different rates of recovery from inactivation. The functional knockout of Kv3.4

eliminates use-dependent AP broadening and induces mechanical allodynia but does not alter thermal sensitivity. These results establish the molecular mechanism responsible for the regulation of use-dependent AP broadening in DRG neurons, which plays a central in the transmission of nociceptive signals at the level of the first synapse in the dorsal horn of the spinal cord.

Disclosures: T. Alexander: None. **A. Alicea-Pauneto:** None. **S. Tymanskyj:** None. **C. Benson:** None. **A. Tan:** None. **Y. Zhang:** None. **L.K. Kaczmarek:** None. **M. Covarrubias:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.14/C53

Topic: B.03. Ion Channels

Title: Mkrn1 regulates protein stability of herg potassium channels

Authors: *Y.-C. FANG[1,2], C.-Y. TANG[1], C.-J. JENG[2];
[1]Dept. of Physiol., Natl. Taiwan Univ., Taipei, Taiwan; [2]Inst. of Anat. and Cell Biol., Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: The human ether-à-go-go-related (hERG) voltage-gated potassium channel, encoded by *KCNH2*, is crucial for maintaining membrane potential in the heart and brain. Mutations in cardiac hERG channels are associated with a lethal ventricular arrhythmia, type 2 long QT syndrome (LQT2). Neuronal hERG channels also play a critical role in maintaining membrane excitability, and are implicated in brain disorders such as epilepsy, Parkinson's disease, and schizophrenia. Most LQT2-causing mutations instigate defective hERG protein homeostasis (proteostasis), leading to enhanced endoplasmic reticulum (ER)-associated degradation and reduced channel protein expression at the cell surface. Herein we aim to elucidate the molecular basis of the ER protein quality control system for hERG channels. We have identified makorin ring finger protein 1 (MKRN1) as a novel E3 ubiquitin ligase participating in ER quality control of hERG. Our biochemical analyses revealed that MKRN1 preferentially modulated protein stability of core-glycosylated hERG via ubiquitin-proteasome degradation. RNA interference knock-down of endogenous MKRN1 resulted in increased cell-surface expression of hERG protein in the mouse HL-1 atrial cardiomyocyte. We further demonstrated that MKRN1 contributes to ER quality control of LQT2-associated hERG mutants. Altogether our findings support the notion that deficient membrane trafficking of disease-related hERG mutants may in part be corrected by down-regulating the E3 ligase function of MKRN1.

Disclosures: Y. Fang: None. **C. Tang:** None. **C. Jeng:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.15/C54

Topic: B.03. Ion Channels

Support: NIH/NIGMS Grant R35GM131896

Title: An activator of voltage-gated K^+ channels Kv1.1 as a therapeutic candidate for episodic ataxia type 1

Authors: *M. PESSIA;
Physiol. and Biochem., Univ. of Malta, Msida, Malta

Abstract: Mauro Pessia, Ilenio Servettini, Giuseppe Talani, Alfredo Megaro, Maria Dolores Setzu, Francesca Biggio, Michelle Briffa, Luca Gugliemi, Nicoletta Savalli, Francesca Binda, Francis Delicata, Gilles Bru-Mercier, Neville Vassallo, Vittorio Maglione, Ruben J. Cauchi, Alba Di Pardo, Maria Collu, Paola Imbrici, Luigi Catacuzzeno, Riccardo Olcese and Maria Cristina D'Adamo. Loss-of-function mutations in the *KCNA1*(Kv1.1) gene cause episodic ataxia type 1 (EA1), a severe neurological disease characterized by cerebellar dysfunction, ataxic attacks, persistent myokymia with painful cramps in skeletal muscles and epilepsy. Precision medicine for EA1 treatment is currently unfeasible, as no drug that can enhance the activity of Kv1.1-containing channels and offset the functional defects caused by *KCNA1* mutations has been approved. Here we uncovered that niflumic acid (NFA), a currently prescribed analgesic and anti-inflammatory drug with an excellent safety profile in the clinic, potentiates the activity of Kv1.1 channels. NFA increased Kv1.1 current amplitudes by enhancing the channel open probability, causing a hyperpolarizing shift in the voltage dependence of both channel opening and gating charge movement, slowing the OFF-gating current decay. NFA exerted similar actions on both homomeric Kv1.2 and heteromeric Kv1.1/Kv1.2 channels, which are formed in most brain structures. We show that through its potentiating action, NFA mitigated the EA1 mutation-induced functional defects in Kv1.1 and restored cerebellar synaptic transmission, Purkinje cell availability, and precision of firing. In addition, NFA ameliorated the motor performance of a *knock-in* mouse model of EA1 and, restored the neuromuscular transmission and climbing ability in *Shaker* (Kv1.1) mutant *Drosophila melanogaster* flies (Sh^5). By virtue of its multiple actions, NFA has strong potential as an efficacious single-molecule-based therapeutic agent for EA1, and serves as a valuable model for drug discovery.

Disclosures: M. Pessia: None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.16/C55

Topic: B.03. Ion Channels

Support: NIH DC007695

Title: Developmental maturation of voltage-gated potassium channels in the medial nucleus of the trapezoid body

Authors: *N. M. BENITES[1], D. T. HELLER[2], E. AMICK[2], A. DAGOSTIN[3], S. M. YOUNG, Jr[4], H. VON GERSDORFF[5], G. SPIROU[2];
[1]Med. Engin., [2]Univ. of South Florida, Tampa, FL; [3]OHSU, Portland, OR; [4]Dept. of Anat. and Cell Biol., Univ. of Iowa, Iowa City, IA; [5]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The principal neurons (PNs) in the medial nucleus of the trapezoid body (MNTB) express a diverse population of voltage-gated potassium (Kv) channels. Kv1, Kv3, and Kv4 subfamilies have been characterized in the PNs of adult mice and developmentally, typically after postnatal day (P)6 when the calyx of Held has formed. However, the expression profiles during the first postnatal week remain poorly understood. Small terminals grow into CHs at P3 and mostly resolve to mono-innervation by P6 (≈75% of PNs). In parallel, PNs input resistance decreases, resting membrane potential (RMP) hyperpolarizes, action potentials (AP) decrease in duration, and AP firing patterns transition from tonic to phasic in response to depolarizing current injections. Our goal is to correlate the functional expression of Kv channel types with these functional phenotypes. Kv1 and Kv4 are low-voltage activated (LVA) K^+ channels, the latter requiring prior hyperpolarization for full activation, associated with steady-state inactivation. LVA channels open near the RMP, influencing the AP threshold. Previous studies have shown an increase in mRNA expression of Kv1 in the first postnatal week. Kv3 channels are high-voltage activated K^+ channels, opening at membrane potentials greater than -10 mV, primarily affecting AP repolarization. In MNTB PNs and other fast-spiking neurons, Kv3.1 channels mediate rapid repolarization due to fast activation and inactivation kinetics. To investigate Kv channel conductance, we employed voltage-clamp protocols using P1-P6 FVB mice. Different Kv conductances were isolated by varying holding potential and sequentially applying channel blockers (dendrotoxin, TEA), calculating currents by subtraction. We provide the initial report of Kv1, Kv3, and Kv4 conductances at P2 and P3, just prior to and at the earliest stage of CH growth. The conductances were normalized by the driving force and fitted using the Boltzmann function, with ranges of -53 mV to 7 mV (Kv1 and Kv4) and -53 mV to 27 mV (Kv3). Analyzing P2&3 MNTB PNs (n = 3), we found that Kv4 exhibited a V50 (half-activation potential) of -32.5 ± 3.4 mV, with a slope of 6.3 ± 3.1. Kv1 showed a V50 of -14.3 ± 7.6 mV and a slope of 11.8 ± 7.5. Kv3 showed a V50 of -4.6 ± 10 mV and a slope of 14.5 ± 11. These conductance differences imply potential influences on the AP threshold, amplitude, and synaptic transmission, as well as intracellular Ca^{2+} signaling during early developmental stages. We have implemented a biophysical model of the PN (NEURON simulation environment) to interpret these data as we track measurements through the first postnatal week and correlate them with current-clamp recordings.

Disclosures: **N.M. Benites:** None. **D.T. Heller:** None. **E. Amick:** None. **A. Dagostin:** None. **S.M. Young:** None. **H. von Gersdorff:** None. **G. Spirou:** E. Ownership Interest (stock,

stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); syGlass.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.17/C56

Topic: B.03. Ion Channels

Support: NIH Grant NS102239

Title: Disease-causing Slack (Kcnt1) potassium channel mutations produce opposite effects on excitability of excitatory and inhibitory neurons

Authors: *J. WU, Y. ZHANG, I. QURAISHI, H. WANG, L. KACZMAREK;
Yale Univ., New Haven, CT

Abstract: The *KCNT1* gene encodes the sodium-activated potassium channel Slack (KCNT1, $K_{Na}1.1$), a regulator of neuronal membrane excitability. Gain-of-function (GOF) mutations of *KCNT1* in humans cause cortical network hyperexcitability and seizures, as well as very severe intellectual disability. The underlying molecular mechanisms have, however, yet to be determined. Using a mouse model of Slack GOF-associated epilepsy expressing the *Slack-R455H* mutation, we have previously shown that both excitatory and inhibitory neurons of the cerebral cortex have increased Na^+-dependent K^+ (K_{Na}) currents and voltage-dependent sodium (Na_V) currents (I_{Na}). Paradoxically, the excitability of excitatory neurons was enhanced by this variant while that of inhibitory interneurons was suppressed. Our current results showed that the expression of Na_V channel subunits, particularly that of $Na_V1.6$, was upregulated and that the length of the axon initial segment (AIS) and of axonal Na_V immunostaining was increased in both neuron types expressing the *Slack-R455H* mutation. These results suggest that the upregulation of Na_V subunits in the mutant neurons contributes to increased Na^+ influx and elevated I_{Na} and K_{Na} currents. Our results further showed that the distance between the soma and the AIS was significantly shorter in excitatory neurons than in inhibitory neurons, but that this was not altered significantly by the *Slack-R455H* mutation in either cell type. These results suggest that proximity of Slack K_{Na} channels to sodium Na_V channels differs in the two types of cells, potentially contributing to the different effects of increased I_{Na} and K_{Na} currents on membrane excitability. Our ongoing experiments aim to test if Slack channels regulate Na_V subunits expression, particularly $Na_V1.6$, through complexing with FMRP/CYFIP, two regulators of mRNA translation. Our study on the coordinate regulation of K_{Na} currents and the expression of Na_V channels may provide a new avenue for understanding and treating epilepsies and other neurological disorders.

Disclosures: J. Wu: None. **Y. Zhang:** None. **I. Quraishi:** None. **H. Wang:** None. **L. Kaczmarek:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.18/C57

Topic: B.03. Ion Channels

Support: CAPES
PROEX
CNPq
FAEPA
FAPESP

Title: Dynamic control of firing by KATPchannels by changes in metabolic ATP in a glycinergic neuron from the dorsal cochlear nucleus of mice

Authors: *D. F. DE SIQUEIRA[1], R. M. LEAO[2];
[1]Dept. of Physiol., Univ. de São Paulo, Ribeirão Preto/SP, Brazil; [2]Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: Hyperactivity of the Dorsal Cochlear Nucleus (DCN) is critical for the development of tinnitus. Cartwheel (CW) neurons are glycinergic interneurons that fire spontaneously producing strong tonic inhibition in fusiform neurons of the DCN. We have recently shown that ATP-sensitive potassium channels (K_{ATP}) control the firing of CW neurons. However, in whole-cell patch clamp experiments, we added phosphonucleotides in the internal solution. Therefore, we intend to know how sensitive K_{ATP} channels are to changes in metabolic ATP, using whole-cell patch clamp recordings from CW neurons in slices from rats (p17-22) with an internal solution devoid of phosphonucleotides. In this solution electrophysiological properties of CW neurons remained stable over 40 minutes (n=10). We observed no significant differences in the analyzed parameters compared to cells recorded with phosphonucleotides. The mithocondrial uncoupler CCCP [1uM] hyperpolarized the membrane potential (-64 ± 1 mV to -71 ± 1 mV; p=0.01; n=6), decreased spontaneous firing (12±2 to 2±1; p=0.005) and generated an outward current similar to that produced by the K_{ATP} channel opener diazoxide, that was inhibited by the K_{ATP} antagonist tolbutamide. Application of an external solution without glucose decreased or stopped spontaneous firing (16±3 Hz to 3±2 Hz; p=0.0009; n=17), decreased the input resistance (p<0.0001), hyperpolarized the membrane potential (-66±1 mV to -75±1 mV; p<0.0001), and generated an outward current reverted by tolbutamide (n=9). We hypothesized that depolarization of cartwheel neurons, the available ATP would decrease by intense Na^+/K^+ ATPase activity. However, we depolarizing the neuron with a 250pA DC current did no increase spontaneous firing and membrane potential but decreased the input resistance (p=0.04; n=10) and generated an outward current. On the other hand, depolarization of CW neurons by 250pA DC current after tolbutamide [100uM] increased the spontaneous firing (11±4 Hz to 27±11 Hz; p=0.008; n=8). Additionally, tolbutamide prevented the development of the outward current, suggesting that, with depolarization, available ATP in the cell decreases in response to increased

firing, leading to the opening of K_{ATP} channels rapid controlling action potential firing, demonstrating that K_{ATP} channels in the DCN may exercise dynamic control over the firing of spontaneous action potential firing which could prevent over excitation and sodium accumulation in the neuron after prolonged firing.

Disclosures: D.F. De siqueira: None. **R.M. Leao:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.19/Web Only

Topic: B.03. Ion Channels

Support: GSU Brains and Behavior Seed Grant
GSU MBD Seed Grant
NIH RO3 NS116327

Title: Pias3 (a.k.a kchap) augments kv4 sumoylation in cardiomyocytes

Authors: *L.-A. JANSEN[1], M. WELCH[2], D. BARO[2];
[1]Georgia State Univ., Loganville, GA; [2]Biol., Georgia State Univ., Atlanta, GA

Abstract: I_{tof} is the first repolarizing K^+ current in the cardiac action potential. Loss of I_{tof} increases action potential duration, which can potentiate cardiac arrhythmias. Kv4.2 and Kv4.3 channels mediate $I_{tof.}$ These channels are expressed in ternary complexes (TC) with KChIP1-4 and DPP6/10 proteins. Post-translational modifications regulate Kv4 surface expression. Enhanced SUMOylation of Kv4.2 at K579 in the TC increases both surface expression and maximal conductance (I_A gmax) of the channel by increasing rab11a-slow recycling of the channel after endocytosis; conversely, phosphorylation at S552 decreases surface expression. E3 SUMO ligases facilitate target SUMOylation by working to 1) bridge the target with Ubc9, the SUMO conjugating protein, and 2) position the donor SUMO using an internal SIM1. No E3 SUMO ligases have been identified for any voltage-gated ion channel. The Protein Inhibitor of Activated STAT3 (PIAS3) was shown to increase Kv4 currents and co-IP with Kv4 channels in rat heart lysates. PIAS3 was shown to be an E3 SUMO ligase for transcription factors; however, the link between PIAS3 and ion channel SUMOylation was never made. We hypothesized that PIAS3 was an E3 SUMO ligase for Kv4 channels. In HEK cells, PIAS3 significantly increased I_A gmax in a rab11a-slow recycling dependent manner. This effect was blocked with a SUMO-deficient Kv4.2-K579R TC. Both Ubc9 and the internal SIM1 of PIAS3 were necessary for its effects on I_A, suggesting its role as an E3 SUMO ligase for Kv4. Further, western blot (WB) experiments showed a PIAS3-induced increase in the SUMOylation of Kv4.2-K579. In addition, our data show that PKA-mediated phosphorylation at S552 blocked the PIAS3-mediated increase in channel SUMOylation and I_A, thereby reducing channel recycling. Our study was extended into the rodent heart where Co-IP and WB experiments

showed that Kv4.2 and Kv4.3 channels were both SUMOylated and co-IP'd with PIAS3. In Situ Proximity Ligation Assays on cultured rat cardiomyocytes demonstrated that acute treatment with TAT-PIAS3, a cell permeable protein, produced a significant ~50% increase in Kv4 SUMOylation density when normalized to PBS control (*p=0.0002*). This effect was eliminated with a SUMOylation deficient TAT-PIAS3. 8-bromo cAMP, a PKA activator, had no effect compared to PBS alone but could block the effects of TAT-PIAS3 on Kv4 SUMOylation density (*p=0.345*). These data are consistent with the hypothesis that PIAS3 acts as an E3 SUMO ligase for Kv4 channels and PIAS3 targets are shaped by the targets phosphorylation status.

Disclosures: L. Jansen: None. **M. Welch:** None. **D. Baro:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.20/C58

Topic: B.03. Ion Channels

Title: NETSseq platform identifies the astrocyte specific potassium channel KCNJ10 (k_{ir}4.1) as a target for the potential treatment of neurodegenerative diseases

Authors: C. L. BENDER, ***J. R. M. HARVEY**, B. OSSOLA, P. J. PICKFORD, D. F. BARKER, V. J. MULLIGAN, K. J. PAGE, M. L. B. CARLTON, N. L. BRICE, L. A. DAWSON;
Cerevance, Cambridge, United Kingdom

Abstract: Neuronal hyperexcitability is a common early pathological feature of many neurodegenerative diseases. The exact mechanisms causing these changes in excitability are not fully characterised; however, abnormal glutamate release and dysfunction of glutamate clearance causes neuronal injury through activation of glutamate receptors resulting in excitotoxicity. Efficient glutamate uptake by astrocytes requires expression of functional glutamate transporters and an intact K^+ electrochemical gradient. Using Cerevance's proprietary Nuclear Enriched Transcript Sort Sequencing (NETSseq) platform, which produces deep sequencing (~12,000 genes) of specific purified cell types from human post-mortem brain tissue, we demonstrated highly specific glial expression of the inwardly rectifying K^+ channel K_{ir}4.1 (encoded by the KCNJ10 gene), along with disease related changes. KCNJ10 is expressed in astrocytes from post-mortem brain tissue of Alzheimer's and Parkinson's disease donors. Network analysis revealed that KCNJ10 is a central hub of many pathways significantly impacted by CNS diseases, including glutamate uptake. Non-selective literature inhibitors, VU0134992 and fendiline, dose-dependently reduced thallium influx (as a surrogate of K^+ influx) in KCNJ10 overexpressing HEK cells and primary mouse astrocytes. In astrocytes, glutamate uptake and transporter-gated currents were blocked by tool inhibitors in a concentration-dependent manner. AAV induced overexpression of KCNJ10 in primary mouse astrocytes showed a robust increase in thallium influx which was blocked with VU0134992. Additionally, a small increase in

glutamate uptake was observed in these cells. Here we demonstrate that NETSseq can identify changes in gene expression that could be relevant to human disease pathology. Expression changes in KCNJ10 may lead to dysfunctional glutamate clearance and consequently neuronal injury by excitotoxicity. Therefore, the enhancement of KCNJ10 function could present a novel approach to prevent neuronal hyperexcitability in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

Disclosures: **C.L. Bender:** A. Employment/Salary (full or part-time):; Cerevance. **J.R.M. Harvey:** A. Employment/Salary (full or part-time):; Cerevance. **B. Ossola:** A. Employment/Salary (full or part-time):; Cerevance. **P.J. Pickford:** A. Employment/Salary (full or part-time):; Cerevance. **D.F. Barker:** A. Employment/Salary (full or part-time):; Cerevance. **V.J. Mulligan:** A. Employment/Salary (full or part-time):; Cerevance. **K.J. Page:** A. Employment/Salary (full or part-time):; Cerevance. **M.L.B. Carlton:** A. Employment/Salary (full or part-time):; Cerevance. **N.L. Brice:** A. Employment/Salary (full or part-time):; Cerevance. **L.A. Dawson:** A. Employment/Salary (full or part-time):; Cerevance.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.21/C59

Topic: B.03. Ion Channels

Support: NIH Grant R01 ES033158 (A.D, A.B)

Title: Effects of toluene on cerebral artery diameter; sex differences and bk channel involvement

Authors: ***A. SHAW**[1], K. NORTH[2], J. D. STEKETEE[3], A. BUKIYA[1], A. DOPICO[4]; [1]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]St. Jude, Memphis, TN; [3]Univ. Tennessee Hlth. Sci. Ctr., MEMPHIS, TN; [4]The Univ. of Tennessee HSC, Coll. Med., Dept. Pharmacol., Memphis, TN

Abstract: Toluene is a volatile organic solvent found in many common household products. In addition to accidental or environmental exposure to these products, toluene intoxication may result from recreational use as individuals inhale toluene to reach a "high". Toluene acute intoxication and cerebral ischemia share many neurological and psychological outcomes. Moreover, computed tomography in humans shows that toluene acute intoxication leads to brain hypoperfusion (Ryu et al., 1998). Remarkably, whether toluene inhalation may alter cerebral artery function has not been investigated. Since cerebral artery constriction is a well-known mechanism leading to brain ischemia, we hypothesized that toluene was a cerebral artery constrictor. To test this, we determined the effect of toluene at different concentrations on the diameter of middle cerebral arteries (MCA) in a rat model in vivo, as the rat cerebrovascular circulation shares many features with that of humans, including the fact that MCA irrigates the largest portion of the brain. Thus, male and female Sprague-Dawley rats (250-350 g) were

anesthetized with ketamine/xylazine mixture to undergo cranial window surgery. MCA diameter images were obtained immediately before rats were placed in an inhalation chamber where either 1.2 mL of toluene or saline was dispensed on a paper towel and the door closed. Rats were left in the chamber for 1, 5, 10, 20, or 30 minutes for toluene to mix into the chamber atmosphere, and parts per million of toluene were determined at the end of each exposure by infrared analyzer. MCA diameter were then obtained at the end of each toluene exposure. In both males and females, toluene evoked a dose-dependent reduction in diameter. In males, average peak arterial constriction reached 8.5% of pre-toluene values and was becoming apparent at about 6,000 ppm. While reaching the same peak as in males, females MCA started constricting only at ≥3,000 ppm toluene. These data document for the first time that toluene inhalation at concentrations used by humans for recreational purposes constricts cerebral arteries in vivo, a drug action that likely mediates, or at least, contributes to toluene-induced brain ischemia. Toluene in vivo action was replicated in isolated, de-endothelialized and ex vivo-pressurized MCA segments, underscoring that circulating and endothelial factors were not involved. Selective pharmacological block of big conductance, Ca^{2+}/voltage-gated K^+ (BK) channels ablated toluene constriction of MCA. In addition, this toluene action was lost in MCA from *KCNMA1*$^{-/-}$ mice, which do not express BK channels. Thus, BK channels mediate toluene-induced MCA constriction.

Disclosures: **A. Shaw:** None. **K. North:** None. **J.D. Steketee:** None. **A. Bukiya:** None. **A. Dopico:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.22/C60

Topic: B.03. Ion Channels

Support: NIH R01MH085927
NIH R01NS109388

Title: Molecular mechanisms of BK channel regulation by melatonin

Authors: *K. VEDANTHAM, L. NIU, Z.-W. WANG;
Neurosci., Univ. of Connecticut Hlth., Farmington, CT

Abstract: Melatonin, secreted by the pineal gland, produces its sleep-promoting effect via two G protein-coupled receptors: MT_1 and MT_2. These two receptors are expressed in a variety of brain structures, including the cerebral cortex, suprachiasmatic nucleus (SCN), and hippocampus. However, the downstream molecular target(s) mediating the sleep effect of melatonin has remained enigmatic. In our recent study (Niu et al., PNAS 2020), we found that melatonin promotes sleep by activating the BK channel SLO-1 through a specific melatonin receptor in *C. elegans*. In addition, we found that the human BK channel Slo1 may be activated by melatonin in the *Xenopus* oocyte heterologous expression system by binding to MT_1 but not MT_2, and that the

activation results from the action of Gβγ subunits. These results suggest that melatonin might also activate Slo1 in mammalian neurons through a specific melatonin receptor. We have embarked on a project of testing this hypothesis by performing several types of experiments. First, we examined the effects of various chimeras of mouse MT_1 and MT_2 on mouse Slo1 single-channel open probability (*Po*) using *Xenopus* oocytes as a heterologous expression system. We found that substitution of the N- but not C-terminus of MT_1 by that of MT_2 abolished the activation effect of melatonin on Slo1, suggesting that the N-terminus of MT_1 is required for Slo1 activation. Second, we isolated synaptosomes from wild-type mice, and determined whether they contained MT_1. We detected MT_1 in them by western blot. Because Slo1 is an important protein at presynaptic sites and its presence in synaptosomes has been confirmed in a previous study, our result suggests that MT_1 and Slo1 likely coexist at presynaptic terminals. Third, we performed co immunoprecipitation (co-IP) assays with transfected HEK293T cells and mouse whole-brain lysates. We observed co-IP of Slo1 with MT_1 and found that the co-IP depended on an intracellular loop (S0-S1 loop) of Slo1, suggesting that the two proteins physically interact. In summary, our available results suggest that mammalian Slo1 may physically interact with melatonin receptors and be activated by melatonin in an MT_1-dependent manner.

Disclosures: **K. Vedantham:** None. **L. Niu:** None. **Z. Wang:** None.

Poster

PSTR454. Potassium Channels

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR454.23/C61

Topic: B.03. Ion Channels

Support: New Jersey Governor's Council for Medical Research and Treatment of Autism predoctoral Fellowship CAUT23AFP015
NIA Grant R01AG060919
NSF Grant 2030348

Title: A Novel Mouse Model To Study Potassium Ion Channel Related Early-Onset Epileptic Encephalopathies

Authors: *A. BORTOLAMI[1], D. P. CROCKETT[2], F. SESTI[3];
[1]Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ; [2]Dept Neurosci. and Cell Biol., Rutgers-Robert Wod Johnson Med. Sch., Piscataway, NJ; [3]Neurosci. and Cell Biol., Rutgers, Piscataway, NJ

Abstract: Potassium channels play a role in controlling the excitability of neurons and can trigger a cascade of signals within the central nervous system. One specific potassium channel, called voltage-gated potassium channel subfamily B member 1 (KCNB1), is associated with integrins (IKCs) and is important for converting its electrical properties into signals that promote cell proliferation and migration. Mutations in the KCNB1 gene are linked to brain disorders. In

particular, a substitution mutation at position 312 in the KCNB1 gene, where arginine is replaced by histidine (Kcnb1R312H), has been found in children with developmental and epileptic encephalopathies (DEEs). Children affected by this disorder experience severe developmental delay and in the majority of cases recurrent seizures. To study this neurological condition, we created a mouse model harboring the Kcnb1R312H gene variant using CRISPR knock-in (KI) technology. In this study, we verified aberrant neurodevelopment and synaptic connectivity. We assessed the behavior of the Kcnb1R312H mouse, focusing on anxiety, motor functions, and complex tasks, in order to establish a reliable model for studying DEEs. Our results indicate that the Kcnb1R312H mouse model offers a valuable tool for gaining a deeper understanding of human potassium ion channels related DEEs.

Disclosures: A. Bortolami: None. **D.P. Crockett:** None. **F. Sesti:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.01/C62

Topic: B.05. Synaptic Plasticity

Support: Howard Hughes Medical Institute
Cullen Foundation

Title: A critical role for CaMKII in behavioral timescale synaptic plasticity in hippocampal CA1 pyramidal neurons.

Authors: *K. XIAO[1], Y. LI[2], R. A. CHITWOOD[2], J. C. MAGEE[2];
[1]Baylor Col. of Med., Houston, TX; [2]HHMI, Baylor Col. of Med., Houston, TX

Abstract: Behavioral timescale synaptic plasticity (BTSP) is a type of non-Hebbian synaptic plasticity reported to underlie place field formation in the hippocampal CA1 neurons. Despite this important function, the molecular mechanisms underlying BTSP are poorly understood. The α-Calcium-calmodulin-dependent protein kinase II (αCaMKII) is activated by synaptic transmission-mediated calcium influx and its subsequent phosphorylation is central to synaptic plasticity. Because the activity of αCaMKII is known to outlast the event triggering phosphorylation, we hypothesized it could be involved in the extended timescale of the BTSP process. To examine the role of αCaMKII in BTSP, we performed whole-cell in-vivo and in-vitro recordings in CA1 pyramidal neurons from mice engineered to have a point mutation at the autophosphorylation site (T286A) causing accelerated signaling kinetics. Here we demonstrate a profound deficit in synaptic plasticity, strongly suggesting that αCaMKII signaling is required for BTSP. This study elucidates part of the molecular mechanism of BTSP and provides insight into the function of αCaMKII in place cell formation and ultimately learning and memory.

Disclosures: K. Xiao: None. **Y. Li:** None. **R.A. Chitwood:** None. **J.C. Magee:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.02/C63

Topic: B.05. Synaptic Plasticity

Support: NINDS 5R01NS065856-12
JSPS KAKENHI grant numbers 23H04244

Title: Rem2 interacts with CaMKII at the synapse to restrict long-term potentiation in the hippocampus

Authors: *R. ANJUM[1], V. R. J. CLARKE[2], Y. NAGASAWA[3], H. MURAKOSHI[3], S. PARADIS[1];
[1]Biol., Brandeis Univ., Waltham, MA; [2]Feinberg Sch. of Med., Northwestern Univ., Evanston, IL; [3]Physiological Sci., The Grad. Univ. for Advanced Studies, Hayama, Japan

Abstract: In order to understand learning and memory at the molecular level, we must uncover the mechanisms underlying synaptic plasticity. Ca2+/calmodulin-dependent protein kinase II (CaMKII) is a well-studied serine-threonine kinase that is highly enriched at the post-synaptic density and is required for multiple types of synaptic plasticity including long-term potentiation (LTP). Our lab has previously shown that Rem2, a Ras-like GTPase from the RGK (Rad/Rem/Rem2/Gem/Kir) protein family, is a potent endogenous inhibitor of CaMKII. We also demonstrated that mutation of two key amino acid residues in the N-terminus (R79/R80) are required for this inhibition. Thus, we sought to determine whether Rem2 inhibition of CaMKII plays a role in LTP. To investigate this question, we recorded field excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA1 area, and induced LTP using tetanus stimulation. We observed enhanced LTP at the Schaffer Collateral-CA1 (SC-CA1) synapse in the absence of *Rem2*; this effect is dependent on Rem2 inhibition of CaMKII. These results suggest that Rem2 normally functions to restrict LTP suggesting an interaction between Rem2 and CaMKII in the postsynaptic density of the synapse. To test this hypothesis, we performed 2-photon fluorescence lifetime imaging (2pFLIM) using mCherry-Rem2 and Clover-CaMKII transfected into organotypic hippocampal slices. An interaction between Rem2and CaMKII for approximately 2 minutes was observed in single spines upon glutamate uncaging. Taken together, our data lead us to propose that Rem2 normally serves as a brake on runaway synaptic potentiation via inhibition of CaMKII activity. Further, the enhanced LTP phenotype we observe upon *Rem2* knockout reveals the existence of a previously unsuspected molecule that regulates CaMKII function at the synapse.

Disclosures: R. Anjum: None. **V.R.J. Clarke:** None. **Y. Nagasawa:** None. **H. Murakoshi:** None. **S. Paradis:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.03/D1

Topic: B.05. Synaptic Plasticity

Title: Shining the Light on Protein Kinase Cs

Authors: *G. OZ, L. COLGAN, Y. HAYANO, R. YASUDA;
Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: Protein kinase C (PKC) isozymes are believed to play distinct roles at different stages of hippocampal structural plasticity and long-term potentiation (LTP) *in vitro*. However, our understanding of their isozyme-specific function in memory acquisition, consolidation, and maintenance *in vivo* has been limited due to the lack of precise inhibitors for PKC isozymes. To address this limitation, we developed a reversible optogenetic inhibitor tool for PKCα (optoPKCIα) and PKCδ (optoPKCIδ). By utilizing these tools, we investigated the effects of transient loss of function of PKCα and PKCδ during LTP induction and memory acquisition. Additionally, we designed a chemogenetic tool to explore the role of PKMζ in LTP and memory maintenance. The combined utilization of both light-inducible and chemogenetic manipulation techniques are significantly enhancing our ability to study these isozymes in the processes of learning and memory.

Disclosures: G. Oz: None. **L. Colgan:** None. **Y. Hayano:** None. **R. Yasuda:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.04/D2

Topic: B.05. Synaptic Plasticity

Title: KIBRA-PKMζ interaction maintains LTP and memory 3: decoupling reverses established late-LTP

Authors: P. TSOKAS[1], C. HSIEH[2], R. E. FLORES-OBANDO[2], J. KREMERSKOTHEN[4], J. E. COTTRELL[3], A. A. FENTON[5], *T. SACKTOR[6];
[1]Anesthesiology, Physiol. and Pharmacology, Furchgott Ctr. for Neural and Behavioral Sci., [2]Physiol. and Pharmacology, Furchgott Ctr. for Neural and Behavioral Sci., [3]Anesthesiol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [4]Univ. Clin. Münster, Münster, Germany; [5]Ctr. for Neural Sci., New York Univ., New York, NY; [6]SUNY Downstate Med. Ctr., Yonkers, NY

Abstract: Here we tested the hypothesis that continual interaction between molecules sustains potentiation at activated synapses to maintain LTP. Tsokas et al., SfN2023, shows that synaptic stimulation facilitates the formation of KIBRA-PKMζ complexes that persist at least 3 h in the maintenance of protein synthesis-dependent late-LTP. To examine whether continual KIBRA-PKMζ interactions maintain late-LTP, we decoupled the molecules using ζ-stat and ζ-trap, two novel, structurally distinct antagonists of KIBRA-PKMζ dimerization. We first decoupled KIBRA from PKMζ using 10 μM ζ-stat, which blocks the KIBRA-binding site in PKMζ, on acute hippocampal slices from 2-4 month-old male mice. Late-LTP was induced by two 100 Hz 1 s trains, 20 s apart. Throughout the experiment we recorded a second pathway as control within the same slice. After establishing late-LTP for 3 h in one pathway, we applied the inhibitor to the bath and recorded responses for 4 h. We found that ζ-stat reverses late-LTP maintenance in the stimulated synaptic pathway without affecting the control pathway. After ζ-stat was washed out for an additional 4 hrs, the potentiation did not return, indicating that the KIBRA-PKMζ coupling is the mechanism that maintains late-LTP. To examine possible off-target effects, we used PKMζ-null mice (Tsokas et al., eLife, 2016; Volk et al., Nature, 2013) that recruit compensatory mechanisms involving other PKCs to sustain persistent late-LTP. In striking contrast to ζ-stat's effect in wild-type mice, the drug had no effect on compensatory, PKMζ-independent late-LTP maintenance in knockout mice lacking PKMζ. To further test that disrupting KIBRA-PKMζ interaction is an effective way to reverse LTP, we used a second dimerization inhibitor, the cell-permeable, myristoylated peptide ζ-trap, which mimics the PKMζ-anchoring sequence in KIBRA. When applied 3 h post-tetanization ζ-trap (10 μM) reversed late-LTP maintenance in wild-type mice, with no effect on baseline synaptic transmission. Like ζ-stat, ζ-trap had no effect on late-LTP maintenance in ζ-knockout mice, lacking PKMζ. In summary, neither inhibitor affected basal synaptic transmission, yet both disrupt the maintenance of enhanced synaptic transmission selectively at activated synapses. Neither antagonist affected compensatory PKMζ-independent maintenance in ζ-knockout mice; thus, both require PKMζ for their effect. These results reveal that two processes maintain LTP: 1) synaptic potentiation by PKMζ, and 2) persistent anchoring of this action to activated synapses by KIBRA.

Disclosures: P. Tsokas: None. **C. Hsieh:** None. **R.E. Flores-Obando:** None. **J. Kremerskothen:** None. **J.E. Cottrell:** None. **A.A. Fenton:** None. **T. Sacktor:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.05/D3

Topic: B.05. Synaptic Plasticity

Support: NIMH R01 MH115304
NIMH R37 MH057068

Title: KIBRA-PKMζ interaction maintains LTP and memory 1: coupling of KIBRA and PKMζ in PSDs.

Authors: *R. E. FLORES-OBANDO[1], C. HSIEH[2], P. TSOKAS[3], J. KREMERSKOTHEN[4], J. E. COTTRELL[5], A. A. FENTON[6], T. C. SACKTOR[7];
[1]Physiol. and Pharmacol., Downstate Med. Ctr., Brooklyn, NY; [2]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., SUNY Downstate Med. Ctr. Col. of Med., Brooklyn, NY; [3]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [4]Univ. Hosp. Münster, Münster, Germany; [5]Anesthesiol., SUNY Downstate, Brooklyn, NY; [6]New York Univ., New York, NY; [7]Physiol. and Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: A fundamental problem in neuroscience is how molecules that last only hours to days can maintain memory that persists weeks to years, an issue raised by Crick (1). As a general solution, Crick proposed that the persistent interaction between molecules at synapses could strengthen transmission in the face of continual protein turnover. PKMζ is known for its crucial role in enhancing synaptic transmission during long-term potentiation (LTP) and long-term memory storage; however, it is unclear how PKMζ targets to postsynaptic sites. A possible mechanism may be through KIBRA (kidney/brain-expressed protein/WWC1), a postsynaptic scaffolding protein genetically linked to human episodic memory performance. KIBRA interacts with the catalytic domain of PKMζ, stabilizing the protein (2). However, a direct interaction between endogenous KIBRA and PKMζ in neurons has yet to be described. We first examined the dendritic distribution of KIBRA and PKMζ in primary cultures of mouse hippocampal neurons using immunocytochemistry. Postsynaptic densities (PSDs) were labeled with PSD-95. The results revealed KIBRA is present in a subset of PSDs, whereas PKMζ is distributed throughout the dendritic shaft and PSDs. Comparing PSDs with and without KIBRA, those with KIBRA show a 3-fold increase in PKMζ accumulation. The direct binding of KIBRA with PKMζ was then investigated by proximity ligation assay (PLA), which detects protein-protein interactions <40 nm within a complex. The results reveal KIBRA-PKMζ complexes in dendritic spines. These findings highlight the potential role of KIBRA in anchoring PKMζ at dendritic spines. In subsequent abstracts (Tsokas P and Hsieh C) the function of this coupling is explored in LTP and spatial memory maintenance.1. F. Crick, Memory and molecular turnover. Nature 312, 101 (1984).
2. A. Vogt-Eisele et al., KIBRA (KIdney/BRAin protein) regulates learning and memory and stabilizes Protein kinase Mzeta. J Neurochem 128, 686-700 (2014).

Disclosures: R.E. Flores-Obando: None. **C. Hsieh:** None. **P. Tsokas:** None. **J. Kremerskothen:** None. **J.E. Cottrell:** None. **A.A. Fenton:** None. **T.C. Sacktor:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.06/D4

Topic: B.05. Synaptic Plasticity

Support: NIMH Grant R37 MH057068
NIMH Grant R01 MH115304
NIH Grant R01 NS108190

Title: KIBRA-PKMζ interaction maintains LTP and memory 4: decoupling disrupts established long-term memory

Authors: *C. HSIEH[1,2], R. E. FLORES-OBANDO[1,2], P. TSOKAS[1,2,3], J. KREMERSKOTHEN[5], J. E. COTTRELL[3], A. A. FENTON[6], T. C. SACKTOR[1,2,4];
[1]Dept. of Physiol. and Pharmacol., [2]The Robert F. Furchgott Ctr. for Neural and Behavioral Sci., [3]Dept. of Anesthesiol., [4]Dept. of Neurol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [5]Dept. of Mol. Nephrology, Univ. Clin. Münster, Münster, Germany; [6]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Here we tested the hypothesis that the persistent coupling of KIBRA (**ki**dney/**bra**in-expressing protein/WWC1) and PKMζ maintains long-term and remote spatial memory by using two new, structurally distinct antagonists of KIBRA-PKMζ interaction: (1) ζ-stat, which blocks the KIBRA-binding site in PKMζ, and (2) ζ-trap, which mimics the PKMζ-binding site in KIBRA. Adult wild-type (WT) and PKMζ-knockout (KO, PKMζ-null) mice were first trained by a rapidly conditioning spatial learning task, active place avoidance. ζ-stat or vehicle was injected bilaterally into hippocampus 1 day after the conditioning. Memory retention was tested 2 days postinjection with shock off. The ζ-stat-injected WT mice showed persistent loss of retention for the location of the shock zone, whereas the vehicle-injected WT mice avoided the shock zone. In contrast, both ζ-stat and vehicle injection did not affect memory retention in the compensating KO mice lacking PKMζ. After the drug was eliminated, the previously ζ-stat-injected WT mice that showed memory loss after the first conditioning were able to form, maintain, and express a new long-term memory to avoid another shock zone with a different set of spatial cues. Like ζ-stat, ζ-trap injection in hippocampus disrupted spatial long-term memory in WT, but not KO mice.
Prior knockdown experiments indicate both PKMζ and KIBRA turn over in neurons within hours to days[1,2]. We examined whether the KIBRA-PKMζ coupling sustains remote spatial memory despite protein turnover. We injected ζ-trap or vehicle bilaterally in hippocampus 4 weeks after conditioning, and remote memory retention was tested 2 days postinjection. The ζ-trap-injected mice showed memory loss of the shock zone location during retention testing, whereas the vehicle-injected mice avoided the shock zone.
Thus, decoupling KIBRA and PKMζ by either blocking the KIBRA-binding site in PKMζ (ζ-stat) or mimicking the PKMζ-binding site in KIBRA (ζ-trap) disrupts long-term memory maintenance. KIBRA-PKMζ decoupling disrupts remote spatial memory, despite PKMζ and KIBRA turnover, in line with Crick's hypothesis[3]. Thus, two processes maintain LTP and memory: 1) synaptic potentiation by PKMζ, and 2) persistent anchoring of this action to activated synapses by KIBRA.
[1] Wang, S., Sheng, T., Ren, S., Tian, T. & Lu, W. Distinct Roles of PKCiota/lambda and PKMzeta in the Initiation and Maintenance of Hippocampal Long-Term Potentiation and Memory. *Cell Rep* **16**, 1954-1961 (2016).
[2] Vogt-Eisele, A. *et al.* KIBRA (KIdney/BRAin protein) regulates learning and memory and stabilizes Protein kinase Mzeta. *J Neurochem* **128**, 686-700 (2014).
[3] Crick, F. Memory and molecular turnover. *Nature* **312**, 101 (1984).

Disclosures: **C. Hsieh:** None. **R.E. Flores-Obando:** None. **P. Tsokas:** None. **J. Kremerskothen:** None. **J.E. Cottrell:** None. **A.A. Fenton:** None. **T.C. Sacktor:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.07/D5

Topic: B.05. Synaptic Plasticity

Support: NIMH R37 MH057068
NIMH R01 MH115304
NIH R01 NS108190

Title: KIBRA-PKMζ interaction maintains LTP and memory 2: persistent coupling in late-LTP

Authors: *P. TSOKAS[1], C. HSIEH[2], R. E. FLORES-OBANDO[2], J. KREMERSKOTHEN[5], J. E. COTTRELL[3], A. A. FENTON[6], T. C. SACKTOR[4];
[1]Physiol. and Pharmacology; Anesthesiol., [2]Physiol. and Pharmacology, Furchgott Ctr. for Neural and Behavioral Sci., [3]Anesthesiol., [4]Physiol. & Pharmacology, Anesthesiology, Neurology, Furchgott Ctr. for Neural & Behavioral Sci., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [5]Dept. of Mol. Nephrology, Univ. Clin. Münster, Münster, Germany; [6]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: How molecules with limited lifespans persistently enhance only activated synapses to maintain LTP and long-term memory remains unclear. One hypothesis, proposed by Crick (Nature, 1984), is that persistent interactions between molecules at synapses strengthen transmission, thus maintaining LTP and long-term memory in the face of protein turnover. Here we used *in situ* proximity ligation assay (PLA) to detect interaction of KIBRA and PKMζ in CA1 pyramidal cells of mouse hippocampal slices during the maintenance of late-LTP. In PLA, pairs of antibodies are linked to oligonucleotides, and if the molecules detected by the antibodies are <40 nm within a complex, the oligonucleotides can be ligated to generate a DNA that is amplified and detected by a fluorescent probe. Acute mouse hippocampal slices were prepared from adult male mice. The Schaffer collateral/commissural fibers were tetanized in CA3 to persistently potentiate field EPSP responses in CA1 *stratum (st.) radiatum*, which were recorded for 3 h. Adjacent slices from the same hippocampus that did not receive tetanization were recorded for the equivalent time. Fixed slices were subsectioned and stained using conventional immunocytochemistry or PLA. We examined the distribution of total KIBRA, total PKMζ, and KIBRA-PKMζ colocalization (assessed by immunofluorescence), as well as KIBRA-PKMζ complexes (detected by PLA). Under basal conditions, KIBRA and PKMζ colocalized in CA1 in both pyramidal cell bodies and synaptic layers. In striking contrast, PLA revealed abundant KIBRA-PKMζ complexes selectively in synaptic layers. Using PLA, we found tetanic stimulation inducing late-LTP facilitated the formation of complexes that persist at least 3 h in late-LTP maintenance in dendrites and spines of *st. radiatum*. In contrast, the complexes did not

increase in CA1 pyramidal cell bodies or regions of dendrites in *st. lacunosum-moleculare* that did not receive afferent stimulation. Tetanic stimulation inducing late-LTP also increased the levels of total KIBRA, total PKMζ, and their colocalization in dendrites and spines in *st. radiatum*. Thus, in line with Crick's hypothesis that protein interactions maintain synaptic potentiation, our experiments reveal that late-LTP-inducing stimulation facilitates the formation of KIBRA-PKMζ complexes in the activated synaptic layer that persist at least 3 h in late-LTP maintenance. To further test this hypothesis, we find that the continual interaction of KIBRA-PKMζ maintains LTP at activated synapses (Tsokas et al., SfN 2023) and long-term memory (Hsieh et al., SfN 2023).

Disclosures: P. Tsokas: None. **C. Hsieh:** None. **R.E. Flores-Obando:** None. **J. Kremerskothen:** None. **J.E. Cottrell:** None. **A.A. Fenton:** None. **T.C. Sacktor:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.08/D6

Topic: B.05. Synaptic Plasticity

Support: NIH/NIA R01 AG055581
R01 AG056622
The Alzheimer's Association grant NIRG-15-362799
The BrightFocus Foundation grant A2017457S

Title: Excitatory neuron-specific repression of AMPKβ2 impairs recognition memory, synaptic morphology, and hippocampal LTP in mice

Authors: *N. A. SWIFT[1], Q. YANG[1], A. MANUEL[2], X. ZHOU[1], G. R. STEINBERG[3], T. MA[1];
[1]Dept. of Intrnl. Medicine, Geriatrics, Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [2]Dept. of Biochem. and Mol. Biol., Wake Forest Univ., Winston-Salem, NC; [3]Div. of Endocrinol. and Metabolism, McMaster Univ., Hamilton, ON, Canada

Abstract: The cellular energy sensor AMP-activated protein kinase (AMPK) has been shown to be integral to synaptic plasticity, learning, and memory. The catalytic α subunit has been shown to be essential for such processes, and its dysregulation has been implicated in diseases characterized by cognitive impairment such as Alzheimer disease; however, little is known about the neuronal effects of the scaffolding β subunit. There exists evidence that AMPKβ has an auto-inhibitory effect on AMPK activity; thus, suppression of one or both AMPKβ isoforms (β1 and β2) could have impact AMPK activity and cognitive function. In this study, the AMPKβ1 or β2 isoforms were conditionally knocked down in the excitatory neurons of C57BL/6 mice (β1 KD, β2 KD). These mice were exposed to a battery of behavioral tests to assess their learning and memory. Additionally, hippocampal *de novo* protein synthesis was assessed via the Surface

Sensing of Translation (SUnSET) assay, and the phosphorylation of various AMPK-related signaling molecules was assessed via western blotting. Furthermore, dendritic structure and function were assessed via synaptic electrophysiology, Golgi staining, and electron microscopy (EM). Analysis of the behavioral test results showed an apparent decrease in the ability of β2 KD (but not β1 KD) mice to discriminate between novel and familiar objects in the novel object recognition behavioral task, indicating an impairment in recognition memory. Interestingly, spatial learning / memory was unaltered in both β1 KD and β2 KD mice as measured by the Morris water maze task. Electrophysiology results revealed an impairment of hippocampal long-term potentiation (LTP) in β2 KD (not β1 KD) mice. Further, dendritic spine maturity was impaired in β2 KD mice, and the abundance and size of postsynaptic densities were altered in β2 KD mice. Moreover, AMPK activity (measured by phosphorylation at the T172 site of AMPKα) was decreased in the hippocampus of β2 KD mice, but unaltered in the β1 KD mice. Overall protein translation, as assessed by SUnSET, was unaltered. Together, these data suggest a previously unrecognized role for AMPKβ2 in regulation of postsynaptic morphology, hippocampal LTP, and recognition memory.

Disclosures: **N.A. Swift:** None. **Q. Yang:** None. **A. Manuel:** None. **X. Zhou:** None. **G.R. Steinberg:** None. **T. Ma:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.09/D7

Topic: B.05. Synaptic Plasticity

Support: NIH Grant AG073823
NIH Grant AG056622
NIH Grant AG082388
A2017457S

Title: Characterization of a novel mouse model with neuronal overexpression of eEF2K

Authors: *H. JESTER[1], Q. YANG[2], N. KASICA[3], X. ZHOU[2], T. MA[2];
[1]Intrnl. Medicine, Gerontology, Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [2]Intrnl. Medicine-Geriatrics, Wake Forest Sch. of Med., Winston-Salem, NC; [3]Bristol Myers Squibb, Nashville, TN

Abstract: Phosphorylation of the eukaryotic elongation factor 2 by its kinase eEF2K results in inhibition of general protein synthesis. Dysregulation of the eEF2K signaling has been implicated in many human diseases. *De novo* or from new protein synthesis is necessary for long-term memory and synaptic plasticity. Aberrantly phosphorylated eEF2 is observed in the brains of Alzheimer's disease patients and people with depression. Nearly all functional studies of eEF2K involve its suppression using transgenic models with eEF2K knockout. Using the

CRISPR technique, we have generated a neuron-specific eEF2K overexpression mouse model (eEF2K-cKI) with the hypothesis that it may recapitulate some disease phenotypes associated with AD. We found that *de novo* protein synthesis, measured via the SUnSET assay, was significantly decreased in the hippocampus of the eEF2K-cKI mice($p=0.0316$). This deficit was also accompanied by impaired hippocampal long-term potentiation (LTP). The eEF2K-cKI mice also showed significant deficits in cognitive function as measured by Novel Object Recognition and Morris Water Maze behavioral assays. Golgi staining revealed a significant decrease in the ratio of mature to immature spines ($p=0.0006$). This novel model of eEF2K overexpression will help to shed light on eEF2K's role in complex disease states like Alzheimer's and Depression.

Disclosures: H. Jester: None. **Q. Yang:** None. **N. Kasica:** None. **X. Zhou:** None. **T. Ma:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.10/D8

Topic: B.05. Synaptic Plasticity

Support: Alzheimer's Association Research Grant (AARG-21-847593)
Italian Minister of Health (GR-2016-02363982)

Title: Engineering LIM kinase 1 to control dendritic spine plasticity and memory

Authors: *C. RIPOLI[1], O. DAGLIYAN[2], P. RENNA[1], F. PASTORE[1], F. PACIELLO[1], R. SOLLAZZO[1], M. RINAUDO[1], M. BATTISTONI[1], S. MARTINI[1], A. TRAMUTOLA[3], A. SATTIN[4], E. BARONE[3], T. SANEYOSHI[5], T. FELLIN[4], Y. HAYASHI[5], C. GRASSI[1]; [1]Universita' Cattolica del Sacro Cuore, Roma, Italy; [2]Karolinska Inst., Stockholm, Sweden; [3]Dept. A.Rossi Fanelli Biochem., Univ. of Rome Sapienza, Roma, Italy; [4]Neurosci. and Brain Technologies, Inst. Italiano di Tecnologia, Genoa, Italy; [5]Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan

Abstract: The LIM (Lin-11/Isl-1/Mec-3)-domain-containing protein kinase 1 (LIMK1) is a serine/threonine kinase that controls actin cytoskeleton dynamics through the phosphorylation and the inactivation of the actin-depolymerizing factors (ADF)/cofilin. Actin is the major cytoskeletal protein in most cells, playing a critical role in many cellular morphofunctional mechanisms. In dendritic spines, LIMK1 translates Rho GTPase signals in response to neuronal activity, driving the expansion of actin cytoskeleton and dendritic spine enlargements. Protein analogs that can be allosterically controlled by safe unnatural cues offer an unprecedented opportunity to investigate causal links between biochemical signalling and neuronal functions. Here, we developed a novel engineered, chemically activatable LIMK1 allowing inducible phosphorylation of cofilin in living cells. Our approach is based on a safe and well-tolerated clinically approved agonist such as rapamycin that i) crosses the blood-brain barrier, ii) activates

the engineered proteins and iii) has well-known beneficial effects on cognition allowing a potential synergic beneficial effect for future translational applications. Engineered LIMK1 maintained the ability to form complexes with its binding partners, and its activation in organotypic hippocampal CA1 pyramidal neurons induced a long-term enlargement of dendritic spines. Interestingly, in transfected neurons, engineered LIMK1 activation boosted the glutamatergic synaptic transmission. Finally, we introduced our engineered LIMK1 into hippocampal neurons of mice exhibiting age-dependent memory decline, and we found that direct manipulation of cofilin dynamics in aged mice leads to enhanced recognition memory, thus suggesting that actin polymerization in dendritic spines positively impacts on memory ability. The engineered memory by an extrinsically disordered LIMK1 supports a direct causal link between actin-mediated synaptic transmission and memory. Moreover, our results showed that targeting LIMK1 may be a promising therapeutic strategy for improving glutamatergic synaptic function in brain disorders, where dendritic spine density and neuronal communication are reduced.

Disclosures: **C. Ripoli:** None. **O. Dagliyan:** None. **P. Renna:** None. **F. Pastore:** None. **F. Paciello:** None. **R. Sollazzo:** None. **M. Rinaudo:** None. **M. Battistoni:** None. **S. Martini:** None. **A. Tramutola:** None. **A. Sattin:** None. **E. Barone:** None. **T. Saneyoshi:** None. **T. Fellin:** None. **Y. Hayashi:** None. **C. Grassi:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.11/D9

Topic: B.05. Synaptic Plasticity

Support: CIHR Foundation Grant #154276

Title: The role of Glycogen Synthase Kinase-3 paralogs in synaptic plasticity and cognitive functions

Authors: *S. KANG[1,3], F. JIN[3], J. WANG[3], J. R. WOODGETT[3,2], J. GEORGIOU[3,4], G. L. COLLINGRIDGE[1,3,4];
[1]Physiol., [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [3]Lunenfeld-Tanenbaum Res. Institute, Mount Sinai Hosp., Toronto, ON, Canada; [4]TANZ Ctr. for Res. in Neurodegenerative Diseases, Univ. of Toronto, Toronto, ON, Canada

Abstract: Glycogen synthase kinase-3 (GSK-3), a serine/threonine kinase, regulates many cellular functions by phosphorylating >100 proteins. GSK-3 exists as two paralogs, GSK-3α and GSK-3β. Despite their structural similarity, they have non-redundant roles in brain function that are yet to be fully determined. GSK-3 plays a pivotal role in synaptic plasticity. Long-term potentiation (LTP) is one type of plasticity involving a persistent increase in the strength of synaptic connections. Previous work from our lab showed that conditional knockout (cKO),

using the *CamKII-cre* deleter line of GSK-3α, but not β, leads to enhanced LTP in adult mice (Ebrahim Amini et al., 2022). In the current study, we carried out *in vivo* behavioural testing to evaluate cognitive function in the cKOs. We have also started on a pharmacological approach to complement our findings. To assess cognitive function, GSK-3α or β floxed mice were crossed with the *CamKII-cre* deleter line which deletes the floxed gene in forebrain neurons cells starting at 4 weeks of age. Both male and female mice were used. Notably, neither GSK-3α nor β cKOs exhibited alterations in locomotor activity or anxiety compared to littermate floxed controls. In pilot results from the puzzle box test, both GSK-3α and β cKO mice showed enhanced executive function exhibiting shorter times to solve the given task compared to the floxed control group (Latency to escape in seconds; control = 65±8, GSK-3α = 38±5, GSK-3β = 29±4, n=12-26). Impaired novel-object recognition memory was observed in both GSK-3α and β cKO mice (Discrimination Index: control = 0.65±0.05 ($p<0.05$*), GSK-3α = 0.52±0.05 (ns) and GSK-3β =0.37±0.09 (ns), n=8-13; one-sample *t*-test to determine whether each group explored the novel object more than chance (0.50)). We also prepared acute hippocampal slices from male and female C57BL6/J mice, for CA3-CA1 field electrophysiology, and applied BRD0705 (GSK-3α preferring) or BRD3731 (GSK-3β preferring), paralog-selective inhibitors of GSK-3. We found ~25% enhanced LTP with the GSK-3α selective inhibitor compared to the vehicle, but only when we used a spaced theta-burst stimulation (sTBS) protocol that induces a form of LTP, known as LTP2, involving the action of PKA and *de novo* protein synthesis. The results suggest inhibition of GSK-3α has the potential to enhance long-term learning and memory. In conclusion, our study provides evidence for the distinct and non-redundant roles of GSK-3α and GSK-3β in regulating LTP and cognitive function.

Disclosures: S. Kang: None. **F. Jin:** None. **J. Wang:** None. **J.R. Woodgett:** None. **J. Georgiou:** None. **G.L. Collingridge:** None.

Poster

PSTR455. LTP and LTD: Protein Phosphorylation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR455.12/D10

Topic: B.05. Synaptic Plasticity

Support: ARC Project Grant (DP190101390)

Title: Phosphorylation of the GluN2B subunit at Ser-1480 controls activity-induced degradation of NMDA receptors

Authors: *S. SIVA DAS, J. TAN, X. YONG, N. BHEMBRE, V. ANGGONO;
Queensland Brain Inst., Brisbane, Australia

Abstract: N-methyl-D-aspartate (NMDA)-type glutamate receptors (NMDARs) mediate the flux of calcium into the postsynaptic compartment, which subsequently triggers the activation of various signalling cascades that underpin multiple forms of synaptic plasticity, learning and

memory. We have recently reported that the GluN2A-containing NMDARs undergo a rapid insertion into the plasma membrane during synaptic potentiation (Yong et al., *Cell Reports*, 2021). However, the fate of GluN2B-containing receptors remains unknown. Using the CRISPR-Cas-9-mediated labelling technique, we found that the surface and total expressions of endogenously labelled GFP-GluN2B were significantly down-regulated in the late phase of synaptic potentiation. Biochemical analysis revealed that GluN2B-NMDARs undergo lysosomal degradation, concomitant with the increase in the phosphorylation of GluN2B at Ser-1480. Furthermore, the expression of GluN2B-S1480A phospho-deficient mutant inhibits glycine-induced degradation of GluN2B containing NMDARs in primary hippocampal neurons. Our results demonstrate that the phosphorylation of GluN2B at Ser-1480 controls the post-endocytic removal of GluN2B-NMDARs, which may have implications for the structural and functional plasticity of synapses.

Disclosures: S. Siva Das: None. **J. Tan:** None. **X. Yong:** None. **N. Bhembre:** None. **V. Anggono:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.01/Web Only

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 5T32NS061788-13
NIH Grant R01NS064025
NIH Grant R01NS105438
NIH Grant R01NS113948

Title: T-type Ca^{2+} channels mediate a critical period of LTP in adult-born granule cells

Authors: *W. M. KENNEDY[1,2], H. LEE[2], J. I. WADICHE[2], L. S. OVERSTREET-WADICHE[2];
[1]Neurobio., UAB Grad. Biomed. Sci., Birmingham, AL; [2]Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: Adult-born granule cells (abGCs) exhibit a transient period of elevated synaptic plasticity that is thought to play a role in hippocampal function. Several mechanisms have been proposed to underlie this critical period of plasticity, including minimal GABAergic synaptic inhibition (Wang et al., 2000), expression of NR2B-containing NMDA receptors (Snyder et al., 2001; Ge et al., 2007), and high intrinsic excitability conferred by T-type Ca^{2+} channels (Schmidt-Hieber et al., 2004). Here we assessed the contribution of inhibition and intrinsic excitability to long-term potentiation (LTP) of synaptic transmission in abGCs using perforated patch recordings. We used adult *Ascl1-Cre^{ER}* mice to identify abGCs during the closing of the critical period at 4-, 6-, and 8-weeks after tamoxifen induction. To induce LTP, we applied a

theta-burst stimulation paradigm to the perforant path while pairing postsynaptic depolarization sufficient to generate a spike. We show that intact synaptic inhibition does not alter the timing of the critical period, with elevated LTP in 4 and 6 week-old GCs compared to 8 week-old and unlabeled mature GCs. Furthermore, blockade of $GABA_A$ receptors enhances LTP to a similar degree in 4 week-old and unlabeled mature GCs, suggesting differential inhibition does not substantially contribute to critical period plasticity. However, the closure of the critical period coincides with a reduction in the contribution of T-type Ca^{2+} channels to intrinsic excitability and blockade of T-type Ca^{2+} channels prevented LTP in 4 week-old abGCs, with no effect on LTP in mature GCs. During theta burst stimulation, T-type Ca^{2+} channels boost NMDAR-mediated synaptic depolarization in 4 week-old abGCs with no effect on NMDAR-mediated depolarization in mature GCs. Together these results suggest that T-type Ca^{2+} channels enhance excitability and promote NMDAR activation to allow LTP induction during the critical period despite strong $GABA_A$ receptor mediated inhibition.

Disclosures: W.M. Kennedy: None. **H. Lee:** None. **J.I. Wadiche:** None. **L.S. Overstreet-Wadiche:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.02/D11

Topic: B.05. Synaptic Plasticity

Support: NRF-2022R1C1C2008960

Title: Stress responsiveness shapes the induction of synaptic plasticity in the lateral habenula

Authors: *H. PARK, C. CHUNG;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: Exposure to acute stress leads to abnormal activation of the lateral habenula (LHb), an epithalamic area important for stress responses. We previously observed dynamic changes in the synaptic efficacy of the LHb with great heterogeneity: a brief, strong theta burst stimulation (TBS) caused moderate long-term potentiation (LTP) only in a subpopulation of LHb neurons. Stress exposure significantly facilitated the induction of LTP in LHb synapses. Given that stress selectively activates certain LHb neurons, and the magnitude of potentiation varies among recorded neurons, we investigated whether stress-responsive neurons exhibit different responses to TBS compared to neurons that are not directly activated by stress. Interestingly, TBS in naïve mice led to a slight decrease in synaptic responses, likely through the activation of $GABA_B$ receptors. Using viral delivery of c-fos-tTA and TRE-EYFP, we successfully labeled stress-responsive neurons within the LHb and recorded excitatory postsynaptic currents (EPSCs) exclusively from these neurons. Upon TBS, the EPSC amplitudes of non-responsive neurons significantly decreased, while those of stress-responsive LHb neurons remained unchanged.

Although the specific molecular or physiological characteristics that determine stress responsiveness are still unknown, our findings suggest that stress-responsive neurons are more readily potentiated or resistant to decreases in response to stimulation. This study, together with previous studies, unveils another mechanism contributing to the enhanced potentiation of the LHb following stress, offering valuable insights into understanding the hyperactivation of the LHb observed in animal models of depression.

Disclosures: H. Park: None. **C. Chung:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.03/D12

Topic: B.05. Synaptic Plasticity

Support: R01 MH123212
R21 AG063193

Title: Tracking plasticity of millions of synapses induced by learning and memory in vivo

Authors: *G. I. COSTE[1], A. R. GRAVES[1,2,3], Z. CHEN[2], T. LI[2], Y. T. XU[1,3], D. E. BERGLES[1,3], J. SULAM[2,3], A. S. CHARLES[2,3], R. L. HUGANIR[1,3];
[1]Neurosci., [2]Biomed. Engin., [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Synaptic plasticity is a central molecular mechanism underlying learning and memory, wherein functional connections between specific neural circuits are dynamically tuned by regulating the expression of AMPA-type glutamate receptors. While over 50 years of research has revealed the biochemical and physiological mechanisms of plasticity, a systems-level understanding of how these changes are distributed within dense synaptic networks to represent memory remains unclear. Visualization of the strength of synapses in vivo has proven difficult, due to their small size and high density. To overcome these obstacles, we have fluorescently tagged endogenous AMPA receptors and performed longitudinal in vivo imaging to directly observe changes in synaptic strength following different forms of learning and memory. We developed a machine-learning algorithm capable of resolving millions of diffraction-limited synapses and reliably tracking their strength at single-synapse resolution. By imaging synaptic networks in behaving animals, we found that learning-induced synaptic potentiation in the retrosplenial cortex is sustained over weeks. Furthermore, the magnitude of potentiation one week after learning was correlated with memory strength, indicating a role of sustained synaptic potentiation in memory storage. Overall, this technique illuminates how learning and memory are encoded in the cortex as changes in AMPA receptor expression within millions of synapses.

Disclosures: G.I. Coste: None. **A.R. Graves:** None. **Z. Chen:** None. **T. Li:** None. **Y.T. Xu:** None. **D.E. Bergles:** None. **J. Sulam:** None. **A.S. Charles:** None. **R.L. Huganir:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.04/D13

Topic: B.05. Synaptic Plasticity

Support: NIH-R15DA049260
BYU College of Life Sciences Undergraduate Research Awards

Title: Ketogenic diet impact on long-term potentiation in the dorsal CA1 hippocampal region in young and adult rodents

Authors: J. R. CHRISTENSEN[1], ***M. P. DEW**[2], E. SAITO[3], B. REED[2], A. EVERETT[2], J. WEIGHT[2], N. VALENTINE[3], C. KEMBERLING[3], B. T. BIKMAN[3], J. G. EDWARDS[3]; [1]Biophysics, [2]Neurosci., [3]Cell Biol. and Physiol., Brigham Young Univ., Provo, UT

Abstract: The ketogenic diet (KD) has gained notoriety over the last few decades, originally for its potential to treat epilepsy. In recent years, the diet has resurged as a weight loss aid, though its effects on the neurological system are not completely understood. We examined the cognitive effects of the KD on behavior and synaptic plasticity, employing CA1 hippocampal long-term potentiation (LTP) as a measure in young (3-8 weeks) Sprague-Dawley rats, as well as young (2-8 weeks) and adult (7 months) C57 mice. For each of these groups, two treatment methods were employed including a 3-4 week high lipid diet to increase ketone bodies *in vivo*, or bathing hippocampal slices in a controlled amount of ketone beta-hydroxybutyrate (BHB)-enriched artificial cerebrospinal fluid (ACSF) to produce a higher concentration of ketones than was produced in rodents *in vivo*. Rodents on the lipid diet only reach ~2mM levels of blood ketones on this diet, less than what can be attained in humans. To ensure scientific rigor researchers were blinded as to which treatment group they were analyzing. Experiments were conducted using field electrophysiology. In both young and adult animals, there were no statistically significant differences in LTP between animals on KD and animals on a control diet. However, in 3-8 week old female rats, we noted that those exposed to 7.5 mM BHB with 2.5 mM glucose for >2 hours demonstrated significantly ($p<0.05$) increased LTP (190 ± 13%; n=13) compared to controls of 0 mM BHB and 11 mM glucose (150 ± 9%; n=13). In contrast, in trials of slices from 2-8 week old mice, we did not observe a difference in LTP between slices exposed to 7.5 mM BHB and 2.5 mM glucose for >2 hours (n=13) compared to controls of 0 mM BHB and 11 mM glucose (n=15). There were no statistically significant differences in the magnitude of LTP between slices from young male and female mice that were exposed to BHB or control ACSF. We plan to repeat BHB-enriched ACSF experiments in adult mice. Additionally, in experiments involving young mice given 3-4 weeks of the KD, behavioral Morris water maze experiments, which involve training an animal to find a submerged platform and test spatial memory, showed no significant ($p>0.05$) difference in time to platform or time in correct quadrant comparing mice treated with the high-fat diet chow (n=11) compared to control chow (n=12). We are currently analyzing Morris water maze data for adult mice given 3-4 weeks of the KD. Overall, our data

suggest examining KD for impact on neurological function such as LTP and memory behavior warrants further investigation.

Disclosures: J.R. Christensen: None. **M.P. Dew:** None. **E. Saito:** None. **B. Reed:** None. **A. Everett:** None. **J. Weight:** None. **N. Valentine:** None. **C. Kemberling:** None. **B.T. Bikman:** None. **J.G. Edwards:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.05/D14

Topic: B.05. Synaptic Plasticity

Support: NIH Grant EY025922

Title: Adrenergic gating of a rapid disconnection of fast-spiking cell circuits in the visual cortex

Authors: *D. SEVERIN[1], C. MORENO[2], C. WESSELBORG[3], S. MEHTA[4], R. LEE[2], A. TANG[4], A. KIRKWOOD[4];
[2]Mind/Brain Inst., [3]CMDB, [4]Neurosci., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Disinhibition is an obligatory initial step in remodeling cortical circuits by sensory experience. Previous investigations on disinhibitory mechanisms in the classical model of ocular dominance plasticity uncovered an unexpected novel form of experience-dependent circuit plasticity. In layer 2/3 of the mouse visual cortex, monocular deprivation (MD) triggers a complete “all-or-none” elimination of connections from pyramidal cells onto nearby parvalbumin-positive interneurons (Pyr→PV). This binary form of circuit plasticity is unique as the remaining connections are unaffected in strength. We aimed to examine the mechanism underlying the “all-or-none” Pyr→PV disconnection, testing some of the elements described for spike timing LTD induction in PV cells. In PV cells, α1-adrenergic receptors enable spike timing LTD independently of the induction mechanism and depending on mGluR5 receptors. Using a combination of standard slices electrophysiology method, optogenetics, and whole-cell recordings from PV and pyramidal cells, we evaluated the modifiability of local excitatory inputs originating from layer 2/3. We found that mGluR5 is necessary for the MD-evoked Pyr→PV disconnection. Also, α1-adrenergic receptor activation, in-vivo and in slices, in conjunction with pre- and post-synaptic activation, enable “all-or-none” elimination of excitatory input onto PV cells. We propose that the α1-adrenergic dependent Pyr→PV disconnection resembles the MD-evoked synapse elimination and mechanistically works according to the pull-push rule.

Disclosures: D. Severin: None. **C. Moreno:** None. **C. Wesselborg:** None. **S. Mehta:** None. **R. Lee:** None. **A. Tang:** None. **A. Kirkwood:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.06/D15

Topic: B.05. Synaptic Plasticity

Support: NSERC RGPIN-2016-05538
CIHR FRN-162179

Title: Distinct roles for ionotropic and non-ionotropic NMDA receptor-mediated signaling in synaptic depotentiation in the rodent hippocampus

Authors: *Q. PAULI[1,2], R. P. BONIN[1,2,3];
[2]Leslie Dan Fac. of Pharm., [3]Univ. of Toronto Ctr. for the Study of Pain, [1]Univ. of Toronto, Toronto, ON, Canada

Abstract: Background: Memories can be modified or forgotten to adapt to changing information. During memory formation, hippocampal synapses undergo long-term potentiation (LTP). Reflecting the dynamic nature of memory, LTP is susceptible to modification post-induction. There is evidence that weakening of potentiated synapses, or synaptic depotentiation, underlies forgetting. However, certain memories are resistant to change. Despite the relevance of depotentiation to adaptive and maladaptive forgetting, the mechanisms gating LTP modification are largely unknown. The N-methyl-D-aspartate receptor (NMDAR) is critically involved in bidirectional changes in synaptic strength by recruiting distinct signaling pathways via ion flux-dependent and -independent mechanisms. Importantly, our lab has shown that non-ionotropic NMDAR (NI-NMDAR) mediated signaling is necessary for depotentiation in the spinal cord (Zhang et al., 2023). Therefore, the present study aimed to test its role in modulating hippocampal LTP. **Methods**: Electrophysiological recordings were used to monitor field excitatory postsynaptic potentials (fEPSPs) at CA3-CA1 synapses in acute hippocampal slices obtained from 7-12-week-old male and female C57Bl/6N mice. LTP was induced using either a spaced or compressed theta burst stimulation (sTBS, cTBS) and subsequently reversed with a 2 Hz low frequency stimulation (LFS). To assess the role of NI-NMDAR signaling in depotentiation, antagonists targeting either the glutamate or glycine binding site of the receptor were bath applied during the LFS. Glutamate site antagonists (APV) were used to prevent receptor activation, whereas glycine site antagonists (7-CK, L689,560) enabled receptor activation while blocking ion flux. **Results**: In agreement with previous studies (Park et al., 2019), we demonstrated that LTP induced with an sTBS, compared to a cTBS, is more resistant to depotentiation. Our preliminary results in male mice also indicate that LTP induced by a cTBS is reduced in the presence of glycine, but not glutamate, site antagonists. Notably, glycine site antagonists appear to block depotentiation of LTP induced by an sTBS. All data was analyzed using paired t-tests comparing the average fEPSP slope prior to and following the LFS (n=5-12 slices/group, drug and vehicle control trials interleaved). Future experiments will test for sex differences in LTP modification. **Conclusions**: Our results reflect a potential dissociation between ionotropic and non-ionotropic NMDAR signaling in different types of synaptic

depotentiation. This confirmatory study will provide novel insights on the synaptic mechanisms of forgetting.

Disclosures: Q. Pauli: None. **R.P. Bonin:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.07/D16

Topic: B.05. Synaptic Plasticity

Support: CIHR PG 156223
NSERC DG 2017-04730
Healthy Brains Healthy Lives
Fonds de Recherche du Québec
Marie Skłodowska-Curie Actions
PID2021-125875OB-I00
SBPLY/21/180501/000064
2023-GRIN-34187
FRSQ CB 254033
CFI LOF 28331
NSERC DAS 2017-507818

Title: Unconventional NMDAR signalling in neocortical synaptic plasticity

Authors: *S. RANNIO[1], V. LI[1], J. BROCK[1], A. THOMAZEAU[2], R. LUJAN[3], P. SJOSTROM[1];
[1]McGill Univ., Montreal, QC, Canada; [2]The Res. Inst. of the McGill Univ. Hlth. Ctr., Montreal, QC, Canada; [3]Crib-Facultad De Medicina, Univ. Castilla-La Mancha, 02006 Albacete, Spain

Abstract: In the textbook view, the NMDA receptor is portrayed as a postsynaptic ionotropic (postNMDAR) coincidence detector in Hebbian plasticity. However, unconventional presynaptic NMDARs (preNMDARs) have been found at primary visual cortex (V1) layer 5 (L5) pyramidal cell (PC)→PC synapses. These preNMDARs signal ionotropically via RIM1αβ to boost high-frequency evoked release, but non-ionotropically via JNK2 to control spontaneous release, independently of frequency. Since preNMDARs govern timing-dependent long-term depression (tLTD) independently of frequency, we hypothesised that this signalling is also non-ionotropic. Using genetic deletion, we also experimentally tested the specific contributions of pre- vs. postNMDARs in synaptic release and plasticity.
To explore NMDAR localisation at V1 L5 PC→PC synapses, we used immunogold electron microscopy in P21 C57BL/6 mice. We found GluN1, 2A and 2B at either side of the synapse, but more 2B presynaptically and more 2A postsynaptically ($p < 0.001$). We injected AAV9-eSYN-mCherry-iCre in neonatal $NR1^{flox/flox}$ mice to sparsely delete NMDARs and used

quadruple patch in P10-19 acute slices to find PC→PC pairs lacking pre- or postsynaptic NMDARs (i.e., preKO or postKO). MNI-NMDA uncaging confirmed absence of NMDARs (postKO 0.3 pA ± 0.4 pA, n=16 vs. control -26.7 pA ± 4.7 pA, n=28, $p < 0.001$). AP5 wash-in did not reduce EPSP amplitude or alter paired-pulse ratio (PPR) in preKO pairs (EPSP 118% ± 12%, n = 6; ΔPPR 0.04 ± 0.13, n = 5), unlike in postKO or control (pooled EPSP 49% ± 8%, n = 10, $p < 0.001$; ΔPPR 0.82 ± 0.26, n = 9, $p < 0.05$), showing that pre- but not postNMDARs regulate release. Furthermore, tLTD was abolished in pairs with preKO or pre- and postKO deletion (96% ± 5%, n = 12), but not in postKO or control (77% ± 5%, n = 10, $p < 0.01$). The JNK2 inhibitor SP600125 blocked tLTD (96% ± 2%, n = 9 vs. tLTD 62% ± 4%, n = 15, $p < 0.001$), whereas homozygous RIM1αβ deletion did not (65% ± 7%, n = 10 vs. tLTD, $p = 0.74$), consistent with non-ionotropic NMDAR signalling. Similarly, neither MK-801 (75% ± 6%, n= 7, $p < 0.01$) nor 7-CK (73% ± 5 %, n = 9, $p < 0.001$) affected tLTD. In agreement with the sparse NMDAR deletion, a JNK2-blocking peptide abolished tLTD when loaded pre- but not postsynaptically (pre and both 59% ± 6, n = 13 vs. post and no 97% ± 5%, n = 8, $p < 0.001$). We will next explore how pre- and postKO impact timing-dependent long-term potentiation as well as PC morphology.
In summary, at V1 L5 PC→PC synapses, pre- but not postNMDARs control release and tLTD. Additionally, preNMDARs signal non-ionotropically in neocortical tLTD.

Disclosures: **S. Rannio:** None. **V. Li:** None. **J. Brock:** None. **A. Thomazeau:** None. **R. Lujan:** None. **P. Sjostrom:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.08/D17

Topic: B.05. Synaptic Plasticity

Support: NIH Grant 5R01MH108342-08

Title: Endogenous neuropeptide Y release attenuates long-term potentiation in the temporoammonic pathway of hippocampus

Authors: Q. LI, ***A. F. BARTLEY**, L. E. DOBRUNZ;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Neuropeptide Y (NPY) has emerged as an important mediator of stress, neuroplasticity and memory processes. However, far less is known about how endogenous NPY release affects long-term synaptic plasticity. The temporoammonic (TA) pathway onto hippocampal CA1 pyramidal cells mediates aspects of learning and memory. Additionally, endogenous release of NPY in response to a physiologically based spike train (PST) modulates short-term plasticity at TA synapses. A PST protocol can induce long-term potentiation (LTP), a primary mechanism underlying learning and memory, at Schaffer collateral synapses. However,

it is unknown if this can occur in the TA pathway. We find that PST stimulation induces robust LTP at TA synapses and is NMDA receptor dependent. However, the use of electrical stimulation alone was not sufficient to consistently generate NPY release in the TA pathway. To improve the efficiency of NPY release, we used NPY Cre/ChR2 mice that express channelrhodopsin 2 in NPY interneurons; this enables us to directly activate NPY cells using photostimulation to cause NPY release. To consistently test the effects of endogenously released NPY on PST induced LTP, we used combined electrical stimulation of the TA pathway and optical stimulation of NPY cells applied simultaneously during the PST protocol. Endogenous release of NPY does not prevent LTP, but it attenuates LTP at TA synapses since blocking the NPY receptors (Y1 and Y2) enhanced the magnitude of LTP. This is the first demonstration of NPY released by physiologically relevant stimulation to modulate LTP. One way that NPY release could reduce the magnitude of LTP is by concurrently inducing long-term depression (LTD). However, photostimulation of NPY interneurons paired with low frequency stimulation of TA synapses did not cause LTD but did cause short-term depression. This study is the first to demonstrate the impact of endogenously released NPY on long-term plasticity in the TA pathway. Together, these data provide a potential link between NPY's effects on synaptic function and its role in regulating hippocampal-dependent behaviors.

Disclosures: **Q. Li:** None. **A.F. Bartley:** None. **L.E. Dobrunz:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.09/D18

Topic: B.05. Synaptic Plasticity

Support: Fellowship 725800 (LAM) from Consejo Nacional de Ciencia y Tecnología, México.

Title: Postnatal dysregulation of glutamatergic transmission mediated by NMDA receptors alters the role of Group I mGlu receptors in the induction of long-term depression at the medial perforant path - granule cell synapse

Authors: *L. A. MÁRQUEZ[1], C. LOPEZ-RUBALCAVA[2], E. GALVAN[2];
[1]Farmacobiología, Ctr. for Res. and Advanced Studies of the Natl. Polytechnic Inst., Mexico City, Mexico; [2]Farmacobiología, Ctr. for Res. and Advanved Studies of the Natl. Polytechnical Inst., Mexico City, Mexico

Abstract: Transient hypofunction of NMDA during postnatal brain development mimics neurophysiological and behavioral hallmarks of schizophrenia, a neuropsychiatric disorder in which the hippocampus and related structures are critically involved. Although evidence suggests that dysregulation of synaptic plasticity is associated with schizophrenia, we still lack information on how this condition affects long-term depression (LTD) that contribute to the

information transfer from the medial perforant path (MPP) to the hippocampus's dentate gyrus (DG) granule cells. Male pup rats were treated with MK-801 (0.2 mg/kg s.c.; P7-P11), and electrophysiological recordings were performed in acute hippocampal slices (P30-P37). In control slices, low-frequency stimulation (LFS) at 3 Hz (900 pulses) induced a persistent LTD that lasted up to 90 min and was sensitive to group II mGlu receptors agonist DCG-IV (5 μM), confirming the MPP origin. In contrast, LFS in MK-801-treated slices induced a transient synaptic depression followed by abnormal synaptic potentiation. We also explored the contribution of group I mGlu receptors in the induction of LTD. Whereas perfusion of mGlu5 receptor antagonist MPEP (10 μM) during LFS induced a weak but persistent LTD, perfusion of mGlu1 receptor antagonist LY-367385 (100 μM) paired with LFS induced transient depression, suggesting a predominant role of mGluR1 in the induction of LTD. Next, we examined the effects of the group I mGluR agonist, DHPG (50 μM), on synaptic transmission. In control slices, DHPG (15 min) induced a stable synaptic depression. By contrast, MK-801-treated slices exhibited weak depression. Together, these results indicate that a decreased functional expression of group I mGlu receptors, probably mGlu1 receptor, contributes to the failure in the induction of LTD in the MPP-DG synapse triggered by neonatal blockade of NMDA receptors. Consequently, this plastic dysregulation in the information transfer from the MPP to DG may contribute to the altered encoding of spatial information associated with psychiatric disorders such as schizophrenia.

Disclosures: L.A. Márquez: None. **C. Lopez-Rubalcava:** None. **E. Galvan:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.10

Topic: B.05. Synaptic Plasticity

Support: NIH/NIAAA grant R01 AA027214
NIH grant UL1TR002529

Title: A novel inhibitory corticostriatal circuit that expresses mu opioid receptor-mediated synaptic plasticity

Authors: *B. MUÑOZ[1], B. K. ATWOOD[1,2];
[1]Pharmacol. and Toxicology, [2]Stark Neurosci. Res. Institure, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Corticostriatal circuits are generally characterized by the release of glutamate neurotransmitter from cortical terminals within the striatum. It is well known that cortical excitatory input to the dorsal striatum regulates addictive drug-related behaviors. We previously reported that anterior insular cortex (AIC) synaptic inputs to the dorsolateral striatum (DLS) control binge alcohol drinking in mice. These AIC-DLS glutamate synapses are also the sole

sites of corticostriatal mu opioid receptor-mediated excitatory long-term depression (MOR-LTD) in the DLS. Recent work demonstrates that some regions of the cortex send long-range, direct inhibitory inputs into the dorsal striatum. Nothing is known about the existence and regulation of AIC-DLS inhibitory synaptic transmission. Here, using a combination of patch clamp electrophysiology and optogenetics, we characterized a novel AIC-DLS corticostriatal inhibitory circuit and its regulation by MOR-mediated inhibitory LTD (MOR-iLTD). First, we found that the activation of presynaptic MORs produces MOR-iLTD in the DLS and dorsomedial striatum. Then, we showed that medium spiny neurons within the DLS receive direct inhibitory synaptic input from the
cortex, specifically from the motor cortex and AIC. Using transgenic mice that express cre-recombinase within parvalbumin-expressing inhibitory neurons, we determined that this specific cortical neuron subtype sends direct GABAergic projections to the DLS. Moreover, these AIC-DLS inhibitory synaptic input subtypes express MOR-iLTD. These data suggest a novel GABAergic corticostriatal circuit that could be involved in the regulation of drug and alcohol consumption-related behaviors.

Disclosures: B. Muñoz: None. **B.K. Atwood:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.11

Topic: B.05. Synaptic Plasticity

Support: Health Research Council (18/245) & (22/177)
New Zealand International Doctoral Scholarship
Brain Health Research Center's Roche Hanns Möhler Doctoral Scholarship

Title: Astrocyte-mediated inter-regional metaplasticity in the hippocampus

Authors: *S. SATEESH, C. ABRAHAM;
Psychology, Univ. of Otago, Dunedin, New Zealand

Abstract: The synaptic plasticity process of long-term potentiation (LTP) is vital for memory formation and overall neural health. However, mechanisms must be in place to prevent pathologically excessive LTP. Such regulation comes partly through metaplasticity, whereby neural activity at one point in time influences later plasticity. We have discovered a unique long-range mode of metaplasticity in the hippocampus, whereby "priming" (2x100Hz) stimulation of axons running stratum oriens in area CA1 selectively inhibits later LTP (4x100 Hz) at not only synapses in stratum radiatum, as previously reported but also in the middle molecular layer (MML) of the dentate gyrus. In contrast to the other forms of metaplasticity, this effect in MML is independent of postsynaptic signaling such as AMPAR, NMDAR, or GABAR activity.

Additionally, there are no known excitatory neuronal connections between CA1 and the dentate gyrus. On the other hand, there is an astrocytic network known to cross the hippocampal fissure. Based on this, we hypothesized that the metaplasticity is in fact, accomplished via intercellular communication with the astrocytic network. To directly test the involvement of astrocytes, we undertook intracellular astrocyte patch clamping and extracellular field potential recordings in the MML of acute hippocampal slices taken from young-adult male Sprague-Dawley rats and 2-7-month-old mice. In rat slices, Ca^{2+} was buffered in individual patched astrocytes by dialyzing EGTA intracellularly while recording local synaptic potentials in MML in the presence of glycine. Priming stimulation in CA1 was delivered 15 min prior to MML LTP induction (4x100 Hz trains). Priming inhibited MML LTP compared to non-primed control (One-way ANOVA, $t_{(21)} = 3.2$, $p = 0.0127$) while buffering astrocytic Ca^{2+} abolished this inhibitory effect on LTP ($t_{(21)} = 0.39$, $p > 0.99$). We further confirmed the involvement of astrocytes by a lack of LTP inhibition in both male and female inositol triphosphate receptor-2 (IP3R2)-knockout mice that do not display intracellular Ca^{2+} release from astrocyte-specific IP3R2-dependent stores ($t_{(12)} = 2.2$, $p=0.052$). Finally, we found that the glial cytokine tumor necrosis factor-alpha (TNFα), acting on TNFα receptor-1, and glutamate acting on GluN2BRs, are critical signaling gliotransmitter molecules. Together, these data demonstrate a novel hippocampus-wide regulation of synaptic plasticity mediated by astrocyte-neuron communication. We propose that such metaplasticity may play an important role in hippocampal information processing while also homeostatically counteracting excitotoxicity under extreme conditions.

Disclosures: S. Sateesh: None. **C. Abraham:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.12/D19

Topic: B.05. Synaptic Plasticity

Support: NIH-R35 GM124824

Title: Regulation of hippocampal long-term depression and learning and memory by a novel endosomal proton activated chloride (PAC) channel

Authors: *K. CHEN, J. YANG, B. LIU, R. HUGANIR, Z. QIU;
Johns Hopkins Univ., Baltimore, MD

Abstract: Proper endosomal homeostasis is essential for the trafficking of AMPA receptors (AMPAR) during synaptic plasticity, a core neuronal process underlying complex behaviors such as learning and memory. Endosomal pH in neurons is a key component of endosomal and neuronal health. Indeed, mutations in sodium proton exchangers (NHEs) and chloride transporters (CLCs), endosomal ion transporters that regulate pH, are both implicated in human and mouse neurological disorders. We have recently identified and characterized the proton

activated chloride (PAC) channel and its role in regulating endosomal acidification in cell lines. Based on this information, and that PAC is highly expressed in the central nervous system, we hypothesized that PAC regulates activity-dependent synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD). To test this, we first confirmed the localization of PAC using immunofluorescence in primary hippocampal neurons and found that PAC traffics to early and recycling endosomes, vesicles that supply AMPARs for synaptic plasticity. We then used electrophysiological patch clamp recordings to determine the functional role of PAC in neurons. Interestingly, we observed significant impairments in LTD in acute hippocampal slices from 5-week-old male and female neuron-specific PAC conditional knockout (cKO) mice (n=7 mice per genotype). To assess the physiological consequences of impaired synaptic plasticity in PAC cKO mice, we conducted several behavioral tests on male PAC cKO mice aged 2-5 months that assess learning and memory. Consistent with the electrophysiological data, PAC cKO mice performed poorly in the Morris Water Maze behavioral assay (n=13-14 per genotype). Finally, to explore cellular mechanisms of PAC in synaptic plasticity, we used a well-established NMDA-induced chemical LTD protocol in primary cortical neurons. NMDA treatment induces an expected 20% decrease in surface AMPAR subunit GluA2 in control neurons treated with scramble shRNA. Notably, acute knockdown of PAC using PAC-specific shRNA abolished the internalization of GluA2 during chemical LTD. Based on these results, we propose a novel role for the endosomal chloride channel and pH sensor, PAC, in regulating synaptic plasticity.

Disclosures: **K. Chen:** None. **J. Yang:** None. **B. Liu:** None. **R. Huganir:** None. **Z. Qiu:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.13/D20

Topic: B.05. Synaptic Plasticity

Support: CONACYT Grant 1010748

Title: Repeated fentanyl administration dysregulates synaptic strength and causes neuroinflammation in the hippocampal CA1 region in rats

Authors: *G. ROCHA-BOTELLO[1], S. L. CRUZ[1], E. J. GALVAN[2];
[1]Pharmacobiology, Ctr. de Investigación y Estudios Avanzados, Ciudad de México, Mexico;
[2]CIE, CINVESTAV, Ciudad de México, Mexico

Abstract: Fentanyl, a potent opioid analgesic and anesthetic, is responsible for the major opioid crisis worldwide. Despite its relevance in pain management, there is scant evidence regarding the effects of fentanyl on synaptic transmission and synaptic plasticity in the central nervous system. Here, we explore the impact of repeated fentanyl administration on rats' synaptic transmission of the hippocampal CA1 region. Male Sprague Dawley rats (200-250 g) received three daily

intraperitoneal injections of fentanyl (0.1 mg/kg) for seven days until completing 19 injections. Two hours after the last dose, brain slices from the dorsal hippocampus were obtained for extracellular recordings or immunofluorescence assays. In the first case, we recorded CA1 population spikes (PS) and field excitatory postsynaptic potentials (fEPSPs). In the second case, we analyzed the immunoreactivity of microglia (CD11b), astrocytes (GFAP), the NLRP3 inflammasome, and neurons (NeuN). Compared to control slices, fentanyl significantly decreased both PS amplitude and fEPSP slope, suggesting decreased somatic excitability and synaptic strength, respectively. Theta-burst stimulation of Schaffer collaterals failed to induce robust long-term potentiation (LTP), suggesting a deterring capability of the glutamatergic synapses to express LTP. Analysis of the paired-pulse ratio showed increased facilitation and decreased GABAergic inhibition, indicating altered presynaptic control of glutamate release and decreased GABAergic inhibition, respectively. On the other hand, fentanyl increased GFAP and neuronal NLRP3 expression, suggesting a neuroinflammatory process that might be involved in the dysregulation of synaptic transmission. In addition to the well-known problems caused by fentanyl misuse, our data show that chronic exposure to this opioid affects the synaptic strength and synaptic plasticity of CA1, causing a disbalance in both glutamatergic and GABAergic transmission that may hinder the cognitive capabilities in which CA1 takes part.

Disclosures: **G. Rocha-Botello:** Other; CONACYT - Becas Nacional 2021-2 CVU 1010748. **S.L. Cruz:** None. **E.J. Galvan:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.14/D21

Topic: B.05. Synaptic Plasticity

Support: R01 plasticity

Title: Arc mediates a novel form of intercellular long-term depression

Authors: *K. SULLIVAN, A. RAVENS, J. EINSTEIN, M. HANTAK, T. SHEPHERD, J. SHEPHERD;
Univ. of Utah, Salt Lake City, UT

Abstract: We recently discovered that *Arc*, a neuronal gene required for synaptic plasticity and memory evolved from an ancient retrotransposon. Arc protein has retained the viral-like properties of the ancestral retrotransposon and is capable of self-assembling into virus-like capsids that encapsulate genetic material. Arc capsids can be transferred cell-to-cell in extracellular vesicles (EVs) and deliver nucleic acids, similar to retroviruses. These findings suggest that evolution exploited viral machinery to facilitate memory formation. However, the function of Arc mediated intercellular communication and the role of virus-like capsids in memory formation is unclear. Here we show that Arc capsid assembly and release occurs during

long-term potentiation (LTP) through direct interaction with the I-BAR protein IRSp53. Using time-lapse imaging we observed colocalization and anterograde trafficking of Arc and IRSp53 puncta in dendrites that are released from neurons during LTP. In addition, biochemically purified IRSp53 protein facilitates Arc capsid assembly *in vitro*. These results suggest that IRSp53 assembles Arc capsids that are released during LTP to mediate intercellular signaling. To determine whether released Arc can regulate synaptic function in recipient neurons, we sparsely transfected Arc or GFP in primary cultured Arc KO neurons. Untransfected dendrites neighboring Arc transfected neurons had low levels of Arc transfer while GFP transfer was not observed. In dendrites where Arc transfer occurred, there was a reduction in surface AMPA receptors (GluA1). In addition, neurons incubated with Arc containing EVs have lower surface GluA1 levels. Whereas EVs from Arc KO neurons had no effect.
Together, our results show that Arc mediates an intercellular form of synaptic plasticity. Neurons that undergo high intensity neuronal activity induce LTP and expression of Arc that associates with IRSp53. IRSp53 acts as a molecular switch that facilitates Arc capsid assembly and release. Arc that is transferred in EVs induces synaptic depression in neighboring dendrites. We propose that neurons incorporated into the memory "engram" that undergo LTP release Arc to increase the "signal-to-noise" of memory circuits by weakening synapses on neurons not active during memory encoding.

Disclosures: **K. Sullivan:** None. **A. Ravens:** None. **J. Einstein:** None. **M. Hantak:** None. **T. Shepherd:** None. **J. Shepherd:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.15/D22

Topic: B.05. Synaptic Plasticity

Support: NIH R01 HL142129

Title: Genetic deletion of inflammatory transcription factor STAT4 protects from the effects of a high-fat/cholesterol/carbohydrate diet on activity-dependent, long-term potentiation (LTP)

Authors: *C. M. HOLLANDER[1], X.-L. ZHANG[1], M. D'SILVA[2], M. Y. KHAN[2], H. MA[1], K. X. YANG[1], J. L. NADLER[2], P. K. STANTON[1];
[1]Cell Biol., [2]Pharmacol., New York Med. Col., Valhalla, NY

Abstract: Signal transducer and activator of transcription 4 (STAT4) is an inflammatory transcription factor activated by various cytokines that mediates inflammatory signaling and development of T helper 1 cells. A high fat/cholesterol/carbohydrate (DDC) diet, which elicits STAT4 activation in response to interleukin-12, leads to increased inflammation, atherosclerosis, and a diabetic metabolic phenotype. These conditions have been identified as risk factors for diseases related to memory impairment and cognitive decline, such as Alzheimer's disease and

vascular dementia, which are also associated with heightened chronic inflammation. However, the role of STAT4 activation in cognitive decline and memory impairment is unknown, raising the following questions: Does STAT4 activation impair long-term, activity-dependent potentiation (LTP) of synaptic plasticity, resulting in memory deficits? If so, can the genetic deletion of STAT4 prevent these memory deficits? To address these questions, we measured the LTP of synaptic transmission, a candidate memory storage mechanism, at Schaeffer's collateral-CA1 synapses in acute hippocampal slices from two strains of mice fed either a normal chow diet or a DDC diet. One strain was a transgenic STAT4 knockout (LDLr-/-STAT4flox LysMCre), which has reduced STAT4 expression under control of the LysMCre promoter. The other strain was littermate controls (LDLr-/-STAT4flox/flox) of the same background, with normal STAT4 expression. When mice were fed a normal chow diet, both strains exhibited similar profiles of paired-pulse facilitation (PPF), basal synaptic transmission and LTP at 3 and 24 weeks of age. However, when subjected to a 16-week DDC diet (from 8 to 24 weeks), the magnitude of LTP was significantly reduced in LDLr-/-STAT4flox/flox control mice, while basal synaptic transmission and PPF remained unaltered. LDLr-/-STAT4flox LysMCre mice that lacked STAT4 were protected from the effects of the DDC diet on LTP, exhibiting magnitudes of LTP levels that did not differ from control mice with normal STAT4 expression. These findings suggest an essential role for STAT4 activation in the impairments of synaptic plasticity and cognitive function induced by a DDC diet. Suppressing STAT4 activation could mitigate aging-associated inflammation, protect against the cognitive and diabetic effects of a DDC diet, and reduce the risk of developing Alzheimer's disease, vascular dementia and other diseases associated with cognitive decline and memory impairments.

Disclosures: C.M. Hollander: None. **X. Zhang:** None. **M. D'silva:** None. **M.Y. Khan:** None. **H. Ma:** None. **K.X. Yang:** None. **J.L. Nadler:** None. **P.K. Stanton:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.16/D23

Topic: B.05. Synaptic Plasticity

Support: NIH Grant R15 MH129932-
Ramapo College Faculty Development Fund

Title: Sex differences in endocannabinoid-mediated synaptic plasticity in the adolescent rat hippocampus

Authors: *C. G. REICH[1], G. AGRAPIDIS[2], K. ALVAREZ[3], M. SOOY[1];
[1]Neurosci., [2]Psychology/Neuroscience, [3]Neuroscience/Biology, Ramapo Col. of New Jersey, Mahwah, NJ

Abstract: Work in our lab demonstrated a robust sex difference of rat CB1 receptor function at hippocampal dendritic GABAergic synapses, whereby female CB1 displays a higher sensitivity to exogenous activation compared to males (Ferraro et al., 2020). Exploring the mechanisms underlying this difference, we observe a mixture of constitutive CB1 activity, tonic eCB production and estrogen-mediated eCB production at adolescent female dendritic synapses. This is consistent with the literature on perisomatic eCB signaling in females. Importantly, these effects are not reported in similar studies of eCB signaling in males. We hypothesize that this sexual divergence across the somatodendritic axis in adolescent hippocampal pyramidal cells translates into differences in eCB-mediated synaptic plasticity. We initially explored potential sex differences in long-term-potentiation (LTP) induction. Field excitatory post-synaptic potentials (fEPSPs) were recorded from CA1 in male and female Sprague-Dawley rat (40-55 days old) hippocampal slices*. Following a 10 min baseline, two weak (normally non-LTP inducing) stimulations (30 Hz, 0.5 sec and 50 Hz, 0.5 sec) were applied with 25 min between each stimulation. Then, a stronger LTP-inducing stimulation (100 Hz x 2, 0.5 sec apart) was applied and responses were allowed 30 min to recover. For drug-based experiments, slices were pre-incubated (>1 hr.) with the drug, which was bath applied throughout the experiment. All sample sizes were n= 5 or greater, the size needed for 80% statistical power. We observed significant sex differences in LTP induction thresholds. During the 30 Hz stimulation, male slices exhibited ~115% increase in response magnitude, whereas female slices increased ~150% over baseline values. Male slices required the 100 Hz stimulation to reach response magnitudes of 150% over baseline values. In female slices, blocking CB1 receptors with AM251 (3 µM) impaired LTP induction with 30 Hz stimulation. In male slices, enhancing tonic levels of 2-AG via inhibition of MAG-lipase (JZL184, 100 nM) enhanced LTP induction with 30 Hz stimulation to ~140% compared to baseline levels. The CB1-dependent lower LTP thresholds in female slices is consistent with enhanced CB1-mediated suppression of GABAergic neurotransmission, thus resulting in greater excitatory drive in female CA1. Furthermore, the decrease in male LTP threshold via an increase in 2-AG tone suggests a lower excitatory drive in male CA1. Our preliminary data support the hypothesis that sex differences in tonic eCB activity are observed in eCB-mediated synaptic plasticity.

Disclosures: **C.G. Reich:** None. **G. Agrapidis:** None. **K. Alvarez:** None. **M. Sooy:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.17/D24

Topic: B.05. Synaptic Plasticity

Support: NIH Grant NS111986

Title: Differential roles of anandamide and 2-AG in hippocampal long-term depression

Authors: F. LEMTIRI-CHLIEH, *E. LEVINE;
Univ. of Connecticut Sch. of Medi Neurosci. Grad. Program, Farmington, CT

Abstract: It is widely accepted that exogenous cannabinoids can impair short-term memory and cognition in humans and other animals. This is likely related to the disruption of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), by the global and sustained activation of CB1 cannabinoid receptors by exogenous agonists. Conversely, the temporally and spatially-restricted release of endogenous cannabinoid ligands may mediate or enhance synaptic plasticity in a synapse-specific manner. The functional roles of endocannabinoids (eCBs) are complex because they can modulate synaptic transmission via suppression of GABA and glutamate release, with opposing effects on postsynaptic excitability. We examined the role of eCB signaling in LTD by recording fEPSPs in the CA1 stratum radiatum in hippocampal slices from juvenile mice. LTD was induced by 1 Hz paired-pulse stimulation of the Schaffer collaterals or by brief exposure to the metabotropic glutamate receptor agonist DHPG. Significant LTD (~50% decrease from baseline) could be induced by either 15 min of 1 Hz electrical stmulation or 10 min of exposure to DHPG. The magnitude of both forms of LTD was significantly reduced by blocking cannabinoid receptor activation with the CB1 receptor antagonist NESS-0327. The roles of the endogenous ligands 2-AG and anandamide were examined by using selective inhibtors of DAG-lipase and NAPE-PLD, respectively. Electrical stimulation-induced LTD was significantly reduced by the NAPE-PLD inhibitor LEI-401, but was not affected by the DAG-lipase inhibitor DO34. DO34, however, significantly reduced DHPG-induced LTD. These results indicate that both stimulation-induced LTD and DHPG-induced LTD require activation of CB1 receptors. Interestingly, the endogenous cannabinoid anandamide is required for sitmulation-induced LTD. 2-AG does not appear to contribute to stimulation-induced LTD, but is required for DHPG-induced LTD.

Disclosures: F. Lemtiri-Chlieh: None. **E. Levine:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.18/D25

Topic: B.05. Synaptic Plasticity

Support: LSRF Postdoctoral Fellowship
NIH Grant NS106031

Title: Behavioral timescale synaptic plasticity in adult mouse cortex

Authors: *C. E. YAEGER, R. MOJICA SOTO-ALBORS, M. HALGREN, V. TANG, M. T. HARNETT;
McGovern Inst. for Brain Research, Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: The predominant model of synaptic plasticity for the cortex—spike-timing dependent plasticity—is unable to account for learning that happens after a single experience or when there is an appreciable delay between a sensory experience and an associated consequence. Single-shot synaptic potentiation driven by dendritic plateau potentials occurs in hippocampus at behaviorally relevant timescales, but it is unclear if this plasticity mechanism is also utilized in cortex. Using in vivo whole cell patch-clamp and Neuropixels electrophysiology, we observed frequent plateau potentials (~50-500 ms duration) and high-frequency burst firing (2-10 spikes at ~80-200 Hz) in L5 neurons of mouse primary visual cortex during active visual processing. We applied these patterns in ex vivo acute slices of adult mouse visual cortex: pairing plateau depolarizations with synaptic stimulation induced rapid long-term potentiation of synapses on L5b pyramidal neurons. Synaptic potentiation occurred after only five pairings and exhibited surprisingly broad pre- and post-synaptic timing intervals of up to two seconds. NMDA receptors were required for potentiation, and single spike trains of similar number and frequency without underlying subthreshold depolarization did not induce potentiation. Plateau-driven plasticity was also input-specific: it effectively potentiated intracortical inputs to basal dendrites but was ineffective for thalamic inputs to proximal apical oblique dendrites. These results demonstrate a mechanism for reshaping neocortical representations in only a few experiences over a behavioral timescale, where the timing of inputs over seconds reflects the level of synaptic weight change. Our findings may represent a biological implementation of supervised, continuous learning in cortical sensory neurons that contribute to adaptive behavior by enhancing perception of salient objects without disrupting underlying sensory function.

Disclosures: C.E. Yaeger: None. **R. Mojica Soto-Albors:** None. **M. Halgren:** None. **V. Tang:** None. **M.T. Harnett:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.19/D26

Topic: B.05. Synaptic Plasticity

Support:
Australian Research Council (ARC) Future Fellowship (FT220100485)
National Honor Scientist Program (NRF-2012R1A3A1050385)
University of Queensland (UQ) Amplify award
ARC Discovery Early Career Researcher Award (DE170100112)
Australian Medical Research Future Fund (Clem Jones Centre for Ageing Dementia Research Flagship Project Grant)
John T. Reid Charitable Trusts
Ian Lindenmayer PhD Top-up Scholarship
UQ International Scholarship

Title: Ubiquitination of the GluA1 subunit of AMPA receptors is required for synaptic plasticity, memory and cognitive flexibility

Authors: S. GUNTUPALLI[1,2], P. PARK[3], D. HAN[3], L. ZHANG[1,2], ***X. YONG**[1,2], M. RINGUET[1,2], D. G. BLACKMORE[1,2], D. J. JHAVERI[1,4], F. KOENTGEN[5], J. WIDAGDO[1,2], B.-K. KAANG[3], V. ANGGONO[1,2];
[1]The Univ. of Queensland, Queensland Brain Inst., Queensland, St Lucia, Australia; [2]Clem Jones Ctr. for Ageing Dementia Research, The Univ. of Queensland, Brisbane, Queensland, Australia; [3]Sch. of Biol. Sciences, Seoul Natl. Univ., Seoul, Korea, Republic of; [4]Mater Res. Institute, The Univ. of Queensland, Brisbane, Queensland, Australia; [5]Ozgene Pty Ltd, Bentley DC, Western Australia, Australia

Abstract: Activity-dependent changes in the number of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors (AMPARs) at the synapse underpin the expression of long-term potentiation (LTP) and long-term depression (LTD), cellular correlates of learning and memory. Post-translational ubiquitination has emerged as a key regulator of the trafficking and surface expression of AMPARs, with ubiquitination of the GluA1 subunit at Lys-868 controlling the post-endocytic sorting of the receptors into the late endosome for degradation, thereby regulating their stability at synapses. However, the physiological significance of GluA1 ubiquitination remains unknown. In this study, we generated mice with a knock-in mutation in the major GluA1 ubiquitination site (K868R) to investigate the role of GluA1 ubiquitination in synaptic plasticity, learning and memory. Our results reveal that these male mice have normal basal synaptic transmission but exhibit enhanced LTP and deficits in LTD. They also display deficits in short-term spatial memory and cognitive flexibility. These findings underscore the critical roles of GluA1 ubiquitination in bidirectional synaptic plasticity and cognition in male mice.

Disclosures: S. Guntupalli: None. **P. Park:** None. **D. Han:** None. **L. Zhang:** None. **X. Yong:** None. **M. Ringuet:** None. **D.G. Blackmore:** None. **D.J. Jhaveri:** None. **F. Koentgen:** None. **J. Widagdo:** None. **B. Kaang:** None. **V. Anggono:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.20/D27

Topic: B.05. Synaptic Plasticity

Support: F31MH132297-01
T32GM008181-33
T32NS063391-15

Title: Transclaustral circuit strength is attenuated by serotonin

Authors: *M. MADDEN[1], B. N. MATHUR[2];
[1]Pharmacol., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Cognitive flexibility deficits are a major contributor to diminished life and therapeutic outcomes across myriad neuropsychiatric disorders including Alzheimer's, depression, and schizophrenia. The claustrum, a subcortical nucleus, connects frontal cortical and parietal cortical network nodes and is required for optimal performance in cognitively demanding tasks. Administration of the classical psychedelic and broad serotonin receptor agonist, psilocybin, acutely depresses claustrum activity, disrupts functional connectivity between the claustrum and cortical networks, and produces long lasting enhancement of cognitive flexibility in humans. How serotonin may influence the claustrum is unknown. We hypothesize that serotonin receptor signaling suppresses frontal cortical input to claustrum. To test this, we employed whole-cell patch-clamp electrophysiology and pharmacology in mouse claustrum slices to assess serotonin receptor subtypes involved in modulation of frontal cortical inputs to claustrum projection neurons. These data reveal a serotonin receptor mediated depression of frontal input to claustrum via serotonin receptor 1b, which produces a functional suppression of a frontal to parietal cortical trans-claustral circuit.

Disclosures: M. Madden: None. **B.N. Mathur:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.21/D28

Topic: B.05. Synaptic Plasticity

Support: NIAAA grant R01 016022
NIDA grant R01 038890
NIDA R01 056113
George Mason University Presidential Scholarship
George Mason University Dissertation Completion Grant

Title: Effect of estrus and estrogen receptor activity on LTP in the dorsomedial striatum

Authors: *V. LEWITUS[1], K. T. BLACKWELL[2];
[2]Interdisciplinary Program in Neurosci., [1]George Mason Univ., Fairfax, VA

Abstract: Estradiol, a female sex hormone and the predominant form of estrogen, has diverse effects throughout the brain including in learning and memory. Estradiol modulates several types of learning that depend on the dorsomedial striatum (DMS), a subregion of the basal ganglia involved in goal-directed learning, cued action-selection, and motor skills. A cellular basis of learning is synaptic plasticity, and the presence of extranuclear estradiol receptors ERα, ERβ, and G protein-coupled estrogen receptor (GPER) throughout the DMS suggests that estradiol may influence rapid cellular actions including those involved in plasticity. To test whether estradiol affects synaptic plasticity in the DMS, corticostriatal long-term potentiation (LTP) was induced using theta-burst stimulation in *ex vivo* brain slices from intact male and female

C57BL/6 mice. Extracellular field recordings showed that female mice in the diestrus stage of the estrous cycle exhibited LTP similar to male mice, while female mice in estrus did not exhibit LTP. Furthermore, antagonists of ERα or GPER rescued LTP in estrus females and agonists of ERα or GPER reduced LTP in diestrus females. In males, activating ERα but not GPER reduced LTP. These results uncover an inhibitory action of estradiol receptors on cellular learning in the DMS and suggest a cellular mechanism underlying the impairment in certain types of DMS-based learning observed in the presence of high estradiol. Due to the dorsal striatum's role in drug addiction, these findings may provide a mechanism underlying an estradiol-mediated progression from goal-directed to habitual drug use.

Disclosures: V. Lewitus: None. **K.T. Blackwell:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.22/D29

Topic: B.05. Synaptic Plasticity

Support: NIH R01MH117130
NIH R21MH116315
NIH R01NS062736
NIH T32GM099608
NIH T32MH082174
Walter Benjamin project 468470832
NIH RF1AG06749

Title: D-serine inhibits non-ionotropic NMDA receptor signaling

Authors: E. V. BARRAGAN[1], M. ANISIMOVA[1], **A. F. NISAN**[1], V. VIJAYAKUMAR[2], R. J. SALAKA[1], K. B. DORE[2], K. ZITO[1], *J. A. GRAY[3,1];
[1]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA; [2]UCSD, UCSD Dept. of Neurosciences, La Jolla, CA; [3]UC Davis, Davis, CA

Abstract: NMDA-type glutamate receptors (NMDARs) are master regulators of synaptic plasticity, the long-term changes in synapse size and strength in response to learning. NMDARs are unique among neurotransmitter receptors in that, in addition to glutamate binding, they also require simultaneous binding of a co-agonist, which can be either glycine or D-serine, for the receptor cation channel to open. Over the past decade however, there has been an increasing appreciation that NMDARs can signal in an ion flux-independent (non-ionotropic) manner upon the binding of glutamate, providing an opportunity to reassess the fundamental role of NMDAR co-agonism. Notably, recent studies have demonstrated that glutamate binding to NMDARs during competitive antagonism of the co-agonist site drives both LTD and spine shrinkage, suggesting that NMDAR co-agonists might play a direct role in regulating non-ionotropic

signaling. To test this hypothesis, we manipulated co-agonist availability in mouse hippocampal slices during the induction of synaptic plasticity using pharmacological and enzymatic scavenging approaches. We found that scavenging endogenous co-agonists facilitates non-ionotropic NMDAR-mediated LTD independent of induction frequency. Surprisingly, we also found that a saturating concentration of D-serine inhibits both non-ionotropic NMDAR-mediated LTD and spine shrinkage. Additionally, using a FRET-based assay in cultured neurons, we found that D-serine completely blocked NMDA-induced conformational movements of the GluN1 cytoplasmic domains supporting an inhibition of non-ionotropic signaling. These results suggest that the developmental increase in D-serine levels at forebrain synapses may act to limit non-ionotropic NMDAR signaling, thereby enhancing synapse stabilization.

Disclosures: E.V. Barragan: None. **M. Anisimova:** None. **A.F. Nisan:** None. **V. Vijayakumar:** None. **R.J. Salaka:** None. **K.B. Dore:** None. **K. Zito:** None. **J.A. Gray:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.23/D30

Topic: B.05. Synaptic Plasticity

Title: Sex differences in mechanisms mediating long-term potentiation at Hippocampus-Nucleus Accumbens synapses

Authors: *A. COPENHAVER[1], T. LEGATES[2,3];
[1]UMBC, Halethorpe, MD; [2]UMBC, Baltimore, MD; [3]Dept. of Physiol., Univ. of Maryland, Sch. of Med., Baltimore, MD

Abstract: Substantial sex differences have been observed in motivated behaviors, yet the neurobiological basis for these differences remains unclear. Motivated behaviors are mediated by the nucleus accumbens (NAc), a key node in the reward system that integrates excitatory and inhibitory information from numerous sources. Excitatory input from the hippocampus (Hipp) drives NAc activity, and the strength of Hipp-NAc medium spiny neuron (MSN) synapses is a critical mediator of reward-related behaviors. Long-term potentiation (LTP) at this synapse in male mice is dependent upon NMDA receptors, a postsynaptic rise in calcium, and CAMKII, and interestingly occurs independently of dopamine receptor activation. We have recently discovered that female mice display similar LTP at Hipp-MSN synapses, but the mechanism underlying LTP in females is NMDA receptor independent which raises important questions regarding how distinct mechanisms may be used to achieve LTP in males and females. Using whole-cell electrophysiology and pharmacology, we have begun characterizing the mechanisms responsible for mediating plasticity at Hipp-NAc synapses in males and females. Our results have elucidated sex-specific molecular mechanisms for LTP in this integral excitatory pathway that mediates motivated behaviors. Continued characterization of sex-specific plasticity changes will provide novel insight into the neuronal mechanism that may explain the widely observed sex

differences in motivated behaviors with significant implications for how we understand brain function in males and females.

Disclosures: A. Copenhaver: None. **T. LeGates:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.24/D31

Topic: B.05. Synaptic Plasticity

Support: Wellcome Trust/DBT India Alliance grant IA/I/12/1/500529
DST-INSPIRE Fellowship 2016/IF60611

Title: Acetylcholine transforms synaptic dynamics for efficient learning during active exploration

Authors: *R. SHARMA, S. NADKARNI;
Biol., IISER-PUNE, Pune, India

Abstract: Acetylcholine is a widely studied neuromodulator critical for memory formation and retrieval. We explore how acetylcholine modulates synaptic activity at the CA3-CA1 synapses of the hippocampus, a region specialized in spatial navigation and episodic memories. We first developed a detailed biophysical to incorporate the effects of M1 and M4 acetylcholine receptors prevalent on the postsynaptic and presynaptic terminals, respectively. Activating M1 receptors in the postsynaptic terminal enhances excitability via complex pathways, including SK channels, IP3 receptors, and Calcium. In contrast, the M4 receptors suppress neurotransmitter release by reducing calcium influx from VDCCs. Our computational model elucidates the strategic interplay of the seemingly contradictory action of M1 and M4 receptors. The M4-mediated reduction in release rate stretches the limited pool of available vesicles and acts as a band-pass filter.The enhanced postsynaptic response by M1 ensures that each released vesicle results in a maximal response. We asked how this collaborative interaction between M1 and M4 impacts learning in response to ongoing activity during navigation. Place cell fires when an animal traverses a particular region of space (place field). The firing pattern of these cells shows a wide but distinct range of rates modulated by the theta-rhythm. We use place cell data reported from rats/mice during active exploration to stimulate synaptic activity and plasticity at Schaffer-collateral (SC) synapses. Our simulations reveal that synaptic plasticity is pronounced selectively around the firing rates of these place cells. Our results suggest that acetylcholine enhances the potentiation because of amplified calcium signaling during a stimulus. This improves firing rate discrimination to encode neuronal activity in synaptic weights better. At the same time, activating both M1 and M4 receptors makes the sensitivity of synaptic plasticity peak at the firing rates most commonly observed in these neurons. We show that acetylcholine transforms

synaptic dynamics for optimal function across various synaptic parameters in an energy-efficient manner.

Disclosures: R. Sharma: None. **S. Nadkarni:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.25/D32

Topic: B.05. Synaptic Plasticity

Support: NIDA P50DA44118
NIMH R01HD101642-02
ONR N00014-21-1-2940
T32 N5045540-19

Title: Microglia depletion disrupts endocannabinoid-dependent LTP within hippocampus

Authors: *J. CHAVEZ[1], J. QUINTANILLA[2], A. A. LE[2], A. MABOU TAGNE[2], D. PIOMELLI[2], G. LYNCH[3], C. M. GALL[2];
[1]Univ. of California, irvine, Irvine, CA; [2]Anat. and Neurobio., [3]Psychiatry and Human Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Microglia are the resident immune cells of the brain and are well-characterized for their involvement in synaptic refinement and responses to a range of insults. However, their involvement in the synaptic mechanisms necessary for learning and memory has not been defined. Motivated by evidence that adolescent cannabinoid (THC) exposure disrupts both microglial reactivity and endocannabinoid (eCB)-dependent synaptic plasticity, we tested if microglia are critical for activity-induced long-term potentiation (LTP) within four synaptic systems in hippocampus including the lateral and medial perforant path innervation of the dentate gyrus, the lateral perforant path innervation of CA3 str. moleculare, and CA3-CA1 innervation of stratum radiatum. In young adult male mice given chow containing Colony Stimulating Factor 1 receptor (CSF1R) antagonist PLX5622 for 10-21 days, numbers of Iba-1-ir microglial cells were reduced by over 90% in hippocampus. This robust microglial depletion did not significantly alter basal synaptic transmission (fEPSP amplitude, I/O curves), or frequency facilitation in response to gamma (40 Hz) stimulation for any of the systems evaluated. However, in PLX-treated mice, there was a selective impairment in LTP for the lateral perforant path (LPP) innervation of the dentate gyrus (i.e., LPP-LTP). Specifically, in response to a single train of high frequency stimulation (HFS), LPP responses were initially potentiated to a comparable degree in PLX- and control chow-treated mice, but in the PLX group responses decayed to near baseline values over the next 10 min (% LTP at 55-60 min post-HFS: Con: 147.7±6.8, PLX: 115.8±6.1; $p<0.003$). PLX treatment had no effect on LTP in the other axonal systems studied. In slices from control mice, PLX (1 μM) infusion did not influence LPP-LTP thereby indicating

that effects of the *in-vivo* treatment reflect microglial depletion and not direct effects of CSFR1 antagonism. We previously showed (Wang et al, 2018) that in Fmr1-KO (Fragile X model) mice impairments in LPP-LTP are offset by treatment with the monoacylglycerol lipase inhibitor JZL184, which increases levels of the eCB 2-AG required for LPP-LTP. Similarly, in slices from PLX-treated mice, JZL184 infusion restored robust LPP-LTP (150.3±3.6%). These results show microglial depletion does not disrupt presynaptic mechanisms that express LPP-LTP and, instead, suggest that the microglia are critical for the production or delivery of 2-AG at the LPP-DG synapse. We are currently evaluating the consequences of microglial depletion on forms of episodic memory that are known to depend on the LPP system.

Disclosures: **J. Chavez:** None. **J. Quintanilla:** None. **A.A. Le:** None. **A. Mabou Tagne:** None. **D. Piomelli:** None. **G. Lynch:** None. **C.M. Gall:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.26/D33

Topic: B.05. Synaptic Plasticity

Support: NIH grant R15DA038092
NIH grant R15DA049260
NIH grant AA020919

Title: Vta gaba neuron inhibitory plasticity type is input specific

Authors: ***B. WU**[1], T. M. NUFER[4], A. BEASLIN[1], H. UREY[1], J. G. EDWARDS[2], S. HOFFMAN[3];
[1]Cell, [2]PDBio, [3]Brigham Young Univ., Provo, UT; [4]Psychiatry, Univ. of Washington, MERIDIAN, ID

Abstract: The ventral tegmental area (VTA) is an essential component of the mesocorticolimbic dopamine (DA) circuit, as well as a primary target of addictive drugs. In response to drug exposure, synaptic connections of the VTA circuit can be rewired through synaptic plasticity, which is thought to be responsible for the pathology of drug dependence. In a recently published study, we observed that the VTA GABA neurons, which regulate VTA DA neuron activity, exhibit either inhibitory long-term potentiation (iLTP) or inhibitory long-term depression (iLTD) in response to a 5Hz stimulus, with iLTD being eliminated by chronic ethanol exposure. In our current study, using whole cell electrophysiology and optogenetics, we further explore the underlying mechanism of the observed duo plasticity phenomenon in the VTA GABA circuit. Since VTA GABA neurons receive GABAergic projections from both inside and outside the VTA, we hypothesize that plasticity type could be due to unique GABAergic input sources. To test this hypothesis, we optogenetically drove three different GABAergic inputs to the VTA, the Lateral Hypothalamus (LH), the rosotromedial tegmental nucleus (RMTg) and the VTA

interneurons. Activation of GABAergic LH and RMTg terminals induces iLTP in response to an optical 5Hz stimulus (iLTP, LH to VTA: P<0.0001 compared to baseline, ANOVA, n=14; iLTP, RMTg to VTA: p<0.0001 compared to baseline, ANOVA, n=6), while activation of the VTA interneurons produces iLTD with the same stimulus (iLTD, VTA to VTA, P<0.0001 compared to baseline, ANOVA, n=12). In addition, plasticity type could correspond to VTA GABA neuron subtypes. Therefore, we used single-cell PCR to examine the gene expression profiles of VTA GABA cell subtype markers to iLTP- and iLTD-exhibiting cells. In summary, our data support that different GABAergic inputs to VTA GABA neurons lead to distinct inhibitory plasticity type, which is likely why the duo plasticity phenomenon was observed.

Disclosures: B. Wu: None. **T.M. Nufer:** None. **A. Beaslin:** None. **H. Urey:** None. **J.G. Edwards:** None. **S. Hoffman:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.27/D34

Topic: B.05. Synaptic Plasticity

Title: Changes in GluA1 and VAMP2 Distribution in Postsynaptic Plasticity: Insights from Developmental Studies

Authors: *T. LANDAAS SKJERVOLD, S. DAVANGER;
Dept. of Mol. Med., Univ. of Oslo, Oslo, Norway

Abstract: Postsynaptic plasticity, a critical driver of neuronal development and function, involves dynamic processes like vesicle biogenesis and receptor recycling within the postsynaptic region. Glutamate receptor subunit composition at synapses strongly influences physiological effects in the postsynaptic spine. Recent studies shed light on AMPA receptors' dynamic behavior during long-term potentiation (LTP) and long-term depression (LTD), vital for synaptic strengthening and weakening. These receptors exhibit rapid movement between synaptic and extrasynaptic sites upon stimulation.While traditionally linked to the presynaptic active zone, emerging evidence suggests that SNARE proteins also contribute to regulating receptor composition in the postsynaptic spine. It is likely that SNARE proteins undergo alterations during chemical stimulation associated with LTP and LTD.In this study, we investigated changes in the distribution of the GluA1 subunit of AMPA receptors following chemical induction of LTP (forskolin) and LTD ((S)-3,5-Dihydroxyphenylglycine). We examined enriched synaptic fractions during postnatal development in the rat hippocampus, utilizing biotinylation of treated hippocampal slices. Our findings revealed a consistent decline in GluA1 levels across different developmental stages. Chemical LTP stimulation led to a direct increase in GluA1 levels within the postsynaptic density (PSD), while other compartments remained unaffected. Surprisingly, chemical LTD stimulation resulted in increased GluA1 concentrations within the PSD, accompanied by decreased lateral membrane concentration.

These observations imply that GluA1 can be incorporated into the PSD under both potentiating and depressing conditions, utilizing distinct trafficking mechanisms.Furthermore, we explored concentration expression of the vesicle-associated protein 2 (VAMP2, synaptobrevin 2) within the PSD. Intriguingly, chemically induced LTP elevated cytosolic VAMP2 concentration. Additionally, we observed reduced PSD expression of VAMP2 following both chemically induced LTP and LTD, indicating the involvement of vesicular machinery in synaptic plasticity.Our findings significantly contribute to our understanding of postsynaptic plasticity. Dysregulation of synaptic transmission and plasticity mechanisms is implicated in the pathophysiology of various neurological disorders that manifest early in life, and the observed alterations in the distribution of AMPA receptor subunits and dynamics of postsynaptic vesicles provide valuable insights into potential mechanisms underlying these diseases.

Disclosures: T. Landaas Skjervold: None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.28/D35

Topic: B.05. Synaptic Plasticity

Support: Ministry of Health (MOH-000641-01)
Ministry of Education Academic Research Fund Tier 3 (MOE2017-T3-1-002)

Title: Synapses in CA2 hippocampal neurons exhibit long-term depression with differing associative properties

Authors: *Z. WANG, L. WONG, S. SAJIKUMAR;
Physiol., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Recent studies revealing the involvement of CA2 hippocampal region on social memory have drawn scientific interest towards this area. In particular, the synaptic plasticity properties of CA2 neurons differ from other neuronal populations and are of great interest. However, studies on electrically-induced long-term depression (LTD), a cellular correlate for memory, in CA2 pyramidal neurons are lacking. In this study, we investigate the presence of, and subsequently characterize LTD in CA2 pyramidal neurons at both synapses with Schaffer collateral inputs (SC-CA2), and synapses with entorhinal cortical inputs (EC-CA2). We found that NMDA receptor-dependent LTD is present at both inputs. Late-LTD at both synapses are protein synthesis dependent and require p75 neurotrophin receptor (p75NTR). Strong LTD-inducing stimulus delivered to SC-CA2 causes subsequent early LTD-induction at EC-CA2 to exhibit late-LTD, but not in the other direction, demonstrating the presence of synaptic tagging and capture in the SC-CA2 to EC-CA2 direction. These results suggest that the two types of synapses could differ in terms of the plasticity-related products (PRPs) involved. Hence, we

applied inhibitors of candidate PRPs during the maintenance phase of LTD to ascertain their involvement at the two types of synapses. Application of ERK inhibitor U0126 and p38 MAPK inhibitor SB203580 had no effect on LTD maintenance in both synapses, and are unlikely to be the PRPs involved. Application of pro-BDNF chelator TrkB-fc prevented LTD maintenance in SC-CA2 synapses but not EC-CA2 synapses. Shotgun proteomics analyses are currently in progress to identify the PRPs involved in LTD maintenance common between both synapses to allow for the observed associativity in the SC-CA2 to EC-CA2 direction. These results reveal interesting differences in the mechanisms underlying CA2 synaptic plasticity, and should prove useful towards enhancing our mechanistic understanding of hippocampal circuitry involving the CA2, and consequently mechanisms for learning and memory.

Disclosures: Z. Wang: None. **L. Wong:** None. **S. Sajikumar:** None.

Poster

PSTR456. LTP and LTD: Pre- and Postsynaptic Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR456.29/D37

Topic: B.05. Synaptic Plasticity

Support: Ministry of Health MOH-000641-00
NUHS Seed Fund NUHSRO/2020/145/RO5+6/Seed-Sep/05
NUSMED-FOS Joint Research Programme
NUHSRO/2018/075/NUSMed-FoS/01

Title: Interrogating modulatory effects of CA2 on the persistence of CA1 plasticity in mice hippocampus

Authors: *M. BIN IBRAHIM, N. KOH, C. LIM, S. SAJIKUMAR;
Yong Loo Lin Sch. of Med., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: The hippocampus plays an integral role in episodic memory, particularly by neurons in CA1 subfield. Beyond the canonical circuitry is the CA2 region, implicated in social memory. There also exists monosynaptic connections from CA2 innervating CA1, which functional relevance is relatively unknown. We aimed to examine how the CA2-CA1 connections can modulate maintenance of functional plasticity models, such as long-term potentiation (LTP) and long-term depression (LTD) in the Schaffer collateral (SC)-CA1 synapses. Field electrophysiology of hippocampal slices from young male C57BL/6 mice was performed to measure synaptic modification over time. Subthreshold stimulation of SC-CA1 synapses exhibit early form of LTP (early-LTP) but not persistent late form of LTP (late-LTP). However, when "primed" by activation of CA2, SC-CA1 synapses exhibit protein synthesis-dependent late-LTP upon subthreshold stimulation within a temporal window. Moreover, CA2 "priming" does not perturb persistence of late forms of LTD when SC-CA1 synapses are stimulated by strong low-frequency stimulation. In fact, weak low-frequency stimulation can promote protein synthesis-

dependent late-LTD in CA2-"primed" SC-CA1 synapses. Lastly, we established a behavioural model where social novelty, which activates CA2, can lead to a persistence in CA1-dependent memory via the weak inhibitory avoidance task. Moving forward, we will integrate chemogenetics into the behavioural assay to ascertain the role of CA2 in memory enhancement. This set of results demonstrate that CA2 connections onto CA1 can influence the synaptic plasticity of CA1, suggesting possible implications of how social behavioural states can modulate persistence of memory.

Disclosures: **M. Bin Ibrahim:** None. **N. Koh:** None. **C. Lim:** None. **S. Sajikumar:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.01/D38

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Swartz Foundation
NIH Grant MH046742

Title: A Study on Activity-Dependent Regulation of Ion Channel Voltage Dependence in the Morris-Lecar Neuron

Authors: *Y. MONDAL[1], E. MARDER[2];
[1]Brandeis Univ., Waltham, MA; [2]Brandeis Univ., Brandeis Univ., WALTHAM, MA

Abstract: Neurons maintain activity profiles over their lifetimes, often years, despite facing environmental perturbations. Experimental studies suggest that neurons maintain their target activity profiles by regulating the number of ion channels they express and their voltage dependences in an activity-dependent way. However, computational studies of maintaining target activity profiles, historically, have focused on how neurons use only the former mechanism. In this study, we develop a mathematical model of the latter mechanism and study how it can be used to help achieve a specified activity profile. To do this, we augment Morris-Lecar neuron containing calcium and potassium currents with both activity-dependent ion channel regulatory mechanisms. Here, activity-dependent regulation of ion channel voltage dependence is implemented by shifting the half-activation of the potassium channel's activation curve. We find that when a target activity profile is specified, namely tonic firing, the ion channel regulatory mechanisms appear to converge to a particular Morris-Lecar neuron, determined by the number of potassium and calcium ion channels it possesses and the location of its potassium channel half-activation - regardless of how many potassium ion channels, calcium ion channels, and what voltage dependence the potassium channel was in the original Morris-Lecar neuron. We call this the "targeted model". We study how robust the targeted model is to environmental perturbations - modelled as changes in extracellular potassium ion concentration. We observe that the targeted model retains its tonic firing profile for a wider range of extracellular potassium ion

concentrations when the neuron is allowed to regulate both the number of ion channels and the voltage-dependence of potassium channels than when either regulation mechanism is present alone. We also examine what happens when environmental perturbations are modelled as current injections commensurate in magnitude to currents created by changes in extracellular potassium perturbations. This study suggests activity-dependent regulation of ion channel voltage-dependence plays a role in increasing the robustness of a neuron's chosen electrical activity profile.

Disclosures: **Y. Mondal:** None. **E. Marder:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.02/D39

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Canadian Institutes of Health Research.

Title: Effects of extracellular conditions on saltatory conduction: improving the double cable axon model.

Authors: *N. ABDOLLAHI[1], S. A. PRESCOTT[2];
[1]Inst. of Biomed. Engineering, Neurosciences and Mental Hlth., Univ. of Toronto, The Hosp. for Sick Children, Toronto, ON, Canada; [2]Neurosciences and Mental Health, Inst. of Biomed. Engineering, Dept. of Physiol., The Hosp. For Sick Children, Univ. of Toronto, Toronto, ON, Canada

Abstract: Myelinated axons propagate spikes efficiently by regenerating spikes at each node of Ranvier, a process known as saltatory conduction. Previous studies have shown that saltatory conduction is affected by the intrinsic properties of the axon, including morphological, passive electrical, and active electrical properties. Besides properties of the axon itself, saltatory conduction may also be affected by extracellular conditions, including its passive electrical properties. Indeed, neurons are routinely modeled with the assumption that extracellular space is connected to ground, resulting in infinite extracellular conductivity; in many cases (including in intact nerve), that assumption is invalid. By extension, transmembrane voltage is often calculated under the assumption that extracellular voltage is zero, but this too is inaccurate. How extracellular current flow under realistically resistive conditions affects spike propagation has been overlooked in previous studies. We found that finite extracellular conductivity affects conduction velocity; specifically, low extracellular conductivity along the fibers (as expected for axons surrounded by other myelinated fibers) speeds up conduction velocity. We explain this result by tracking the flow of current intracellularly and extracellularly. Moreover, this effect interacts with intrinsic axon properties, meaning certain intrinsic factors like myelin thickness can have a large or small effect on velocity depending on the extracellular resistivity. Overall,

our results demonstrate that the resistivity of the extracellular space impacts spike propagation and must be considered, especially when trying to gauge the importance of other, intrinsic factors. This research was supported by a Foundation Grant from the Canadian Institutes of Health Research.

Disclosures: N. Abdollahi: None. **S.A. Prescott:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.03/D40

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Human Brain Project (HBP SGA 3 / 1.15)

Title: Derivation of a protocol agnostic current clamp (PACC) description for the cross-analysis of diverse neuronal electrophysiological databases

Authors: *J. BALLBÉ Y SABATÉ[1], M. CALICE[1], M. GAJOWA[2], D. MARINAZZO[3], L. J. GRAHAM[1];
[1]Ctr. Giovanni Borelli - CNRS UMR 9010 - Univ. Paris Cité, 45 rue des Saints Pères, Paris 75006, France; [2]Dept. of Mol. and Cell Biol., Univ. of California, Berkeley, CA; [3]Dept. of Data Analysis, Ghent Univ., Ghent, Belgium

Abstract: Cellular models for biophysically mimetic network simulations draw on intracellular recordings of neurons under various configurations, most generally current clamp. Of particular interest is establishing functional electrophysiological types (e.g. regular-adapting, fast-spiking and bursting), and then referencing exemplars from these types to define model parameters. Data for this task can be found in public domain databases, often including recordings coupled with other information, for example transcriptomic or morphologic. To leverage this information across databases on the basis of the electrophysiology from diverse protocols, we describe a pipeline to extract protocol agnostic measures, requiring only that the data includes voltage responses to long (> = 500 milliseconds) current steps that reasonably capture a cell's dynamic range. The pipeline on a given cell consists of: 1) trace selection and pre-processing, 2) standard spike and firing measures on individual traces, 3) ensemble measures over all traces, e.g. adaptation of spike width, fAHP and frequency, based on spike index, response duration, and threshold dependence on prior hyperpolarization, as well as functional parameterization (threshold, gain, saturation, cut-off) of Hill-sigmoid I/O fits. These measures constitute a PACC vector, characteristic of a cell independent of its database. We apply the pipeline on databases including the Allen Institute Cell Types Database (primarily mouse V1, *in-vitro* (Teeter et al., 2018; Gouwens et al., 2019,2020)), the Lantyer database (mouse SS layer II/III, *in-vitro* (Lantyer et al., 2018)), and our own (cat, rat and mouse V1, *in-vivo*). Consistent with the assumption that neurons of a given type have membrane properties that scale with cell area, current-dependent

parameter variance is reduced after re-scaling by input resistance. This is particularly noticeable for comparing *in-vitro* to *in-vivo* recordings, and suggests that the impact of increased background synaptic activity *in-vivo* on the resting input resistance can account for certain differences with the *in-vitro* state. For our own database, which includes neurons recorded under both current clamp and dynamic clamp, the PACC vector is correlated with the impact of inhibition on firing as measured under dynamic clamp. This "Rosetta stone" mapping between fundamentally different configurations allows the prediction of conductance-dependent properties for neurons recorded exclusively under current clamp, whatever the data source.

Disclosures: J. Ballbé Y Sabaté: None. **M. Calice:** None. **M. Gajowa:** None. **D. Marinazzo:** None. **L.J. Graham:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.04/D41

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NSF-GRFP 138517

Title: Membrane mechanics dictate axonal morphology and function

Authors: *J. GRISWOLD[1], C. T. LEE[3], M. BONILLA-QUINTANA[4], R. PEPPER[1], S. RAYCHAUDHURI[1], P. RANGAMANI, 92093[3], S. WATANABE[2];
[2]Dept. of Cell Biol., [1]Johns Hopkins Univ., Baltimore, MD; [4]Mechanical and Aerospace Engin., [3]UCSD, La Jolla, CA

Abstract: Neurons are known for their intricate cellular morphology. Axons in particular are exceptionally long (100-1000 mm) and ultrathin (100 nm). Their cable-like morphology is essential for conduction of electrical signals, or action potentials, throughout the brain and body. Thus, it has been long assumed that axons are tubular structures with occasional synaptic varicosities. However, our work has challenged this assumption. Using high-pressure freezing to preserve membrane morphology for electron microscopy or super-resolution imaging of live neurons, we performed ultrastructural analysis of axons in Caenorhabditis elegans motor neurons, mouse hippocampal neurons, and human cortical neurons. We discovered that axons are not simple tubes but rather exhibit a pearls-on-a-string morphology through their entire length, with the pearls being ~250 nm and the strings ~70 nm in diameter. This morphology is reminiscent of membrane tubes undergoing tension-driven instability. Consistent with this notion, the pearled area becomes smaller when hyperosmotic solution is applied and larger when hypoosmotic solution is applied. Interestingly, pharmacological perturbation of the cytoskeleton did not greatly alter axon morphology, suggesting that membrane mechanics drives axon morphology. In further support of this, increasing the membrane fluidity by cholesterol depletion from the plasma membrane led to a shrinking of the pearled membrane regions. Functionally,

when axon morphology is altered with pharmacological cholesterol depletion, action potential velocity decreases. Similarly, neuronal stimulation that induces plasticity alters the pearled axon morphology. Our *in silico* modeling further supports our experimental data that membrane mechanics can cause pearled axon morphology and that pearled morphology greatly impacts action potential conductance. These data have revealed for the first time that axons are pearled not tubular, and that pearled axon morphology has an important functional role in neuronal activity and plasticity.

Disclosures: J. Griswold: None. **C.T. Lee:** None. **M. Bonilla-Quintana:** None. **R. Pepper:** None. **S. Raychaudhuri:** None. **P. Rangamani:** None. **S. Watanabe:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.05/D42

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH NINDS F31 NS129377 to J.P.M.
NIH NINDS R01 NS119977 to E.M.G.
March of Dimes Basil O'Connor Research Award to E.M.G.
Support of the Linse/Heckert Family to E.M.G.

Title: Targeted pharmacologic blockade of aberrant Na+ current in a human induced pluripotent stem cell-derived neuron model of SCN3A neurodevelopmental disorder

Authors: *J. P. MERCHANT[1,2], G. QU[6], J. C. CLATOT[6], S. T. PHAM[3], L. M. DEFLITCH[6], D. J. FREDERICK, Jr[4], X. ZHANG[6], J. LI[4], S. A. ANDERSON[4,7], E. M. GOLDBERG[1,5,6,7]; [1]Dept. of Neurosci., [2]Neurosci. Grad. Group, [3]Sch. of Arts and Sci., [4]Dept. of Psychiatry, [5]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [6]Div. of Neurology, Dept. of Pediatrics, [7]The Epilepsy NeuroGenetics Initiative, The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Variants in the gene *SCN3A*, which encodes the voltage-gated sodium (Na+) channel α subunit Nav1.3, are associated with *SCN3A* neurodevelopmental disorders (*SCN3A*-NDD), a spectrum that includes epilepsy and malformation of cortical development (MCD). *SCN3A* is highly expressed in the embryonic forebrain; however, it remains unclear how genetic variation in *SCN3A* leads to epilepsy and other pathology, in part due to a lack of advanced model systems available for study.
To investigate the mechanisms underlying *SCN3A*-NDD, we used CRISPR/Cas9 gene editing to modify a control human induced pluripotent stem cell (iPSC) line to express the recurrent pathogenic *de novo* heterozygous missense variant *SCN3A* c.2624T>C (p.Ile875Thr); we also generated an iPSC line from a patient harboring the same variant as well as a CRISPR-corrected isogenic control line. Using the Ngn2 rapid induction protocol, we generated glutamatergic forebrain-like neurons (iNeurons) and confirmed by RT-qPCR that Na+ channel subunit

transcript expression in iNeurons mirrors that seen in early human brain development. We then performed whole-cell patch-clamp recordings to determine the effect of the *SCN3A*-p.Ile875Thr variant on endogenous Na+ current and on cellular excitability of iNeurons. We found that iNeurons generated from both variant-expressing lines exhibited markedly increased slowly-inactivating/persistent Na+ current relative to control and corrected patient lines, which was partially but specifically blocked by the Nav1.3-selective antagonist ICA-121431. *SCN3A*-p.Ile875Thr iNeurons displayed a more hyperpolarized voltage threshold for action potential generation - consistent with increased persistent current - which was increased by ICA-121431 at sub-micromolar concentrations. A prominent subset of *SCN3A*-p.Ile875Thr iNeurons displayed irregular firing patterns with paroxysmal bursting and plateau-like potentials with transition to action potential failure. Paradoxically, ICA-121431 reversibly blocked these plateau-like potentials and led to an increase in maximal steady-state firing frequency in iNeurons exhibiting this feature. However, consistent with typical action as a Na+ channel blocker, ICA-121431 decreased excitability of control iNeurons. Our findings demonstrate that an iPSC-derived neuron system models the trajectory of Na+ channel expression in the developing brain and reveal a profound impact of the *SCN3A*-p.Ile875Thr variant on neuronal physiology, thus providing insight into the mechanistic underpinnings of *SCN3A*-NDD.

Disclosures: **J.P. Merchant:** None. **G. Qu:** None. **J.C. Clatot:** None. **S.T. Pham:** None. **L.M. DeFlitch:** None. **D.J. Frederick:** None. **X. Zhang:** None. **J. Li:** None. **S.A. Anderson:** None. **E.M. Goldberg:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.06/D43

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH/NIDA Grant 0255-B401

Title: Paradoxical induction of lateral habenula burst-firing by inhibitory synaptic inputs

Authors: *M. ISHIKAWA, J. WANG, P. J. KENNY;
Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The lateral habenula (LHb) is thought to play an important role in stress-related behavioral disorders such as major depression. LHb neurons show three major modes of intrinsic activity: silent, tonic, and bursting. Stress induces hyperexcitability of LHb neurons and increases their propensity to engage in burst-firing, which facilitates the expression of depressive-like behaviors. The control of LHb burst-firing by synaptic inputs is still not fully understood. Basal forebrain (BF) neurons project to the LHb, but their role in controlling LHb activity is poorly understood. In this study, we used optogenetic and conventional electrophysiological techniques to investigate synaptic mechanisms of action potential firing in

LHb neurons. We demonstrate that excitatory and inhibitory synaptic inputs from BF can modulate firing patterns of LHb neurons. Specifically, we found that glutamatergic inputs from BF can trigger tonic and bursts of action potentials in LHb neurons. Using acute pressure application of GABA, we found that GABA induces hyperpolarization of LHb neurons, which is followed by LHb burst-firing. Similarly, optically stimulating GABAergic inputs from BF hyperpolarized LHb neurons followed by "rebound" burst-firing. Strikingly, optical stimulating glutamatergic inputs from BF blocked GABA-induced LHb bursting activity. These findings suggest that excitatory and inhibitory synaptic inputs from BF exert remarkably complex control of LHb activity. Future studies will investigate whether stress disrupts synaptic inputs from BF to LHb to precipitate depression-related behavioral abnormalities.

Disclosures: M. Ishikawa: None. **J. Wang:** None. **P.J. Kenny:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.07/D44

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: R01AG066489

Title: Exploring locus coeruleus sex differences in using in vivo electrophysiology

Authors: *R. RAE[1], L. MCMAHON[2];
[1]Med. Univ. of South Carolina (MU Neurosci. Inst. - Grad., Charleston, SC; [2]Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: The Locus Coeruleus (LC) is a small nucleus of approximately 12,000 neurons per hemisphere in humans. Despite its small size, it produces over 90% of noradrenaline for the brain. Noradrenaline is involved in various functions related to arousal, including attention, learning, stress, fear, mood, and other fundamental processes, making the LC a crucial brain region. Literature shows the LC exhibits sexual dimorphism in its anatomy and during development. However, only one study has explored the physiologic impact of estrogen on LC neurons, demonstrating that estrogen application in vitro significantly reduced LC neuron firing. Not only does this raise questions of potential sex differences between males and females, but it also highlights the need to investigate how LC firing may change across the estrous cycle. The current study assesses single unit activity of noradrenergic locus coeruleus (LC) firing patterns in 4-month-old wild-type Fischer male and female rats. In vivo electrophysiology recordings with an Omniplex recording system (Plexon) under anesthesia are employed. Recordings measure neuronal activity under basal conditions and in response to 10mA footshock stimulation which elicits burst firing in LC neurons. Single unit activity is differentiated with Plexon's Offline Spike Sorter, and unique units are assessed between groups. Analysis is done extracting interspike interval (ISI) and firing rate of single units, additional analysis is done to quantify

properties of bursting patterns (burst duration, spikes per burst, and interburst interval). During the anesthetized recordings, location validation was conducted using pharmacologic inhibition of noradrenergic neuron activity with the α2A antagonist clonidine. Subsequently, post-recording validation was performed utilizing Nissl stain to assess location of a lesion that is created at the end of each recording. The current data show that females have significantly increased interspike interval and a decrease in spike frequency during basal activity. While preliminary analysis reveals no significant differences in bursting patterns between sexes, there is a trend. Because female rats have been estrous cycle tracked via vaginal lavage, stratification into estrus phases may uncover differences within females. These data suggest that estrogen acts as a potent neuromodulator of noradrenergic LC neurons, providing valuable insights into the physiology of this brain region.

Disclosures: R. Rae: None. **L. McMahon:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.08/D45

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: R01DA041705

Title: Four distinct electrophysiological phenotypes of midbrain dopaminergic neurons explained using computational modeling

Authors: *C. J. KNOWLTON[1], J. MANKEL[2], J. ROEPER[3], C. C. CANAVIER[4];
[1]Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA; [2]Goethe-University Frankfurt, Frankfurt, Germany; [3]Goethe Univ. Frankfurt, Johann Wolfgang Goethe-University Frankfurt, Frankfurt, Germany; [4]LSU Hlth. Sci. Ctr., LSU Hlth. Sci. Ctr. New Orleans: Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Midbrain dopamine (DA) neurons are pacemakers *in vitro* and display continuous tonic activity *in vivo* (1-8 Hz) interrupted by short high frequency bursts (15-100 Hz) and pauses. We model a compressed dynamic firing range DA population (DA_{CR}) and an extended dynamic range (DA_{ER}) based upon the maximum sustained frequency exhibited in response to depolarization. We also model ramp rebound (DA_{ramp}) and burst rebound (DA_{burst}) populations based on the qualitative differences in post-hyperpolarization responses. Combinations of these two categories result in four different electrophysiological phenotypes of intrinsic dynamics that are associated with distinct axonal projection targets, implying participation in different circuits. The difference between extended and compressed dynamic range in the models, per our previous results (Knowlton et al 2021), is due to less long-term Na_V channel inactivation in the former, and the slow rate of recovery from inactivation of K_V4 distinguishes ramp from rebound burst responses. Poisson process synaptic input was applied to simulate a balanced state *in vivo*. The

compressed dynamic range neurons are far more regular than extended dynamic range both under simulated *in vitro* and *in vivo* conditions, largely due to the slow conductances evoked during their deep AHP. DA_{CR_ramp} projecting to dorsomedial striatum and the lateral shell of the nucleus accumbens have strong coupling to both high and low threshold calcium channels. Due to those couplings, these cells have the deepest after-hyperpolarization (AHP), lack of frequency sensitivity to CaV1.3 in accordance with (Shin et al 2022), robust ramp responses, and sensitivity in pacing frequency to HCN (unpublished results), and the lowest CV among all four populations under the balanced state. DA_{CR_burst} projecting to dorsolateral striatum have an intermediate AHP and frequency sensitivity to CaV1.3. The burst response depends on a largely uncoupled Ca_V3 de-inactivated during hyperpolarization combined with faster inactivation of KV4.3. Counterintuitively but in accordance with experiment, HCN block accentuates the burst response due to more effective recruitment of CaV3. In all subtypes HCN block does not change the ramp versus rebound response but increases the latency. DA_{EX_ramp} projecting to the medial shell and core of the nucleus accumbens have broader, lower amplitude spikes due to smaller conductances for Na_V and KDR and the slowest recovery from inactivation of K_V4. DA_{EX_burst} projecting from the substantia pars lateralis to the tail of the striatum are similar to DA_{CR_burst} with less NaV long-term inactivation and shallower AHP due to reduced SK coupling.

Disclosures: **C.J. Knowlton:** None. **J. Mankel:** None. **J. Roeper:** None. **C.C. Canavier:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.09/D46

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NS003134

Title: Dopaminergic neurons in the substantia nigra pars lateralis: a distinct cellular subpopulation with unique physiological properties and synaptic connectivity.

Authors: *L. SANSALONE, Z. KHALIQ;
NINDS, Bethesda, MD

Abstract: Midbrain dopamine neurons (DA) located in the ventral tegmental area (VTA) project to the ventral striatum where they contribute to reward processing while substantia nigra pars compacta (SNC) DA neurons innervate the dorsal striatum (DS) and play a key role in motor control. DA neurons of the substantia nigra pars lateralis (SNL) convey learning information to the tail of the striatum (TS) during aversive and threatening behaviors. In contrast to the well-studied DA neurons in the VTA and SNC, the physiological properties SNL DA neurons remain largely undefined. We tested the firing and synaptic properties of SNL DA neurons. We identified SNL neurons based on their expression of calbindin (Calb1) and VGlut2 (Slc17a6).

We found that SNL DA neurons exhibit highly irregular spontaneous firing compared to SNC DA neurons (*CV ISI, SNC: 4.0 ± 1.7 %, n = 13; SNL: 28.8 ± 15.4 %, n = 29*). In addition, they display higher maximal firing rates in part due to a smaller Ca^{2+}-dependent potassium (SK) conductance. Moreover, SNL DA neurons display a significantly higher frequency of mEPSC compared to SNC DA neurons (*mEPSC frequency, SNL: 14.3 ± 9.8 Hz, n = 8; SNC: 1 Hz ± 0.5 Hz, n=8*), suggesting that they receive higher tonic levels of synaptic glutamate. Finally, we tested modulation of the firing activity of SNL DA neurons by excitatory synaptic afferents arriving from distinct basal ganglia nuclei. We found that pedunculopontine (PPN) and subthalamic nucleus (STN) differentially innervate SNL compared to SNC DA neurons. Finally, we raise the possibility that SNL DA neurons activity is modulated by different STN and PPN subpopulations compared to SNC DA neurons which could explain their distinct role in aversive/threatening behaviors compared to motor-related tasks.

Disclosures: L. Sansalone: None. **Z. Khaliq:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.10/D47

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Neuromodulatory mechanisms of rebound timing and gain control in dorsolateral striatum-projecting dopamine substantia nigra neurons

Authors: *S. STOJANOVIC[1], J. ROEPER[2];
[1]Inst. of Neurophysiol., Frankfurt am Main, Germany; [2]Inst. of Neurophysiol., Goethe-University Frankfurt, Frankfurt, Germany

Abstract: Rebound properties of dopaminergic (DA) midbrain neurons exhibit significant variation among distinct subpopulations. In this study, we focused on the unique characteristics of DA neurons in the substantia nigra (SN) that project to the dorsolateral striatum (DLS). In vitro experiments revealed that these neurons displayed a spontaneous bimodal distribution of rebound timing. We quantified rebound timing as the latency to spike following the termination of a hyperpolarizing current injection (control: median rebound delay: 329.1 ms, n= 24, N= 7). Additionally, rebound gain was assessed by the transient post-inhibitory firing response, which ranged from single spikes to high-frequency bursts (control: median rebound firing rate: 3.7 Hz, n= 24, N= 7).
By employing selective ion channel blockers, we defined the underlying biophysical mechanisms controlling rebound in DLS-projecting DA SN neurons. Inhibition of Kv4, GIRK2, SK3, or K-ATP channels uniformly accelerated rebound timing compared to the control condition (median rebound delay: AmmTX3: 32.3 ms, p= 0.0002, n= 14, N= 3; Tertiapin-Q: 98.1 ms, p= 0.0017, n= 17, N= 3; Apamin: 114.9 ms, p= 0.0015, n= 19, N= 3; Glibenclamide: 105.0 ms, p= 0.001, n= 20, N= 3, respectively). Notably, inhibition of SK3 channels proved to be the most effective in

enhancing rebound gain (Apamin: median rebound firing rate: 38.04 Hz, $p < 0.0001$, $n = 19$, $N = 3$). Conversely, inhibition of T-type calcium channels, but not L-type calcium channels, eliminated the bimodal rebound timing and reduced rebound gain.
Furthermore, our investigation revealed a strong influence of various G protein-coupled receptors (GPCRs) on the rebound properties of DLS-projecting DA SN neurons. Inhibition of tonically active D2R and $GABA_B$ receptors removed the intrinsic bimodality of rebound timing. Similar outcomes were observed when noradrenergic and muscarinergic GPCRs were activated. In contrast to mitochondrial calcium uniporter inhibition, the GPCR stimulation results were recapitulated either by enhancing intrinsic calcium buffering (1 mM BAPTA: median rebound delay: 170.4 ms, $p = 0.0267$; median rebound firing rate: 12.9 Hz, $p < 0.0001$, $n = 17$, $N = 4$) or by inhibiting ER calcium pumps (CPA: median rebound delay: 133.7 ms, $p = 0.0321$; median rebound firing rate: 19.1 Hz, $p < 0.0001$, $n = 16$, $N = 2$).
To test the functional relevance of rebound regulation in vivo, we are currently exploring a projection-specific approach utilizing AAV9-based overexpression of calbindin-D28k, a calcium-buffering protein. This approach aims to decouple the T-to-SK channel crosstalk and to induce rapid rebound spiking exclusively in DLS-projecting DA SN neurons.

Disclosures: S. Stojanovic: None. **J. Roeper:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.11/D48

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH NIGMS T32 GM142520

Title: Muscarinic activation inhibits rebound firing in SNc neurons through a non-T-type mechanism

Authors: *M. L. BEAVER, R. C. EVANS;
Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Subpopulations of dopaminergic neurons of the substantia nigra *pars compacta* (SNc) that play a key role in the initiation of movement and motor control are differentially vulnerable to degeneration in Parkinson's disease (PD). The most vulnerable neurons are known to exhibit rebound activity in response to strong inhibition. This rebound involves T-type calcium channels and may be important for information processing within the basal ganglia. Cholinergic neurons of the pedunculopontine nucleus (PPN) provide the primary source of muscarinic activation in the SNc and also degenerate in PD. However, there is a gap in our knowledge regarding how loss of cholinergic signaling from the PPN affects information processing in SNc neurons. Here, we use *ex vivo* whole-cell electrophysiology and two-photon calcium imaging to explore the effects of muscarinic activation on SNc neurons and the mechanisms by which they occur. The SNc

population most vulnerable to neurodegeneration is characterized by rebound firing and large hyperpolarization-activated afterdepolarizations (ADPs) that are mediated by T-type calcium channels. Our data show that application of a non-selective muscarinic acetylcholine receptor agonist, Oxotremorine (OxoM), onto these rebound-ready SNc neurons decreases their ability to rebound after inhibition and decreases the size of the ADP. These OxoM effects occur in both the presence and absence of synaptic blockers (AP-5, NBQX, Gabazine, and CGP55845), suggesting that OxoM is acting post-synaptically and affecting SNc neuron intrinsic properties. Because dopaminergic neurons contain predominately M5 muscarinic receptors, which have been shown to strongly inhibit the $Ca_V3.3$ subtype of T-type calcium channels in expression systems, we patched SNc neurons in $Ca_V3.3$ knockout mice and measured the effects of muscarinic activation. We found a similar decrease in rebound firing and ADP size, suggesting that the OxoM effect is not $Ca_V3.3$-mediated. Additionally, application of the non-selective T-type calcium channel blocker TTA-P2 prior to OxoM does not occlude the effect of OxoM on decreasing rebound firing. We performed similar experiments using a BAPTA-containing internal solution. Here, we observe a similar effect of OxoM on rebound firing and ADP size, suggesting that the OxoM effect is not calcium-mediated. Muscarinic activation is known to increase calcium in dopaminergic neurons, but it is not known if this effect is similar in all dopaminergic subtypes. Using two-photon calcium imaging, we show that the OxoM-mediated calcium increase occurs across anatomically-defined SNc populations.

Disclosures: **M.L. Beaver:** None. **R.C. Evans:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.12/D49

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Showalter Research Trust
Purdue Big Idea Challenge 2.0 on Autism
NIH Grant R01NS117585
NIH Grant R01NS123154
FamilieSCN2A foundation for the Action Potential Grant
NSF GRFP fellowship DGE-1842166
Indiana Spinal Cord & Brain Injury Research Fund
Indiana CTSI funded, in part by UL1TR002529
P30CA082709
PCCR Grant P30CA023168
Walther Cancer Foundation
PIDD
PIIN

Title: Reversing social impairments in an autism-associated mouse model of Scn2a deficiency

Authors: *J. ZHANG, X. CHEN, M. EATON, B. A. DEMING, M. S. HALURKAR, J. WU, Y.-E. YOO, Y. ZHAO, K. WETTSCHURACK, Z. QUE, M. I. OLIVERO ACOSTA, Y. YANG; Medicinal Chem. and Mol. Pharmacol., Purdue Univ., West Lafayette, IN

Abstract: Genetic variants in the voltage-gated sodium channel Na$_V$1.2 (encoded by the *SCN2A* gene) are strongly linked with autism spectrum disorder (ASD), epilepsy, as well as other neurodevelopmental disorders. Recent large-scale genetic studies in humans have revealed *SCN2A* carrying loss-of-function (LoF) and protein-truncating mutations as the leading monogenic cause of ASD. Since impairment in social functioning is a major disease hallmark of ASD, thus, it is essential to investigate how *Scn2a* deficiency leads to social deficits. Conventional complete knockout of *Scn2a* in mice is perinatal lethal, while *Scn2a*$^{+/-}$ mice display mild or even a slight increase in social behaviors. We thus developed a viable homozygous mouse model with severe Na$_V$1.2-deficiency by gene-trap strategy. Using this mouse model, we revealed that these mice display major social impairments. As an imbalance between excitation and inhibition (E/I) has been suggested as a common mechanism in ASD, we recorded striatal medium spiny neurons (MSNs) using patch-clamp electrophysiology and found an elevated E/I ratio for the transmissions projected onto these neurons. Notably, we observed a corrected E/I ratio and rescued social deficits by a global restoration of *Scn2a* expression via adeno-associated virus (PHP.eB-AAV) mediated genetic intervention with flippase (FlpO) in adulthood. Interestingly, while striatum-specific restoration of *Scn2a* expression did not alleviate impaired sociability, restoration of *Scn2a* in the striatum-projecting circuits via retrograde AAV vector alleviated social deficits, indicating the transmissions onto these MSNs play a critical role in the behavioral pathology associated with Na$_V$1.2-deficiency. Using *in vivo* calcium imaging (Inscopix) of freely moving mice as a surrogate to monitor neuronal activity, we perform additional studies to further understand how Na$_V$1.2 deficiency perturbs the neuronal functions and brain circuits resulting in social deficits. Taken together, our study is expected to provide behavior to circuitry of the reversibility of social impairments in Na$_V$1.2-deficient mice, providing a framework for the development of novel interventions to alleviate social deficits related to *SCN2A*-deficiency.

Disclosures: J. Zhang: None. **X. Chen:** None. **M. Eaton:** None. **B.A. Deming:** None. **M.S. Halurkar:** None. **J. Wu:** None. **Y. Yoo:** None. **Y. Zhao:** None. **K. Wettschurack:** None. **Z. Que:** None. **M.I. Olivero acosta:** None. **Y. Yang:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.13/D50

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant DA051450

Title: Indirect pathway spiny projection neuron excitability is increased in the dorsolateral striatum after protracted abstinence from methamphetamine self-administration in female but not male mice

Authors: *S. CHOI[1], H. N. METHIWALA[2], S. GRAVES[3];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Pharmacol., [3]Univ. of Minnesota Twin Cities, MINNEAPOLIS, MN

Abstract: Methamphetamine (meth) is a potent psychostimulant with rates of abuse on the rise in the US (NSDUH 2021). The dorsolateral striatum (DLS) is densely innervated by substantia nigra pars compacta dopamine neurons and has been implicated in maladaptive drug-seeking behaviors. For example, inactivation with baclofen/muscimol (Rubio *et al., J Neurosci*, 35:5625, 2015) and non-selective dopamine receptor antagonism with -flupenthixol (Vandershuren *et al., J Neurosci*, 25:8665, 2005) in the DLS attenuates stimulant-seeking behaviors in rodent models. However, the effect of stimulant exposure, and in particular meth exposure, on DLS neuronal function is unclear. The dorsal striatum is primarily composed of D1 dopamine receptor expressing direct (dSPNs) and D2 dopamine receptor expressing indirect pathway spiny projection neurons (iSPNs). We have previously shown that repeated administration of meth resulted in changes in iSPN excitability in the dorsomedial striatum (Choi *et al., Sci Rep*, 12:12116, 2022). To determine the impact of meth exposure on DLS iSPN excitability, adult (>8 weeks of age) male and female mice expressing tdTomato and eGFP under the control of *Drd1a* and *Drd2* receptor regulatory elements, respectively, were implanted with chronic indwelling jugular vein catheters and trained to self-administer meth (0.1 mg/kg/infusion) for 2 hours/day for 10 days using a fixed ratio 1 schedule of reinforcement during the light cycle; saline-yoked control subjects were run in parallel. After acute (1-4 days) or protracted (21-24 days) abstinence, *ex vivo* brain slices entailing the DLS were prepared, iSPNs identified based on eGFP fluorescence, and intrinsic excitability assessed using whole-cell patch clamp techniques consistent with our prior study (Choi *et al., Sci Rep*, 12:12116, 2022). During acute abstinence from meth self-administration, DLS iSPN intrinsic excitability was unchanged in both male and female mice. However, after protracted abstinence, DLS iSPN intrinsic excitability was increased in female (two-way ANOVA current X treatment interaction $F_{(25,350)}=1.597$, $p<0.05$; *post-hoc* analysis showed significant differences at 380-480 pA) but not male subjects. These data indicate that meth self-administration results in sex-specific and abstinence-dependent hyperexcitability of DLS iSPNs. Further study is necessary to examine the behavioral consequences of meth induced changes in iSPN function.

Disclosures: S. Choi: None. **H.N. Methiwala:** None. **S. Graves:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.14/D51

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Brain Initiative Grant NS110059
NSF Fellowship DGE-1644868

Title: Fast-spiking interneurons in dorsomedial and dorsolateral striatum show bidirectional changes in excitability with acquisition of goal-directed behavior and transition to habitual responding

Authors: *V. L. HALL[1], M. HAZLETT[1], E. ROBINSON[2], A. WEST[1], N. CALAKOS[3]; [1]Dept. of Neurobio., [2]Dept. of Neurol., [3]Dept. of Neurology/Neurobiology, Duke Univ. Sch. of Med., Durham, NC

Abstract: Fast-spiking interneurons (FSIs) are a sparse cell type within striatal microcircuitry yet exert powerful influence over striatal output. FSIs are wrapped by a specialization of the extracellular matrix known as perineuronal nets (PNNs), shown to be permissive for experience-dependent plasticity. Prior work from our lab demonstrates that FSIs undergo plasticity with habit learning, broadly and bidirectionally influence striatal projection neuron firing, & are required for habitual responding. The behavioral transition from goal-directed to habitual responding is known to differentially rely on dorsomedial (DMS) & dorsolateral (DLS) striatal regions, but relatively little is known about how plasticity is expressed within these regions over this goal to habit transition. Here, we investigate region-specific striatal FSI plasticity through this behavioral continuum. Replicated across 2 blinded cohorts, intrinsic membrane excitability significantly differed between goal-directed and habitual mice, with DLS FSIs from habitual animals being more excitable ($F(4,92)=2.250, p=0.0136$, n=12 & 13 cells). Despite divergent roles in supporting goal vs habit behavior, we found that increased FSI excitability was associated with habitual behavior similarly in both DLS & DMS ($F(4,92)=7.283, p<.0001$, n=13 & 12 cells). Even with these commonalities, electrophysiological data indicate that DMS & DLS appear to deploy distinct ion channel mechanisms to modify FSI excitability. Intriguingly, we found a bidirectional model of FSI plasticity in both subregions where FSI excitability is most similar between naïve & habitual states, indicating that acquisition of goal-directed behavior introduces a transient dip in FSI excitability – updating working models for habit learning & expression. In accordance with this finding, the emergence of goal-directed behavior correlates with degradation of PNNs surrounding FSIs in both the DMS ($t(34)=3.838$, $p=.0005$) & DLS ($t(34)=2.041$, $p=.0491$, n=4 and 5 mice) – potentially working to create a permissive environment for plasticity and the rearrangement of contributing ion channels. Together, these findings identify FSIs within both the habit-associated DLS and goal-directed DMS as a site of plasticity during habit learning that bidirectionally modify their excitability such that FSIs in the goal-directed state are distinct from those in both naïve and habitual conditions, which is contrary to working models in the field and highlights the importance of examining region-specific contributions of FSIs to striatal output across behavior.

Disclosures: V.L. Hall: None. **M. Hazlett:** None. **E. Robinson:** None. **A. West:** None. **N. Calakos:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.15/D52

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Metabolic signaling to GnRH neurons: NPY and α-MSH interactions

Authors: *N. MANSANO, S. WRAY, S. WRAY;
Cell. and Developmental Neurobio. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: GnRH neurons are essential for fertility, a process modulated by numerous extrinsic and intrinsic cues. As such, GnRH neurons integrate incoming signals from a wide range of systems involved in ensuring reproductive success. For example, GnRH neurons express receptors related to food intake, such as, the orexigenic hormone Neuropeptide Y (NPY) and anorexigenic hormone proopiomelanocortin (POMC), potentially linking metabolic function and reproduction. Although it has been reported that GnRH neurons respond independently to NPY and α-melanocyte-stimulating hormone (α-MSH, product from POMC) in adult mice, it's unclear how these circuit might interact. This study will identify the relationship between α-MSH and NPY signaling in GnRH neurons using calcium imaging of GnRH cells maintained in explants and *in situ* recording will be done in brain slices. In addition, immunocytochemical analysis will be performed examining the expression of NPY receptor and α-MSH receptor combinations in GnRH cells (Y1R , Melanocortin 4 (MC4) and 1(MC1)). For calcium imaging experiments, recordings were divided into 5 min periods, with an amino acid blocker added after the first serum-free media period to delineate direct from indirect responses via interneurons. The amino acid blocker (AAB) is a cocktail containing inhibitors to ionotropic glutamatergic receptors (-Cyano-7-nitroquinoxaline-2,3-dione (CQNX, 10μM), D-2-Amino-5-phosphonopentanoic acid (D-AP5, 10μM)) and GABA A receptor antagonist (-Bicuculline methiodide (BIC, 10μM), cell types known to be present in this model system. All recordings were terminated with a 40 mM KCl stimulation to ensure the viability of the cells. The changes of fluorescence over time were measured in single GnRH neurons with iVision and analyzed with MATLAB (Mathworks, Natick, MA). Preliminary results indicate that after AAB, calcium oscillations decreased in GnRH cells after exposure to NPY (10nM) ($P<0.0001$), consistent with the literature. Notably, after 5 min washout ~13% of GnRH cells responded with an increase in activity to α-MSH (50nM, n=10/81 cells from 2 explants). To verify the effect was due to α-MSH and not a recovery from NPY exposure, the same paradigm was repeated without addition of α-MSH. No significant difference was detected in the 5thperiod, indicating that the response in the previous experiment was related to α-MSH exposure. Further experiments will be performed using specific agonists and receptor antagonists. These results indicate that GnRH cells can respond to NPY and α-MSH, integrating signals from circuits between metabolic and fertility.

Disclosures: N. Mansano: None. **S. Wray:** None. **S. wray:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.16/D53

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NS027881
DA034748

Title: Hypocretin/orexin (H/O) receptors are required for development of a decreased soma size and reduced excitability in H/O neurons following two weeks of daily morphine injections

Authors: *E. BERRY[1,2], E. N. HUHULEA[2], P. SHEU[2], R. MCGREGOR[3], M. ISHIBASHI[4], J. M. SIEGEL[5], C. S. LEONARD[2];
[2]Dept Physiol, [1]New York Med. Col., Valhalla, NY; [3]UCLA, Sherman Oaks, CA; [4]Hamamatsu Univ. Sch. of Med., Hamamastsu, Japan; [5]Dept Psychiat, Univ. California Los Angeles, North Hills, CA

Abstract: H/O receptor antagonists are emerging therapeutics for substance use disorders and are currently being tested in clinical trials. These drugs dampen behavioral signs of addiction and withdrawal and prevent induction of excitatory synaptic plasticity in VTA dopamine neurons following single injections of cocaine or morphine (M) in preclinical studies. Recent studies indicate that H/O neurons themselves undergo profound structural changes following chronic opioid use, which increases the number of immunopositive H/O neurons, and decreases their soma size by ~ 20% in humans and mice. Correspondingly, we found that two weeks of daily morphine (M; 50mg/kg) injection produces a striking decrease in excitability of H-type, but not D-type H/O neurons using whole-cell patch recordings in brain slices from orexin-EGFP mice. Following M treatment H-type neurons had an elevated rheobase, smaller and broader action potentials and a reduced ability to sustain repetitive firing without changes in their passive properties. These changes required more than a single M injection, and were reversed after 4 W of spontaneous withdrawal, suggesting a functional plasticity in intrinsic properties in H-type neurons.In the present study, we investigated the role of H/O receptors in mediating this structural and functional plasticity induced by M in H/O neurons. We first measured soma size of H/O immunoreactive mice from mice lacking either one or both H/O receptors following two weeks of daily injections of S or M (50 mg/kg; n = 5 for each genotype and treatment). We found that M-treated H/O receptor wild type mice showed ~15% decrease in H/O soma size, as expected. In contrast, the absence of either, or both, H/O receptors prevented the decrease in H/O soma size in the M- treated groups. This indicates that both H/O receptors are required for the M-induced structural changes in H/O neurons.We next measured excitability of H/O neurons from orexin-EGFP mice in three treatment groups. Each group got an IP injection of solvent (Sv) or the dual H/O receptor antagonist TCS1102 (30mg/kg; TSC), followed by sq injection of S or M (50mg/kg) each day for 2 W. Results from the Sv/M recordings indicate that both D and H-type H/O neurons showed reduced excitability. Importantly, we found that there was no reduction in excitability in recordings from the TCS/M group. Thus, signaling through both H/O receptors is required for H/O neuron structural and function plasticity. Collectively, this work provides further support for the use of H/O receptor antagonists in blocking drug-induced brain plasticity.

Disclosures: **E. Berry:** None. **E.N. Huhulea:** None. **P. Sheu:** None. **R. McGregor:** None. **M. Ishibashi:** None. **J.M. Siegel:** None. **C.S. Leonard:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.17/D54

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIMH Grant 119456

Title: Direct effects of corticosterone on AgRP neuron activity

Authors: *A. KARIMI MOGHADDAM[1], Y. CHEN[1], X.-Y. LU[2];
[1]Med. Col. of Georgia at Augusta Univ., Augusta, GA; [2]Neurosci. & Regenerative Med., Med. Col. of Georgia At Augusta Univ., Augusta, GA

Abstract: The arcuate nucleus (ARC) located in the hypothalamus plays a crucial role in stress response andbehavioral regulation. Our recent research has revealed that chronic unpredictable stress (CUS), a stressmodel known to induce anhedonia and behavioral despair, exerts inhibitory effects on agouti-related protein(AgRP)-expressing neurons within the ARC. The reduced activity of AgRP neurons can be attributed toboth intrinsic and synaptic inhibition. CUS increases the activity in the hypothalamo-pituitary-adrenal(HPA) axis, leading to sustained elevation of corticosterone levels. To elucidate the direct impact ofcorticosterone on AgRP neuron firing, we conducted patch-clamp recordings on brain sections obtainedfrom AgRP reporter mice. AgRP neuron activity was assessed before and after exposing the neurons to abath application of corticosterone. Our findings demonstrated that treatment with 100 nM corticosterone, aconcentration observed during stress, diminished the firing rate of AgRP neurons. On-going investigationsaddress the receptor specificity and intracellular mechanisms that underlie the effects of corticosterone onAgRP neuron function.

Disclosures: **A. Karimi Moghaddam:** None. **Y. Chen:** None. **X. Lu:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.18/D55

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01DK102918
NIH Grant F32DK120298
NIH Grant RF1AG059778

Title: Altered inhibitory neurotransmission in AgRP neurons as a causal factor for diet-induced obesity.

Authors: A. KORGAN[1], W. WEI[2], S. L. MARTIN[3], Z. J. D. BRIDGES[3], C. C. KACZOROWSKI[4], ***K. O'CONNELL**[3];
[1]CU Anschutz Med. Ctr., Aurora, CO; [2]Georgia State Univ., Atlanta, GA; [3]The Jackson Lab., Bar Harbor, ME; [4]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Neurons coexpressing the neuropeptides agouti-related peptide (AgRP) and neuropeptide Y (NPY) are critical for the regulation of appetite and energy balance. Consistent with this, the intrinsic activity of these neurons is tightly correlated with nutritional state - AgRP neuronal activity is high after fasting and low in satiated animals. Output of these neurons responds quickly to food cues in a process that does not require post-ingestive signals, suggesting there is strong “top-down” modulation of AgRP neurons. We previously showed that a high-fat diet (HFD) induces persistent hyperexcitability of AgRP neurons that precedes weight gain, suggesting that diet-induced dysfunction of AgRP neurons is a causal event in the development of obesity. In this study, we investigated the impact of HFD and diet-induced obesity (DIO) on synaptic modulation of AgRP neurons from male and female mice. We show that excitatory glutamatergic neurotransmission is unaltered by weight gain in HFD-fed mice and remains sensitive to modulation by food deprivation. Paradoxically, we found that inhibitory GABAergic neurotransmission is increased in AgRP neurons from DIO mice and that AgRP neurons are no longer inhibited by GABA or optogenetic stimulation of the GABAergic vDMH → ARH circuit. There was a significant depolarizing shift in the reversal potential of the GABA-evoked Cl^- current, suggesting the dysfunction in GABA-mediated inhibition is due to changes in Cl^- homeostasis in the postsynaptic AgRP neurons. Consistent with this, pretreatment of brain slices from DIO mice with the NKCC1-inhibitor bumetanide completely restored GABA-induced inhibition in AgRP neurons and overexpression of *Slc12a5* (encoding KCC2) using viral gene therapy completely prevented weight gain in mice fed HFD for 5 months. Based on these results, we propose a model in which inhibitory, but not excitatory, neurotransmission is altered by consumption of HFD and is associated with changes in the function of KCC2 in postsynaptic AgRP neurons and nominating KCC2 as a novel therapeutic target for obesity.

Disclosures: A. Korgan: None. **W. Wei:** None. **S.L. Martin:** None. **Z.J.D. Bridges:** None. **C.C. Kaczorowski:** None. **K. O'Connell:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.19/Web Only

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01DK102918
NIH Grant F32DK120298
NIH Grant RF1AG059778

Title: Paternal allele influences high fat diet-induced obesity in a C57BL/6J x PWK/PhJ mouse reciprocal cross

Authors: A. KORGAN[1], *Z. BRIDGES[2], S. L. A. MARTIN[2], T. ZHAO[2], C. C. KACZOROWSKI[3], K. O'CONNELL[2];
[1]Psychiatry, Univ. of Colorado Anschutz Med. Sch., Aurora, CO; [2]The Jackson Lab., Bar Harbor, ME; [3]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Previous research has identified significant influence of parent-of-origin in driving allele specific expression (ASE) in the mouse brain. The arcuate nucleus of the hypothalamus (ARH) regulates many facets of homeostasis, including food intake and bodyweight, and has been identified as a putative 'hot-spot' for ASE in F_1 hybrid mice. Gene set enrichment analysis revealed enrichment for genes regulating neuronal projection and development and synapse organization. Within the ARH, several populations of neurons orchestrate metabolic homeostasis. Agouti-related peptide (AgRP)/Neuropeptide Y (NPY) expressing neurons are essential integrators of information and drive orexigenic responses, while ablation results in anorexia and starvation. HFD feeding and diet-induced obesity (DIO) alter the function of AgRP neurons, resulting in persistent activation of these neurons, which become refractory to modulation by physiological cues of hunger or satiety (e.g., ghrelin, leptin). Therefore, diet-induced dysfunction of AgRP neurons may represent a causal event in pathogenesis of obesity that precedes changes in hormonal sensitivities (leptin, insulin, ghrelin) or body weight. Here, we replicate previous findings describing ASE differences in DIO resiliency and susceptibility in B6xPWK and PWKxB6 F_1 hybrid mice, respectively. Beyond previously described influence of imprinted gene expression in adipose tissue, we quantify neuronal activity and synaptic plasticity in ARH AgRP neurons, along with differences in food preference. While other hypothalamic and extra-hypothalamic brain regions contribute to feeding differences in F_1 hybrid mice, the current study lays a foundation for functional differences in neuronal activity regulating the integration of 'top-down' and 'bottom-up' signals driving feeding behavior and bodyweight gain. Altogether, this study highlights the importance of utilizing genetically diverse mice to elucidate mechanisms underlying complex traits such as feeding behavior and DIO resilience and susceptibility.

Disclosures: A. Korgan: None. **Z. Bridges:** None. **S.L.A. Martin:** None. **T. Zhao:** None. **C.C. Kaczorowski:** None. **K. O'Connell:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.20/D56

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R01CA268125

Title: Origins of in vivo spontaneous neuronal activity induced by chemotherapy in rats

Authors: *T. C. COPE[1], S. N. HOUSLEY[2], J. A. VINCENT[3], J. REED[2], P. NARDELLI[2]; [2]Sch. of Biol. Sci., [1]Georgia Inst. of Technol., Atlanta, GA; [3]Neurosci. Cell Biol. and Physiol., Wright State Univ., Dayton, OH

Abstract: Spontaneous or unprovoked spiking of neurons induced by chemotherapy likely contributes to neuropathic pain, muscle spasms/cramps, and paresthesia. Although the site(s) where spiking, aka spontaneous activity (SA) originates in neurons, whether in human or other animals, remains undetermined *in vivo*, available evidence points to ectopic sites, e.g. dorsal root ganglia (DRG). We tested this notion in anesthetized adult Wistar rats (n=14) in terminal experiments one day after treatment with the platinum-based chemotherapy agent oxaliplatin (20-30mg/kg i.p.). We estimated SA origin for individual neurons by applying spike triggered averaging (STA). For all individual low threshold somatosensory neurons supplying muscle and glabrous skin (n=20), spikes averaged from nerves in the periphery preceded the triggering SA recorded in dorsal roots at fixed latencies (1.63 ± 0.43 ms) slightly longer than those electrically evoked in the periphery (1.62 ± 0.42 ms). These data consistently place SA origin neither in DRG nor along axons traversing the peripheral nerve, but instead near to or at normal zone(s) of mechanosensory evoked action potential initiation. For motoneurons (MNs, n=8), action potentials averaged in ventral roots preceded SA trigger spikes discriminable in EMG at fixed latencies (1.67 ± 0.38 ms) consistent with SA initiation at the axon initial segment and intrinsic to MNs since results were replicated with dorsal roots cut. We posit that SA is not ectopic under our experimental conditions but arises instead from the normal sites of spike initiation whether for low threshold somatosensory or MNs. These non-ectopic sites seem most relevant in examining molecular events and targeting treatment for SA.

Disclosures: T.C. Cope: None. **S.N. Housley:** None. **J.A. Vincent:** None. **J. Reed:** None. **P. Nardelli:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.21/D57

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: CIHR PJT-162404
Natural Sciences and Engineering Council of Canada

McGill Initiative in Computational Medicine team grant
Globalink Graduate Fellowship

Title: Modulation of SK channels via calcium buffering tunes intrinsic excitability of parvalbumin interneurons in neuropathic pain: A computation and experimental investigation

Authors: *X. MA[1], L. S. MIRAUCOURT[3], H. QIU[2], R. SHARIF NAEINI[2], A. KHADRA[2]; [1]Integrated Program in Neurosci., [2]Dept. of Physiol., McGill Univ., Montreal, QC, Canada; [3]Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Parvalbumin-expressing interneurons (PVINs) play a crucial role within the dorsal horn of the spinal cord by preventing touch inputs from activating pain circuits. In both male and female mice, nerve injury decreases PVINs' output via mechanisms that are not fully understood. In this study, we performed targeted whole-cell patch-clamp recordings from tdTomato-expressing cells in the acute spinal cord slices from PVcre;Ai14 male mice and the electrical properties were quantified using ElecFeX (Ma et al., 2023), a MATLAB-based graphical user interface toolkit recently developed by our lab. We showed that PVINs from nerve-injured male mice change their firing pattern from tonic to adaptive. To examine the ionic mechanisms responsible for this decreased output, we employed a reparametrized Hodgkin-Huxley (HH) type model of PVINs, which predicted (1) the transition to adaptive firing is due to an increased contribution of small conductance calcium-activated potassium (SK) channels, and (2) this effect is enabled by the impairment of intracellular calcium buffering systems. Analyzing the dynamics of the HH-type model further revealed that a generalized Hopf bifurcation differentiates the two types of state transitions observed in the transient firing of PVINs. This predicted mechanism holds true when embedding the PVINs model in the circuit model of the spinal dorsal horn. To experimentally validate this predicted mechanism, we used pharmacological modulators of SK channels and demonstrated that (1) tonic firing PVINs from naïve male mice become adaptive when exposed to an SK channel activator, and (2) adapting PVINs from nerve-injured male mice return to tonic firing upon SK channel blockade. Our work provides important insights into the cellular mechanism underlying the decreased output of PVINs in the spinal dorsal horn after nerve injury and highlights potential pharmacological targets for new and effective treatment approaches to neuropathic pain. **References**: Ma, Xinyue, et al. "ElecFeX: A user-friendly toolkit for efficient feature extraction from single-cell electrophysiological recordings." bioRxiv (2023): 2023-05.

Disclosures: X. Ma: None. **L.S. Miraucourt:** None. **H. Qiu:** None. **R. Sharif Naeini:** None. **A. Khadra:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.22/D58

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NINDS 1R15NS125560

Title: Using dynamic clamp to determine the contribution of resurgent and persistent sodium currents in Purkinje neuron excitability

Authors: *S. BROWN, J. RANSDELL;
Miami Univ., Oxford, OH

Abstract: The resurgent component of voltage-gated sodium currents (I_{NaR}) is often associated with high rates of neuronal firing. I_{NaR} is revealed on membrane repolarization when a population of voltage-gated sodium (Nav) channels recover from fast inactivation into an open/conductive gating state allowing "resurgent" Na^+ influx. Markov models can be used to explore how ion channel gating states, and the transitions between those states, are reflected in the properties of ionic currents. In Ransdell et al., 2022, a novel Nav channel Markov model was developed that faithfully reproduces the properties of I_{NaR} measured in mouse cerebellar Purkinje neurons. In the present study, we developed several Markov Nav conductance models with differing proportions of I_{NaR} and I_{NaP}, as well as alterations in the kinetics of I_{NaR} decay. We used dynamic clamp to test how the original Markov model, as well as these manipulated models affect the firing properties of adult Purkinje neurons. While previous studies have linked I_{NaR} to high rates of repetitive firing, we found the addition of I_{NaR} via dynamic clamp does not significantly affect Purkinje neuron firing frequency or the action potential waveform. I_{NaP}, however, was identified to be a critical determinant of Purkinje neuron firing rates. Through manipulations of the Nav conductance Markov models, we found occupancy in the "slow-inactivated" kinetic state inversely scales peak I_{NaR} and I_{NaP}. For instance, promoting occupancy in the slow-inactivated state will reduce I_{NaP} and increase I_{NaR}, and reducing occupancy in the slow-inactivated kinetic state enhances I_{NaP} and reduces peak I_{NaR}. Together with our dynamic-clamp studies, these results suggest I_{NaR} may reflect a mechanism by which the magnitude or proportion of I_{NaP} is regulated through Nav channel slow inactivation. For instance, preventing Nav channel slow-inactivation will increase I_{NaP} (while reducing I_{NaR}) and cause an immediate increase in Purkinje neuron firing rates.

Disclosures: S. Brown: None. **J. Ransdell:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.23/D59

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: MSIT Grant 2016R1D1A1A02937282
MSIT Grant 2018R1A5A2025964
MSIT Grant 2022M3E5E8017970

Title: Homeostatic plasticity of Purkinje cell excitability balances fear-related memory

Authors: *J. LEE[1,2], S. KIM[1,2], D. JANG[2,3], M. JANG[1,2], M. BAK[1,2], H. SHIM[1,2], Y.-S. LEE[1,2,4,5], S. KIM[1,2,4,5];
[1]Physiol., [2]Biomed. Sci., [3]Brain and Cognitive Sci., [4]Seoul Natl. Neurosci. Res. Inst., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [5]Wide River Inst. of Immunol., Seoul Natl. Univ., Hongcheon, Korea, Republic of

Abstract: In the brain, two forms of plasticity, synaptic and intrinsic plasticity, are known to be neural substrates for learning and memory. The balance between synaptic transmission and intrinsic excitability is important because abnormality in homeostatic plasticity causes severe diseases such as epilepsy and chronic pain. Here, using patch-clamp recordings, we investigated homeostatic plasticity in the cerebellum related to fear memory. We found that the intrinsic excitability of Purkinje cells (PCs) decreases after auditory fear conditioning, which is known to potentiate the synapse between parallel fiber (PF) and PC. Furthermore, we found that PCs in the fear-conditioned group show a slower speed of prepotential depolarization during spontaneous firing and delayed medium afterhyperpolarization. Regardless of whether the fear memory is formed or not, the activity of PCs evoked by PF stimulations was not significantly different. Depression of excitability may homeostatically regulate enhanced synaptic input. Moreover, temporally limited optogenetic manipulation revealed that abnormal activity of PCs during the early consolidation period impairs normal freezing behavior. These results show that information related to fear memory is enhanced by synaptic potentiation, but counter-balanced by decreased intrinsic excitability to maintain the stable PC activity and sustain the fear memory in a normal range.

Disclosures: J. Lee: None. **S. Kim:** None. **D. Jang:** None. **M. Jang:** None. **M. Bak:** None. **H. Shim:** None. **Y. Lee:** None. **S. Kim:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.24/D60

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Characterizing a synaptic connection between BLA and TRN

Authors: *M. C. BAUER[1], J. S. HAAS[2];
[1]Lehigh Univ. Biol. Sci., Bethlehem, PA; [2]Lehigh Univ., Lehigh Univ., Bethlehem, PA

Abstract: The ability to recognize and direct attention towards threatening stimuli is vital for survival and can be acquired rapidly. The basolateral amygdala (BLA) is required for fear learning and redirects attention towards relevant stimuli through bidirectional plasticity of amygdalar responses during fear learning (Gründemann, 2020). However, the BLA itself is not responsible for gating attention to the sensory surround. That function is thought to be accomplished by the thalamic reticular nucleus (TRN). The TRN acts as an inhibitory interface

between the sensory environment and the cortex. It receives input from thalamocortical neurons that relay sensory information from thalamus to cortex, and from corticothalamic neurons that send projections back to thalamocortical neurons. A link between the BLA and TRN could provide a circuit mechanism for directing attention to emotionally salient sensory stimuli during fear learning, but evidence for such a contact remains sparse. Recent work showed that BLA activation amplifies tone responses in auditory cortex, acting through TRN (Aizenberg et al., 2019). Here, we used viral tracing, optogenetics, and electrophysiological techniques to identify the synaptic foundation of this connection. After injecting channelrhodopsin into BLA, we recorded from single neurons in brain slices containing TRN. Optogenetic stimulation of BLA terminals drove a multi-component excitatory response in TRN neurons, with both fast and slow components, as well as spikelets and dendritic calcium spikes. Consistent with previous results, CNQX only partially blocked fast excitatory responses (Lee et al., 2010). Our data leads us to conclude that this synapse is glutamatergic and mediated by both AMPA and NMDA receptors. Observations of spikelets implies that BLA stimulation drives spikes in electrically coupled neighbors. We hypothesize that the synapse we describe between the BLA and TRN could play avital role in fear learning. Understanding the function and plasticity of this synapse provides a basis for further research into how it contributes to human emotional-attentional processes and neurological dysfunction.

Disclosures: **M.C. Bauer:** None. **J.S. Haas:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.25/D61

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Singapore Ministry of Education Academic Research Fund MOE-T2EP30121-0032
National Medical Research Council Open-Fund Individual Research Grant NMRC-OFIRG21jun-0037
National Research Foundation Competitive Research Programme NRF-CRP17-2017-04
Duke-NUS Signature Research Program Block Grant
Young Individual Research Grant NMRC-OFYIRG22jul-0028
Sunway University, Malaysia
National Neuroscience Institute, Singapore
Canadian Institute for Advanced Research, Canada

Title: Gut microbial metabolites regulate anxiety-related behaviors via amygdala neuronal hyperexcitability in mice

Authors: *W. YU[1], Y. XIAO[1], A. JAYARAMAN[2], Y.-C. YEN[1], S. PETTERSSON[2], H. JE[1];
[1]Duke-Nus Grad. Med. Sch. Singapore, Singapore, Singapore; [2]ASEAN Microbiome Ctr., Natl. Neurosci. Inst., Singapore, Singapore

Abstract: Changes in gut microbiota composition have been linked to anxiety behavioral traits in rodents. However, the underlying neural circuit mechanisms that link gut microbiota and its metabolites to anxiety-behavioral traits remain unknown. Using male C57BL/6J germ-free (GF) mice, devoid of exposure to living microbes, we observed increased anxiety-related behavior and fear response. In addition, the immediate early gene c-Fos expression was significantly increased in the basolateral amygdala (BLA) of GF mice. This phenomenon is attributed to the enhanced intrinsic excitability and spontaneous synaptic activities of BLA pyramidal neurons resulting from their reduced small conductance calcium-activated potassium (SK2) channel currents. Colonization of GF mice with gut microbes or treatment with the microbial-derived metabolite indole reduced anxiety-related behavior and normalized the excitability of BLA pyramidal neurons. Our data demonstrate a distinct molecular mechanism whereby gut microbes/microbe metabolite indole regulate functional changes in the BLA neurons, directly connected to an old evolutionary conserved defense mechanism of anxiety-related behavior in the host animal.

Disclosures: W. Yu: None. **Y. Xiao:** None. **A. Jayaraman:** None. **Y. Yen:** None. **S. Pettersson:** None. **H. Je:** None.

Poster

PSTR457. Intrinsic Properties and Modulation of Neuronal Firing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR457.26/Web Only

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: Air Force Office of Scientific Research Grant FA9550-20-1-0061

Title: Correlation of Spontaneous Electrical Activity and Ca^{2+} Transients Measured Simultaneously in GCaMP6f-Expressing Mouse Chromaffin Cells

Authors: *L. YANG[1], C. VIOLA[1], T. W. GOULD[2], N. PROCACCI[1], G. L. CRAVISO[1], N. LEBLANC[1];
[1]Pharmacol., [2]Physiol. and Cell Biol., Univ. of Nevada, Reno, Reno, NV

Abstract: Adrenal chromaffin cells (ACC) are neural crest-derived cells that share many properties with sympathetic neurons. They play a crucial role in releasing catecholamines through exocytosis during the “flight or fight response”. Previous research using electrophysiological techniques on murine ACC revealed complex spontaneous firing patterns, characterized as regular or bursting action potential (AP) patterns. Spontaneous elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) have also been reported. This study aims to explore how spontaneous fluctuations in $[Ca^{2+}]_i$ correlate with spontaneous APs in murine ACC. This was assessed by performing simultaneous Ca^{2+} fluorescence imaging and membrane potential

measurements in whole-cell current clamped ACC using the perforated patch clamp technique. We observed that approximately 27% of the ACC exhibited spontaneous fluctuations in $[Ca^{2+}]_i$ in wild-type (wt) mouse ACC loaded with Calcium Green-1 dye. However, these fluctuations were small in amplitude and challenging to track over time due to photobleaching. Isolated murine ACC expressing the genetically-encoded Ca^{2+} indicator GCaMP6f in a Sox10 Cre-dependent manner were instead used to enhance the signal-to-noise ratio for monitoring $[Ca^{2+}]_i$. A similar percentage of ACC expressing GCaMP6f exhibited spontaneous Ca^{2+} events (25%) with considerably higher amplitudes and little evidence of photobleaching. In nystatin perforated patched ACC, 4 of 10 cells displayed spontaneous AP spikes exhibiting both regular and bursting firing patterns of APs, consistent with those described in the literature. The more quiescent cells (60%) displayed few or no AP spikes. We did not detect robust increases in $[Ca^{2+}]_i$ in ACC displaying a quiet pattern of electrical activity although slow fluctuations in resting $[Ca^{2+}]_i$ were observed near resting membrane potential (RMP; ~ - 35 to -40 mV). Slow regular spiking patterns (2.5-2.8 Hz) of APs were also not accompanied with large fluctuations in $[Ca^{2+}]_i$. In contrast, more intense bursting patterns (3.1-4.2 Hz) of APs were accompanied by an increase in $[Ca^{2+}]_i$ in the ACC. Spontaneous hyperpolarization following a bursting pattern reduced both the frequency of AP spikes and the magnitude of Ca^{2+} transients. Additionally, within the same ACC, we noticed dynamic switching between regular and bursting spiking patterns. In conclusion, this preliminary study indicates that spontaneous Ca^{2+} transients in mouse ACC are regulated by the frequency of spontaneous APs and the level of RMP. Future research will be undertaken to unravel the molecular mechanisms that drive spontaneous ACC activity and its role in neuromodulation.

Disclosures: L. Yang: None. **C. Viola:** None. **T.W. Gould:** None. **N. Procacci:** None. **G.L. Craviso:** None. **N. Leblanc:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.01/D62

Topic: B.07. Network Interactions

Support: NINDS 5R01MH117149-03
NIMH 5R01MH117149-02
Alfred P Sloan Foundation
Southwestern Medical Foundation

Title: Chronic circuit-level dynamics in the developing mouse brain

Authors: *R. PENDRY[1], L. QUIGLEY[2], L. J. VOLK[1], B. E. PFEIFFER[3];
[1]Neurosci., UT Southwestern Med. Ctr., Dallas, TX; [2]UT Southwestern Med. Ctr., DALLAS, TX; [3]Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Studying circuit-level function in mice across early development, including juvenile and early adolescent stages, can provide a better understanding neurological disorders which manifest behaviorally during this period, such as ASD or schizophrenia. However, performing *in vivo* electrophysiological recordings in juvenile mice is particularly challenging due to technical, surgical, and behavioral hurdles. Here, we present a novel adaptation of *in vivo* tetrode micro-drive construction, a surgical implantation method, and post-surgery recovery strategies which allow us to perform *in vivo* tetrode recordings in awake, freely behaving mice beginning at age p19. We can further perform daily, chronic recordings in the same mouse from p19 through early adulthood (p60 and beyond). Our methodology supports recording both local field potential and single unit activity in a large number of spatially distributed brain regions, and here we present simultaneous bilateral recordings in anterior cingulate cortex, primary auditory cortex, primary somatosensory cortex, inferior colliculus, and both dorsal and ventral hippocampus. Via these large-scale recordings, we can quantify the development of intra- and inter-regional communication via oscillation power and coherence. We report initial findings of network-level oscillatory and single unit changes which occur in this early developmental window in freely behaving WT mice and several mouse models related to neurodevelopmental disorders, including KIBRA-KO and FMRP-KO mice. Our method establishes a framework for studying how network dynamics are altered in genetic mouse models of neurological disorders.

Disclosures: **R. Pendry:** None. **L. Quigley:** None. **L.J. Volk:** None. **B.E. Pfeiffer:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.02/D63

Topic: B.07. Network Interactions

Support: DFG SFB 1089
DI 1721/3-1 KFO219-TP9
Human Brain Project SGA1 720270
Human Brain Project SGA1 785907

Title: Locomotion induced by medial septal glutamatergic neurons is linked to intrinsically generated persistent firing

Authors: *E. L. MAROSI[1], K. KORVASOVÁ[2], F. LUDWIG[3], H. KANEKO[1], L. SOSULINA[1], T. TETZLAFF[4], S. REMY[1], S. MIKULOVIC[1];
[1]Leibniz Inst. for Neurobio., Magdeburg, Germany; [2]Fac. of Mathematics and Physics, Charles Univ., Prague, Czech Republic; [3]German Ctr. for Neurodegenerative Dis., Bonn, Germany; [4]Jülich Res. Ctr., Jülich, Germany

Abstract: Medial septal (MS) glutamatergic neurons expressing type 2 vesicular glutamate transporter (VGluT2) are active during theta oscillation and locomotor activity. Optogenetic

activation of these neurons at theta frequencies (4-12 Hz) entrains hippocampal theta oscillation and sustains locomotion outlasting the stimulus duration. However, the specific cellular and circuit mechanisms responsible for triggering the onset and supporting the maintenance of persistent locomotion and associated theta oscillation remain elusive. To address these questions, we used Neuropixels probe to measure the circuit activity of MS neurons during voluntary locomotion on a linear treadmill, while simultaneously recording hippocampal local field potential. Through optogenetic targeting of VGluT2 neurons using either theta frequency-related or brief transient optical stimulation, we reliably induced persistent locomotion and hippocampal theta. By analyzing the activity of optically tagged putative VGluT2 neurons related to the locomotion onset, we compared the circuit activity recorded during evoked versus voluntary running epochs. Additionally, we discovered persistent spiking of MS neurons that lasted several seconds following the stimulation. By blocking MS synaptic transmission pharmacologically, we still observed persistent locomotion and the underlying persistent spiking upon photoactivation of the VGluT2 neurons. To further test the involvement of synaptic receptors and effect of extracellular Ca^{2+} concentration on MS VGluT2 neurons persistent activity, we employed *in vitro* MS slice preparation using multi-electrode array system. Altogether, our results indicate that the intrinsic excitability of MS VGluT2 neurons drives persistent activity, which in turn contributes to the maintenance of prolonged locomotion and indirectly drives hippocampal theta rhythm.

Disclosures: **E.L. Marosi:** None. **K. Korvasová:** None. **F. Ludwig:** None. **H. Kaneko:** None. **L. Sosulina:** None. **T. Tetzlaff:** None. **S. Remy:** None. **S. Mikulovic:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.03/D64

Topic: B.07. Network Interactions

Support: R21MH117687
P20 GM103446
P20 GM139760

Title: Optogenetic theta stimulation of the medial septum facilitates spatial working memory by enhancing hippocampal - medial prefrontal cortex theta synchrony

Authors: *Z. M. GEMZIK, A. L. GRIFFIN;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: The medial septum (MS) is required for the generation of the hippocampal (HPC) theta rhythm (4-12Hz), which is necessary for spatial working memory (SWM): the ability to process and maintain spatially-relevant information over a temporal gap (Mizumori et al., 1990). Previously our lab has shown that optogenetic suppression of MS delivered during the delay

period of a delayed non-matched to position task disrupted choice accuracy, suggesting that MS activity is necessary for the maintenance of spatial information during SWM tasks (Gemzik et al., 2020). We hypothesized that driving MS-generated theta during the delay period of SWM task would facilitate the maintenance of spatial information and enhance SWM task performance. Recent work from our lab has examined the effects of optogenetic MS theta stimulation delivered during the delay period of a SWM-dependent delayed alternation task. We first trained rats (N=10) to perform a delayed alternation task with a 10 sec delay. After rats reached asymptotic performance, we injected an excitatory optogenetic viral vector (AAV5-hSyn-hChR2-EYFP) into the MS and implanted an optogenetic fiber above the injection site. We challenged SWM demand by adding a long (30 second) delay period in addition to the short (10 second) delay. We found that MS theta stimulation facilitated choice accuracy especially for the longer delay. In support of our hypothesis, the delay-dependent decrease in choice accuracy was eliminated by delivering MS theta stimulation during the delay period (rmANOVA: laser color x delay length, $F(1,9) = 11.484$, $p = 0.008$; post-hoc Holm test), suggesting that MS theta stimulation facilitated spatial working memory. To investigate the circuit-level mechanisms responsible for this facilitation, we next recorded from medial prefrontal cortex (mPFC) and dHPC to examine the impact of MS theta stimulation on mPFC-dHPC theta coherence. As predicted, during times of MS theta stimulation, dHPC-mPFC coherence levels were significantly higher during 20 second bouts of MS theta stimulation when compared to 20 second bouts of no stimulation (paired t-test; $t(14)=-4.95$, $p < 0.001$). These findings support the hypothesis that MS theta stimulation facilitates SWM by increasing HPC-mPFC theta synchrony, allowing the mPFC-dHPC circuit to optimally process and organize task-relevant information on a theta frequency timescale.

Disclosures: Z.M. Gemzik: None. **A.L. Griffin:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.04/D65

Topic: F.07. Biological Rhythms and Sleep

Support: F31MH125640

Title: Manipulation of OLMα2 interneurons during offline consolidation disrupts hippocampus-dependent spatial and contextual learning.

Authors: *M. FRAZER[1], C. ROMAN-ORTIZ[2], E. MOSTAFA[1], G. R. POE[3];
[1]UCLA, Los Angeles, CA; [2]USC, Los Angeles, CA; [3]Dept. of Integrative Biol. and Physiol., UCLA Chapter, Los Angeles, CA

Abstract: Offline consolidation is a critical component of the formation and storage of memories. Disruption of sleep or normal oscillatory activity associated with offline states results

in impaired behavior, particularly in hippocampus-dependent tasks. To better understand circuit mechanisms underlying the role of memory consolidation, we have focused on a subpopulation of hippocampal interneurons, oriens-lacunosum moleculare (OLM) cells expressing the α2 nicotinic acetylcholine receptor, to manipulate during post-learning offline periods in order to observe subsequent impact on spatial and contextual memory. We targeted either DREADDs or an mCherry control to the OLMα2 cells, and injected animals with CNO following acquisition of the novel object place recognition (NOPR) task and a fear conditioning paradigm involving both hippocampus-dependent and independent learning. Inhibition of dorsal hippocampal (dHPC) OLM cells in male OLMα2-cre mice expressing hM4Di (n = 8) resulted in a significant decrease in preference for an object moved to a novel location as compared to OLMα2-mCherry mice (n = 7). This suggests an impaired ability to recall previously learned spatial relationships. Similarly, the recall of contextual information associated with fear conditioning is disrupted in animals following offline inhibition of dHPC OLM cells. Freezing in these animals was inhibited upon reintroduction into the fear context, while freezing in response to the coterminal tone cue was not significantly reduced from OLMα2-mCherry animals. This result suggests that hippocampal-dependent contextual information was not well-consolidated, while non-hippocampal cued information remained unaffected. Electrophysiology was recorded during the offline periods using parietal cortical screws, hippocampal LFP electrodes, and wire in neck muscles to measure muscle tone. No differences in the amount of time spent in each sleep state (waking, NREM, and REM) were detected between the hm4Di and mCherry controls, though the presence of CNO resulted in an increase in NREM sleep in both groups. In OLM-hm4Di mice, spectral analyses of hippocampal electrodes showed a decrease in theta (5 to 10 Hz) power, particularly in waking and REM sleep. These studies suggest a significant role for OLM interneurons in offline memory processing, possibly through the maintenance of the hippocampal theta rhythm. Further studies focusing on behavioral state-specific modulation of OLMα2 activity will continue to delineate the role played by these cells in sleep-dependent consolidation.

Disclosures: **M. Frazer:** None. **C. Roman-Ortiz:** None. **E. Mostafa:** None. **G.R. Poe:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.05/D66

Topic: B.08. Epilepsy

Support: AES Predoctoral Award (SF)
NIH R01NS116357
AES Junior Investigator Award (TS)
CURE Taking Flight Award

Title: Early and late desynchronization of hippocampal and entorhinal-hippocampal circuits in chronically epileptic mice

Authors: *Y. FENG[1], L. PAGE-HARLEY[2], K. DIEGO[2], Z. DONG[2], S. I. LAMSIFER[2], Z. CHRISTENSON WICK[2], L. M. VETERE[2], P. A. PHILIPSBERG[2], Z. T. PENNINGTON[2], I. SOLER[1], J. SCHNIPPER[2], A. JURKOWSKI[2], D. J. CAI[2], T. SHUMAN[2];
[1]Neurosci., [2]Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Abstract: Temporal lobe epilepsy is one of the most common types of epilepsy in adults and causes pervasive memory impairments which significantly impact patients' quality of life. In previous studies, we found that the hippocampus (HPC) becomes desynchronized in pilocarpine-treated epileptic mice, but it remains unclear how upstream inputs from medial entorhinal cortex (MEC) are altered following epileptogenesis. Cognitive processes require precise communication between circuits suggesting that altered timing between MEC and HPC may contribute to the cognitive deficits associated with TLE. In addition, we have found that progressive spatial memory deficits emerge in epileptic mice between 3 and 8 weeks after pilocarpine treatment. In this project, we tested whether MEC-HPC synchronization is disrupted in these mice before and after progressive memory deficits emerge. We performed simultaneous *in vivo* electrophysiology with 512-channel silicon probes in HPC and MEC of head-fixed epileptic and control mice running in virtual reality. We recorded at two timepoints (3 and 8 weeks after pilocarpine) to capture synchronization changes during the progression of memory impairments. Dual-region recording reveals that progressive HPC desynchronization emerges early on at 3wk post pilocarpine, with reduced theta power and altered interneuron phase locking to theta. In contrast, upstream MEC layer 3 (MEC3) excitatory cells are disrupted later in the development of epilepsy, with reduced theta phase locking relative to CA1 theta. Interestingly, we identified 2 distinct populations of MEC3 excitatory units, with only one population showing disrupted phase preference. We also examined theta coherence within and between regions, and found similar results. Theta coherence within the HPC was reduced in epileptic mice as early as 3wk after pilocarpine, while theta coherence in MEC and between MEC-HPC was only reduced at the later time point, 8wk after pilocarpine. Together, this data reveals three main points. **First**, both HPC and MEC are desynchronized in the chronic phase of pilocarpine-induced epilepsy. This is indicated by changes in theta power, theta coherence, and the phase locking of single units to theta oscillations. **Second**, HPC desynchronization emerges earlier than MEC, matching the timeline of seizure onset and hippocampal interneuron loss. **Third**, we identified late onset progressive impairments in the synchrony between the MEC-HPC, as well as within the MEC. These data indicate that epilepsy drives multiple, dissociable changes in HPC-MEC circuits with distinct time courses.

Disclosures: Y. Feng: None. **L. Page-Harley:** None. **K. Diego:** None. **Z. Dong:** None. **S.I. Lamsifer:** None. **Z. Christenson Wick:** None. **L.M. Vetere:** None. **P.A. Philipsberg:** None. **Z.T. Pennington:** None. **I. Soler:** None. **J. Schnipper:** None. **A. Jurkowski:** None. **D.J. Cai:** None. **T. Shuman:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.06/D67

Topic: B.07. Network Interactions

Support: NIH Grant R01NS054281

Title: Synaptic mechanisms of theta-nested gamma oscillations in the medial entorhinal cortex

Authors: *B. D. WILLIAMS[1], F. R. FERNANDEZ[1], C. C. CANAVIER[2], J. A. WHITE[1]; [1]Biomed. Engin., Boston Univ., Boston, MA; [2]Cell Biol. and Anat., LSU Hlth. Sci. Ctr. New Orleans: Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: The medial entorhinal cortex (MEC) performs critical functions in spatial navigation, learning, and memory. Many neurons in layer 2/3 of the MEC fire at periodic locations which generate a grid-like (‘grid cells’) firing pattern when traversing an open field. A network-wide theta (4-12 Hz) frequency oscillation modulates the firing rates of these grid cells. Additionally, higher frequency gamma (40-140 Hz) oscillations are nested within the network theta oscillation and are hypothesized to increase the synchrony of grid cell firing (Reifenstein et al. 2012 *PNAS* 109:6301-6306). Two different (but not mutually exclusive) mechanisms have been proposed to generate gamma oscillations. In the pyramidal-interneuron network gamma (PING) model, pyramidal cells excite local interneurons which provide inhibitory feedback. Alternatively, the interneuron network gamma (ING) model proposes that inhibition among tonically firing interneurons can synchronize. To investigate the mechanisms of theta-nested gamma oscillations, we used intracellular recordings in acute slices to measure synaptic currents in stellate, pyramidal, and fast spiking cells during theta frequency optogenetic stimulation of local excitatory neurons in transgenic CaMKIIα-ChR2 mice. The local field potential (LFP) was simultaneously recorded to capture theta-nested gamma oscillations in the network during a PING mechanism. We found that fast spiking interneurons receive excitatory synaptic inputs followed by feedback inhibition which oscillates in the gamma frequency range. Both excitatory and inhibitory inputs are highly correlated with theta-nested gamma oscillations in the LFP and oscillate at the same gamma frequency, consistent with a PING mechanism. Stellate and pyramidal cells receive gamma frequency feedback inhibition, but not excitation, in agreement with minimal recurrent excitatory connections found in the MEC (Fuchs et al. 2018 *Neuron* 89:194-208). Theta-nested gamma oscillations in the LFP occur simultaneously with the inhibitory currents received by all cell types, further revealing the timing of synaptic events relative to the network activity. We also measured inhibitory synaptic currents in all cell types during optogenetic stimulation of local fast spiking interneurons in transgenic PV-ChR2 mice. Similarly, we found that all cell types received synchronous inhibitory currents at gamma frequencies generated through an ING mechanism. However, ING-driven gamma was faster than the feedback generated through PING. We speculate that PING and ING mechanisms likely contribute to the generation of different frequency gamma oscillations in the MEC.

Disclosures: B.D. Williams: None. **F.R. Fernandez:** None. **C.C. Canavier:** None. **J.A. White:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.07/D68

Topic: B.07. Network Interactions

Support: R37MH08572
T32HD060600
F31MH122068
R01MH112143
P30 CA008748-48

Title: Loss of Engrailed1/2 in Atoh1-derived cerebellar excitatory neurons impairs spatial working memory functional connectivity between prefrontal cortex and hippocampus in mice

Authors: Y. LIU[1], M. FOX[2], B. CORREIA[3], A. LEE[4], A. L. JOYNER[5], ***D. H. HECK**[1]; [1]Biomed. Sci., Univ. of Minnesota Duluth, Duluth, MN; [2]Anat. and Neurobio., Univ. of Tennessee Hlth. Sci. C Neurosci. Grad. Program, Memphis, TN; [3]Neurosci., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [4]Neurosciece Program, Weill Cornell Grad. Sch. of Med. Sci., New York, NY; [5]Developmental Biol. Program, Sloan-Kettering Inst., New York, NY

Abstract: Spatial working memory (SWM) is a cognitive skill supporting survival relevant behaviors, such as foraging for food and finding escape routes. In rodents the medial prefrontal cortex (mPFC) and dorsal hippocampal CA1 region (dCA1) are jointly necessary for SWM. SWM in rodents can be tested by measuring spontaneous alternations during free exploration of a plus maze. Any sequence of four arm entries without repetition is considered a spontaneous alternation and in healthy mice typically ~30-40% of sequences are spontaneous alternations. Previous studies in rodents have shown that SWM-based decisions about maze-arm entry is associated with increased coherence of local field potential (LFP) oscillations between the mPFC and dCA1, commonly interpreted as increased functional connectivity between the two structures. We recently showed that stimulation of Purkinje cells in cerebellar lobulus simplex disrupts SWM performance and the decision-related increase in mPFC-dCA1 coherence. Here we report a similar deficit in mice that have a partial loss of excitatory neurons (eCN) in the medial and intermediate cerebellar nuclei due to loss of Engrailed1/2 expression in all *Atoh1*-derived eCN and granule cells (*Atoh1-En1/2* conditional knockout or KO). SWM performance was tested in 5 KO and 5 control (CT) mice in a plus maze while simultaneously recording neuronal activity in the mPFC and dCA1 using implanted tetrodes. Electrophysiological signals were recorded with an eCube Server and data were analyzed offline in Matlab. The number of arm visits during 12 min of exploration was similar between CT and KO mice. However, the percentage of spontaneous alternations was significantly lower in KO (19.2 ± 3.5) compared to CT (29.7 ± 1.0; Two-Sample t-test, $p < 0.05$) mice.Analysis of LFP amplitude and coherence was time-aligned to the completion of SWM decision making, i.e., all four paws entered the newly chosen maze arm. In CT mice, LFP peak activity did not reflect the decision outcome. In contrast, KO mice showed higher LFP peak values during correct decisions compared to incorrect decisions. In CT mice, coherence between the mPFC and dCA1 significantly increased

in a broad frequency range (12-200 Hz) during SWM decision making, with higher coherence values observed during correct decisions. KO mice showed a decrease in mPFC-dCA1 decision-related coherence in the 30-200 Hz range. Both CT and KO mice showed increased mPFC-dCA1 coherence, irrespective of decision outcome, in the theta, alpha, and beta frequency ranges. These results are consistent with previous finding, suggesting a role of the cerebellum in SWM and in coordinating SWM decision-related mPFC-dCA1 coherence.

Disclosures: Y. Liu: None. **M. Fox:** None. **B. Correia:** None. **A. Lee:** None. **A.L. Joyner:** None. **D.H. Heck:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.08/D69

Topic: B.07. Network Interactions

Support: National Sciences and Engineering Research Council of Canada (grant RGPIN-2018-06291), Canadian Institutes of Health Research PJT- 183862

Title: Respiratory rhythms and limbic harmonies: unraveling potential connections in memory consolidation and olfactory processing

Authors: *J. DESROSIERS, D. BASHA, S. CHAUVETTE, I. TIMOFEEV;
Médecine, Univ. Laval, Québec, QC, Canada

Abstract: Sleep is recognized as crucial for memory consolidation, and olfaction has been implicated in this process. The limbic system, consisting of interconnected regions: the entorhinal cortex, subiculum, and hippocampus (CA1), plays a vital role in short-term memory and replays acquired memories during sleep to be stored in neocortex for long-term memory consolidation. However, the neuronal activities underlying synchrony between olfactory and limbic system are not well explored. We recently investigated activities of hippocampo-thalamo-cortical network in relation to respiratory rhythm (Basha et al., 2023, Scientific Reports). Here we investigate cellular activities of olfactory bulb neurons in relation to respiratory rhythm and their synchronization with limbic system. We conducted intracellular recordings and labelling in vivo in the olfactory bulb, cortical EEG and local field potential (LFP) recordings in the subiculum and CA1 of anesthetized male C57BL/6 mice using ketamine-xylazine anesthesia. We analyzed membrane potential trajectories, synaptic potentials and spiking of olfactory bulb neurons in relation to the time and phase of (a) respiration and (b) cortical slow oscillation. Because membrane potential traces could contain movement artifact related to respiration, we also extracted spontaneous individual synaptic events and correlated them with either respiratory rhythm or slow oscillation. We recorded from 40 neurons in the olfactory bulb and confirmed the location of 6 of those neurons morphologically. Out of these, 35 neurons showed clear relation to respiratory rhythm, 16 neurons showed strong synchrony with slow oscillation. There was some

overlap in recorded activities. A significant number of neurons (n=11) showed synchronous activity with both slow oscillation and respiratory rhythm. 40 % of cells started depolarization at ~ -100º and reached maximum ~ +70º. 12% of cells started depolarization at -30º and reached maximum ~ +100º. 30% of cells started depolarization at the maximum of inspiration and reached maximum ~ +150º (end of expiration). Our study demonstrates that the respiratory rhythm induces rhythmic activities in olfactory bulb implying that the olfactory system influences limbic/cortical systems with a frequency of respiration. The fact that more than 25% of olfactory neurons also correlated with cortical slow oscillation points to the presence of cortical feedback toward olfactory system which might be important 'controller' of sleep-dependent memory consolidation.

Disclosures: J. Desrosiers: None. **D. Basha:** None. **S. Chauvette:** None. **I. Timofeev:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.09/D70

Topic: F.07. Biological Rhythms and Sleep

Support: NIH grant R01-DC-016364
NIH grant R01-DC-018539
NIH grant T32 NS047987

Title: Respiratory coupling of human hippocampal ripples during sleep

Authors: *A. SHERIFF[1], G. ZHOU[1], J. MORGENTHALER[1], J. M. ROSENOW[2], S. SCHUELE[1], G. LANE[1], C. ZELANO[1];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: During non-rapid eye movement (NREM) sleep, neural oscillations at a range of frequencies propagate across neural networks. In humans, nested sleep oscillations include slow oscillations (~0.5-4 Hz) that originate in neocortical networks and are coupled to UP/DOWN states of neuronal activity. Slow oscillations occur with sleep spindles (8-16 Hz). In turn, sleep spindles couple with hippocampal ripples (80-120 Hz). Altogether, the orchestration of these nested sleep oscillations is related to memory consolidation. Recent findings in rodents show respiration coordinates UP/DOWN state transitions, and stimulation of olfactory sensory neurons evokes stronger responses downstream during sleep than wake. These findings support the possibility that, given the lack of thalamic relay between the periphery and primary olfactory cortex, respiration may serve as an underlying sleep oscillation. Specifically, since humans breathe at a frequency (~0.1-0.3 Hz) that is slower than slow oscillations in sleep, it is possible respiration is a novel nested sleep oscillation. We test this hypothesis in humans (n=5, 4 females) using recordings of respiration, scalp EEG, and intracranial local field potentials (LFPs) during sleep. We focus here on respiratory coupling with hippocampal ripples but will consider other

nested sleep oscillations as well. Sleep stages were scored using scalp EEG with a focus on identifying N2 sleep segments. Pathological and electrical artifacts were detected and removed based on previously reported parameters. Ripples were identified in all bipolar-referenced hippocampal electrodes using established automated detection algorithms. To assess respiratory-ripple coupling, hippocampal LFPs and respiratory signals were aligned to ripple peaks for each hippocampal electrode within each subject and averaged across ripple epochs. Significant alignment of ripple peaks to distinct phases of respiration was found at distinct respiratory phases using the Rayleigh's test ($p < 0.05$) of non-uniformity. Furthermore, using modulation index with a permutation test of significance based on surrogate phase-amplitude distributions, respiratory phase-amplitude coupling could be seen in the hippocampus at ripple frequency within each subject. Comparisons of ripple features during inhale vs. exhale and the co-occurrence of respiratory-ripple coupling with other nested sleep oscillations will also be characterized.

Disclosures: **A. Sheriff:** None. **G. Zhou:** None. **J. Morgenthaler:** None. **J.M. Rosenow:** None. **S. Schuele:** None. **G. Lane:** None. **C. Zelano:** None.

Poster

PSTR458. Oscillations in Hippocampal-Cortical Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR458.10/E1

Topic: B.07. Network Interactions

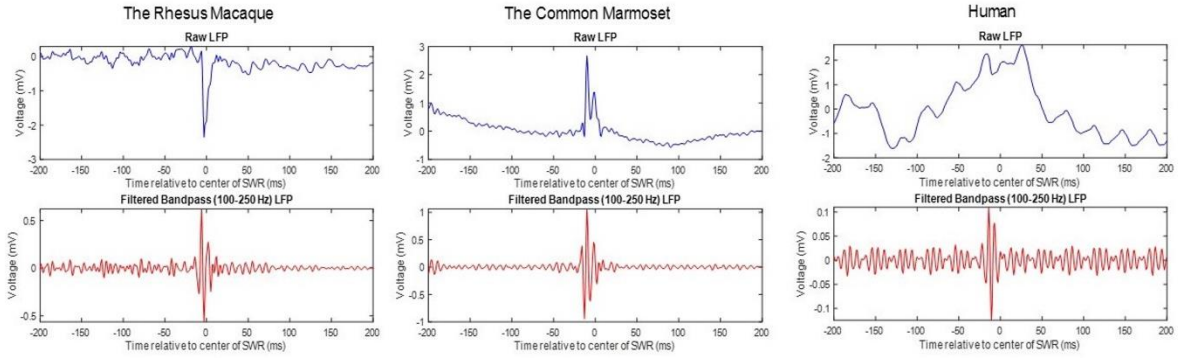
Support: Wallace Coulter Foundation
Canadian Institutes for Health Research (CIHR)
Western Chair in Autism

Title: Detection of Hippocampal Sharp Wave Ripples across three different primate species: the rhesus Macaque, the common Marmoset, and Humans

Authors: *C. OTERO[1], D. BUITRAGO PIZA[2], B. CORRIGAN[2], N. MORTAZAVI[2], M. KHAKI[2], J. C. MARTINEZ-TRUJILLO[2], J. RIERA[1];
[1]Biomed. Engin., Florida Intl. Univ., Miami, FL; [2]Schulich Sch. of Med. and Dentistry, Dept. of Physiol. and Pharmacol. and Western I, Univ. of Western Ontario, London, ON, Canada

Abstract: Sharp wave ripples (SWR) are characterized by a combination of low frequency and high frequency oscillations of Local Field Potentials (LFP) usually originating within the CA1 region of the hippocampus. SWRs have been reported to be associated with the transfer of information from hippocampus subfields to other brain regions involved in memory. Automatic detection of SWR during electrophysiological recordings of LFPs in behaving animal models and humans has been challenging. This has been mostly due to variations in electrode types across species, as well as detection protocols. In rodents, most studies are done with high-impedance electrodes which are helpful in measuring fine-scale neural activity but have

limitations in detecting large-scale network dynamics across regions. Many open-access algorithms for sharp wave detection are developed for rodent data, and it is unknown whether they are optimal for primates. The objective of this study is to address the issues of heterogeneity in recording methods and algorithms by applying a unified approach across species for SWR detection. We recorded LFP data from Marmosets (n=2) (aged 4-6yo), Macaques (n=2) (aged 7-10yo), and Humans (n=8) (aged 35-50yo), using different types of electrodes. In marmosets, we used brush arrays (Microprobe Inc, MD, USA). In macaques, we used FHC platinum-iridium electrodes (FHC Corp, ME, USA), and in humans, we used microcontact Ad-Tech electrodes (Ad-Tech Medical Inc, WI, USA). We collected the data and used signal processing techniques (e.g., sub-sampling and filtering) to homogenize the LFP recordings. We compared the algorithm proposed by Ripplenet (Hagen et al., 2021; RippleNet: a Recurrent Neural Network for Sharp Wave Ripple "SPW-R" Detection) with the current standard of using only the frequency feature to detect ripples. We successfully detected SWRs across the three species, as seen by the figure attached with one ripple example for each species. However, with varying results between algorithms displaying the need for a homogenous approach, this is effective and applicable to primates.



Disclosures: C. Otero: None. **D. Buitrago Piza:** None. **B. Corrigan:** None. **N. Mortazavi:** None. **M. Khaki:** None. **J.C. Martinez-Trujillo:** None. **J. Riera:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.01/E2

Topic: B.08. Epilepsy

Title: The Specificity of electroencephalography to hyperventilation in epileptic patients

Authors: *I. KHACHIDZE[1], N. ADAMASHVILI[2], L. CHANTADZE[3], M. ADVADZE[4];
[1]I. Beritashvili Ctr. of Exptl. Biomedicine. SEU, Tbilisi, Georgia; [2]IVANE BERITASHVILI

CENTER OF EXPERIMENTAL BIOMEDICINE, Tbilisi, Georgia; [4]Med., [3]SEU Georgian Natl. Univ., Tbilisi, Georgia

Abstract: Introduction: Electroencephalography (EEG) responses to hyperventilation test (HPT) are heterogeneous, provoke different interpretations and are still the subject of investigation. There is no clear evidence about the type of pathological EEG pattern and safety of prolonged hyperventilation in epileptic patients. In the previous study, we described pathological EEG responses on hyperventilation (PERH) in patients with different CNS disorders. HPT effect in epileptic patients has practical/theoretical implications in neuroscience and neurology. The study aimed to investigate PERH by onset time, the age and sex of epileptic patients using first online EEG database in Georgia EEGHUB.GE uploaded to European Open Science Cloud (EOSC) Methods: EEGHUB.GE composes EEG recordings of 4087 patients with various CNS dysfunction, aged 1-75 years, men/women following ethical standards. EEG recording includes background activity and functional samples (Photo-stimulation and Hyperventilation-lasted 3 minutes). Previous studies investigated 2186 patients to detect PERH. We revealed PERH in 985 patients with different CNS disorders. Current research studies PERH types by the time of manifestation, age and sex of epileptic patients Results: The study showed the patients' neurological and psychiatric disorders. Epilepsy predominated 405 (41%) compared to other disorders. Three types of PERH–PERH I (main rhythm disorganization), PERH II (paroxysmal discharges), PERH III (epileptiform activity) detected in epileptic patients. Distribution of PERH based on time of manifestation, age and sex of epileptic patients was shown: In 405 patients – PERH I was statistically significant at 76% (309) $p<0.001$ compared to PERH II and III. PERH I by age in all minutes prevailed in 7-18 years. PERH-I in all minutes by sex was not significant. PERH I,II,III detected at the first minute in 363 (90%) patients. PERH I 281(77 %) was statistically significant at the first-minute $p<0.002$, compared to PERH II, III. PERH I was statistically significant at the first minute -281(91%) $P<0.001$ compared to second and third minutes. PERH I at the first minutes was not significant by age. PERH I at the first minute was not significant by gender.
Conclusion: The brain's electrical activity response to HPT depends on the onset time in epileptic patients and facilitates accurate diagnosis of epilepsy. EEG disorganization revealed at the first minute of HPT. Therefore in such cases, it is advisable not to expand HPT after the first minute. The effect of HPT on the EEG should support in diagnosis and treatment of epilepsy. EEGHUB.GE provides wide opportunities in research and medicine to study NS dysfunction

Disclosures: I. Khachidze: None. **N. Adamashvili:** None. **L. Chantadze:** None. **M. Advadze:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.02/E3

Topic: B.08. Epilepsy

Support: NIH Grant R01 NS065957
NIH Grant R15 AT008742
NIH Grant R15 NS066392

Title: Changes in multiple CSF amino acids correspond with antiseizure effects of the ketogenic diet in pediatric epilepsy

Authors: D. N. RUSKIN[1], D. BOISON[3], J. D. GEIGER[4], M. DAHLIN[5], ***S. A. MASINO**[2]; [1]Trinity Col., Hartford, CT; [2]Trinity Col., HARTFORD, CT; [3]Rutgers, New Brunswick, NJ; [4]Dept Pharmacol/Physiol/Therapeut, Univ. North Dakota, GRAND FORKS, ND; [5]Karolinska Hosp., Stockholm, Sweden

Abstract: The low carbohydrate, high fat ketogenic diet can be an effective anticonvulsant treatment in some patients with pharmacoresistant epilepsy. Its mechanism(s) of action, however, remain uncertain. We performed metabolomic analysis of cerebrospinal fluid samples taken before and during ketogenic diet treatment in five patients with optimal response (100% seizure remission) and five with no response (0% seizure improvement) to search for differential diet effects on amino acids (AAs) and dipeptides in these two groups. Responders and non-responders were similar in age range and included males and females. Seizure types and the etiologies or syndromes of epilepsy varied widely, and did not appear to differ systematically between responders and non-responders. 116 AAs and dipeptides were quantified. Prediet v. during ketogenic diet analysis revealed forty of these were changed significantly by ketogenic diet in optimal responders, whereas only four were significant in non-responders (a significant difference in frequency between groups (χ^2 $p < 0.00001$). Of proteinogenic amino acids, most decreased, including the three aromatic AAs, while glycine increased. Branched-chain AAs did not change although many of their metabolites significantly increased. Related to glutathione metabolism, many γ-glutamylated AAs significantly decreased, whereas the cystathionine metabolites α-hydroxybutyrate, α -aminobutyrate, and α -ketobutyrate increased. Concerning group differences in prediet metabolite profiles, optimal responders had lower levels of several γ-glutamylated AAs and all three branched-chain AAs compared to non-responders. These metabolites may therefore be biomarkers of the efficacy of any attempted ketogenic diet antiseizure treatment. The data suggest that a stronger metabolic response to the ketogenic diet relates to a better anticonvulsant response (Masino et al. 2021, Nutr. Metab.); such variability is likely due to inherent biological factors of individual patients.

Disclosures: D.N. Ruskin: None. **D. Boison:** None. **J.D. Geiger:** None. **M. Dahlin:** None. **S.A. Masino:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.03/E4

Topic: B.08. Epilepsy

Support: National Science Foundation GRFP DGE-1842487
University of Southern California Annenberg Fellowship
USC WiSE Top-Off Fellowship
National Science Foundation under the Career Award CPS/CNS-1453860
NSF CCF-1837131
NSF MCB-1936775
NSF CMMI-1936624
NSF CNS-1932620
U.S. Army Research Office (ARO) under Grant No. W911NF-17-1-0076
U.S. Army Research Office (ARO) under Grant No. W911NF-23-1-0111
DARPA Young Faculty Award
DARPA Director Award, under Grant Number N66001-17-1-4044
2021 USC Stevens Center Technology Advancement Grant (TAG) award
Intel faculty award
Northrop Grumman grant

Title: Fractional-order model-based framework for autonomous seizure detection and mitigation in medically refractory epilepsy

Authors: *E. REED[1], Y. WANG[1], K. ALZAMEL[1], P. BOGDAN[1], S. PEQUITO[2], A. ASHOURVAN[3];
[1]USC, Los Angeles, CA; [2]Uppsala Univ., Uppsala, Sweden; [3]Univ. of Kansas, Lawrence, KS

Abstract: Epilepsy, one of the most prevalent neurological disorders worldwide, affects many patients who cannot be effectively treated with medication. With surgery success rates ranging from 30% to 70%, physicians have turned to neurostimulation devices as an alternative treatment for medically refractory epilepsy. However, achieving seizure freedom remains a challenge, primarily due to the inability to accurately pinpoint seizure onsets. This limitation hampers the precise administration of neurostimulation to prevent impending seizures. Consequently, efforts are underway to discover digital biomarkers capable of autonomously identifying seizure onsets, enabling the development of effective seizure control strategies.Here we investigate a model-based framework designed to detect and mitigate seizures. Specifically, we used fractional calculus to map intracranial electroencephalography (iEEG) to a mathematical model. By analyzing the mean-squared error between the model and the iEEG data, we establish the suitability of fractional-order models for representing iEEG signals. Next, we examine the eigenvalues of the model and quantify the energy in the iEEG signals before and after the clinically defined seizure onset. Our findings demonstrate that the model becomes unstable when at least one eigenvalue surpasses a magnitude of 1. By examining the iEEG data from eight patients with similar seizure types, we observe a consistent alignment between the instability of the mathematical model and the clinically identified seizure onset. These results support the concept of critical slowing in epilepsy. Moreover, we evaluate the efficacy of a recently proposed mitigation strategy by testing it on the same patient datasets. This strategy aims to stabilize fractional-order models estimated from iEEG data by assessing their stability at 1-second intervals and applying calculated stimulation to restore signal stability. Our analysis confirms that this mitigation strategy reduces the energy of the iEEG signals to pre-ictal levels in all eight patients. Together, extensive testing affirms the efficiency of calculating the stability of fractional-order models for autonomously determining seizure onsets. We evaluate a novel

stimulation strategy that stabilizes fractional-order models and validate its effectiveness on the same patient datasets. This work establishes a foundation for adopting an algorithmic and mathematical framework in clinical settings, offering the potential to enhance the quality of life for medically refractory epileptic patients.

Disclosures: **E. Reed:** None. **Y. Wang:** None. **K. Alzamel:** None. **P. Bogdan:** None. **S. Pequito:** None. **A. Ashourvan:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.04/E5

Topic: B.08. Epilepsy

Support: NIH NS094399-06

Title: Big Data approaches to characterize pathological High Frequency Oscillations in human intracranial EEG

Authors: ***J. LIN**[1], M. ZOCHOWSKI[2], K. SHEDDEN[3], S. GLISKE[5], W. STACEY[4]; [2]Physics and Biophysics, [3]Statistics, [4]Neurol., [1]Univ. of Michigan, Ann Arbor, MI; [5]Neurosurg., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: High Frequency Oscillations have been identified as a potential biomarker of epileptic tissue for over 20 years. However, HFOs are also produced by normal brain activity, and it has been very difficult to distinguish when they are indicative of pathological activity. A major challenge with this question is that there is no clear 'gold standard' with which to compare. One approach to this problem is to search for natural clusters in the data, but most prior work has dealt with limited datasets that do not allow robust clustering. In this work, we used an automated algorithm that collected millions of HFOs from all patients at the University of Michigan for 8 years. This dataset allows us to use clinical definitions to search for differences between different stratifications of 'normal' and 'abnormal'. Here, we perform a retrospective comparison between the actual resected volume (RV), which is a direct analog of surgical outcome, and the remaining non-resected tissue through a suite of electrographic and spectral features derived from HFO specific waveforms. Our classifier reveals a good separation between resected volume HFOs and that of the remaining tissues in good outcome patients. Furthermore, clustering of the HFO features reveals a natural separation of RV HFOs indicating epilepsy specific HFOs. We compared these results with identification of fast ripples (250-500 Hz) alone, which have been considered by some studies to be more specific for epileptic tissue. Fast ripples were not better than traditional HFOs (80-500 Hz) at distinguishing the RV. These results identify a set of HFO features that are associated with good outcome when they are resected, which we propose are a diagnostic biomarker of epileptic tissue, and could be used as a potential tool for guiding surgical resection.

Disclosures: **J. Lin:** None. **M. Zochowski:** None. **K. Shedden:** None. **S. Gliske:** None. **W. Stacey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Licensing agreement with Natus Medical.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.05/E6

Topic: B.08. Epilepsy

Support: ERC-Syn 2020-26
INCR Predilepsy

Title: Patient specific scalp EEG simulation of focal and generalized epilepsy

Authors: *P. BENQUET[1], M. YOCHUM[1], E. KÖKSAL ERSÖZ[1], A. KAMINSKA[3], R. NABBOUT[4], I. MERLET[1], P. VAN BOGAERT[5], F. BARTOLOMEI[6], M. KUCHENBUCH[7], F. WENDLING[2];
[2]LTSI, [1]INSERM U1099 -LTSI, Rennes, France; [3]Clin. Neurophysiol., Hôpital Necker Enfants Malades, AP-HP, Paris Univ., Paris, France; [4]Reference Ctr. for Rare Epilepsies, Dept. of Pediatric Neurology, Member of EPICARE Network,, Hôpital Necker Enfants Malades, AP-HP, Paris Univ., paris, France; [5]Dept. of Pediatric Neurol., CHU d'Angers, LARIS, Univ. d'Angers, Angers, France; [6]INSERM & Inst. De Neurosciences Des Systè, Marseille, France; [7]Pediatric and Genet. Department, CHU, Nancy, France

Abstract: Context: Scalp electroencephalography (EEG) is the gold standard method for recording interictal and ictal activity in epileptic patients. Dynamic and topography of EEG signals are patient-specific. They depends on the individual's brain anatomy, type of the lesion, propagation of the epileptic activity within large-scale epileptic networks. A key question arises: how can we interpret epileptiform events from a neurophysiological perspective? To answer this, we developed a whole brain model based on neuronal physiology that can be used to interpret patient-specific EEG signals.
Methods. We developed a whole brain neuro-inspired computational model based on neural masses. Each laminar neural mass model (NMM) of the neocortex consists of Glutamatergic and GABAergic (VIP, PV, SST and NGFC subtypes) neurons. Cellular connectivity and kinetics properties of post synaptic potentials were fitted on physiological knowledge. The brain model consists of a large-scale network of 82 interconnected NMM based on the human connectome. Forward problem is used to simulate high density EEG.
Results. Comparison with real data allowed us to reproduce IEDs shape with high degree of realism on scalp EEG in focal epilepsy. Simulations produced hypotheses about cell-related and network-related mechanisms underlying the generation of scalp-recorded epileptic spikes. Simulation of absence seizures reveals a major role of the associative subcortical-cortical loop in

the generation of the 3 Hz spike-wave patterns. High density EEG recordings in child during absence seizure and sources reconstruction allowed us to reproduce the individual specific patterns.
Conclusion. Physiological models have a high face, integrative and explanatory value that can be tuned to be patient specific.

Disclosures: P. Benquet: None. **M. Yochum:** None. **E. Köksal Ersöz:** None. **A. Kaminska:** None. **R. Nabbout:** None. **I. Merlet:** None. **P. Van Bogaert:** None. **F. Bartolomei:** None. **M. Kuchenbuch:** None. **F. Wendling:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.06/E7

Topic: B.08. Epilepsy

Title: Characterizing idiopathic generalized epilepsies: a functional connectivity hdEEG study

Authors: *E. FENOGLIO[1], E. TARTARA[2], R. ESPOSTO[3], E. LEUCI[3], C. A. GALIMBERTI[2], R. DE ICCO[4], M. SEMPRINI[1];
[1]Rehab Technologies, Inst. Italiano di Tecnologia, Genoa, Italy; [2]Ctr. per lo Studio e la Cura dell'Epilessia e Servizio di Elettroencefalografia Clinica, UC Neurofisiopatologia, IRCCS Fondazione Mondino, Pavia, Italy; [3]Scuola di Specializzazione in Neurologia, Dept. di Scienze del Sistema Nervoso e del Comportamento, Univ. degli Studi di Pavia, Pavia, Italy; [4]Dept. of Neurol. and Neurorehabilitation, IRCCS Fondazione Mondino, Pavia, Italy

Abstract: Idiopathic generalized epilepsies (IGE) are chronic syndromes with a variable prognosis, yet the distinctions between different electroclinical presentations remain unclear [Scheffer et al., 2017]. Functional connectivity studies have identified specific brain systems (thalamic-cortical, default-mode-network, sensory-motor cortical areas) as crucial in IGE [Clemens et al., 2013]. However, existing literature employs low-density EEG or combined methods with fMRI, focusing on individual electroclinical subtypes. Limited studies have explored EEG connectivity across all syndromes using low-density EEG, revealing potential specific connectivity "endophenotypes" [Clemens et al., 2012]. This study investigates resting-state functional connectivity in different syndromes of IGE using high-density EEG (hdEEG) recordings. Moreover, it explores connectivity patterns related to variables like photosensitivity (PPR) and clinical course. Data was collected using a 128-channel EEG system from 40 individuals with IGE and 30 healthy controls. 3 epochs of 20s were manually selected for each participant to exclude ictal recordings and minimize artifacts. Epochs underwent standard preprocessing steps. Then, source localization was conducted using the eLORETA method. A set of 30 regions of interest (ROIs), representing 6 distinct networks and 2 thalamic-related ROIs [Samogin et al., 2020; Moeller et al., 2011], were chosen. Data normality was assessed with one-sample Kolmogorov-Smirnov test. To evaluate the impact of different conditions on brain

connectivity, a one-way repeated measures analysis of variance (ANOVA) was employed for each frequency band. Post hoc analysis was performed using the Fisher Least Significant Difference method. The significance level for all analyses was set at 0.05. We found a higher functional connectivity in IGE conditions versus healthy subjects in low frequency bands, mostly involving thalamic-cortical and cortico-cortical connections. Moreover, we identified a "shared" pattern across syndromes, as well as syndrome-related specific patterns of abnormal functional connectivity. Presence of PPR associates with network-specific increased connectivity, while absence of PPR, is characterized by connectivity resembling healthy controls. We also found band-specific hallmarks of clinical course, such as freedom from seizures post-therapy and seizure-free periods < 5 years, especially involving the thalamus, affirming its role in seizure susceptibility. Our findings could advance understanding of IGE's pathophysiology in terms of network dysfunctions, possibly aiding in personalized care options.

Disclosures: E. Fenoglio: None. **E. Tartara:** None. **R. Esposto:** None. **E. Leuci:** None. **C.A. Galimberti:** None. **R. De Icco:** None. **M. Semprini:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.07/E8

Topic: B.08. Epilepsy

Title: Diagnostic and Prognostic Value of Serum S100b Levels in Patients with Status Epilepticus: A Retrospective Cohort Study

Authors: *S. KOH, G. KIM, H. KIM, B. KIM, J. CHOI;
Dept. of Brain Sci., Ajou Univ. Sch. of Med., Suwon-si, Korea, Republic of

Abstract: Status epilepticus (SE) is a life-threatening neurological emergency with challenging diagnosis due to variable clinical presentations and lack of reliable biomarkers. S100b, a calcium-binding protein predominantly expressed in astrocytes, has shown promise as a potential biomarker for seizure-related injury and neuroinflammation. This retrospective cohort study aimed to investigate the diagnostic and prognostic value of serum S100b levels in patients presenting with seizure or seizure-like symptoms, particularly focusing on SE.From Jan 2016 to Mar 2023, 197 patients were included. Serum S100b levels were significantly higher in the SE group (0.1 [0.06 – 0.15] ng/mL) compared to no seizure (0.04 [0.03 – 0.06] ng/mL) and resolved seizure (0.07 [0.05 – 0.1] ng/mL) groups ($p < 0.001$). Within the subset of resolved seizures, a tendency toward higher S100b levels was observed in frequent seizures (0.11 [0.06 – 0.15] ng/mL) compared to single (0.07 [0.05 – 0.1] ng/mL) seizure ($p = 0.0745$). In the SE group, no significant difference in S100b levels was observed between SE and refractory SE (0.1 [0.06 – 0.15] ng/mL vs. 0.09 [0.07 – 0.18] ng/mL, $p = 0.9448$).Within the SE patients, etiological classification did not show significant differences in S100b levels, including acute SE (n = 38), progressive SE (n = 4), remote SE (n = 32), and unknown etiology SE (n = 11) (0.1 [0.07 - 0.22]

vs. 0.07 [0.05 - 0.16] vs. 0.09 [0.06 - 0.14] vs. 0.12 [0.09 - 0.14], respectively, p = 0.8019). Moreover, serum S100b levels could not distinguish the different semiologies of SE (focal SE: 0.1 [0.09 – 0.12], generalized convulsive SE: 0.1 [0.06 – 0.22], non-convulsive SE: 0.09 [0.06 – 0.16], p = 0.8832). Prognostically, S100b levels did not correlate with mortality, hospital stay (r = -0.0796, p = 0.4691), or treatment response (r = 0.0068, p = 0.9505) in the SE group.This study provides insights into the diagnostic and prognostic value of serum S100b levels in patients with seizure or seizure-like symptoms, specifically in relation to status epilepticus. Serum S100b levels were found to differ significantly among different seizure groups and demonstrated potential as a diagnostic marker for status epilepticus. However, serum S100b levels did not significantly correlate with mortality, length of hospital stay, or treatment response in the SE group. Further research is warranted to identify additional prognostic markers and unravel the complex mechanisms underlying status epilepticus.

Disclosures: **S. Koh:** None. **G. Kim:** None. **H. Kim:** None. **B. Kim:** None. **J. Choi:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.08/E9

Topic: B.08. Epilepsy

Title: The usefulness of TSPO-PET in focal epilepsy

Authors: *M. IZUMOTO[1], M. MUKAI[2], Y. IWATANI[6], H. M. KHOO[3], N. TANI[3], K. ISOHASHI[4], S. NABATAME[2], T. YANAGISAWA[3,5], S. OSHINO[3], H. KATO[4], H. KISHIMA[3], K. KAGITANI-SHIMONO[6];
[1]Osaka Univ., Suita-city, Osaka-prefecture, Japan; [2]Dept. of Pediatrics, [3]Dept. of Neurosurg., [4]Dept. of Nuclear Med. and Tracer Kinetics, [5]Inst. for Advanced Co-creation Studies, Osaka Univ., Suita, Japan; [6]Dept. of Child Develop., United Grad. Sch. of Child Development, Osaka Univ., Suita, Japan

Abstract: Introduction: Neuroinflammation is a key factor in the pathophysiology of various chronic neurological diseases, including epilepsy, but the neuroimage to visualize neuroinflammation has not been used in clinical management. Recent studies have found that microglia were activated in the surgical specimens of focal epilepsy and played an important role in the pathophysiology of animal epilepsy models. Positron emission tomography (PET) imaging with translocator protein (TSPO) binding radioligands is the most widely used to assess microglial activation. In this study, we investigated the usefulness of TSPO-PET in clinical epilepsy care and its advantages over other neuroimaging. Methods: We examined TSPO-PET of [11C] DPA713 in patients with intractable unifocal epilepsy at Osaka University Hospital. The PET image was co-registered to the individual’s magnetic resonance imaging (MRI), and the standardized uptake value ratios (SUVr) in volumes of interest (VOI) were calculated by comparing to the identical area in the contralateral lobe as the reference region. The positive

uptake VOI was considered if its SUVr was over 1.2. The results of TSPO-PET were compared with MRI, [18F] fluorodeoxyglucose (FDG)-PET, and magnetoencephalography (MEG). This study was approved by the Institutional Review Board of Osaka University Hospital. Results: 21 patients (median age: 14 years, male/female: 12/9) were included in this study. 76 % of them showed increased uptake of [11C] DPA713 in the focal epileptogenic lesion, although the sensitivities of MRI, FDG-PET, and MEG were 43 %, 60 %, and 68 %, respectively. The TSPO uptake areas were concordant with all epileptogenic lesions found by MRI and FDG-PET. The SUVr value showed no differences according to seizure frequency and duration of epilepsy. Conclusions: This study indicates that TSPO-PET can detect neuroinflammation in focal epilepsy and has a high sensitivity in delineating epileptogenic zones and a high concordance with epileptic focus found by other modalities. This PET scan might help clinical decisions including surgery in focal epilepsy care.

Disclosures: M. Izumoto: None. **M. Mukai:** None. **Y. Iwatani:** None. **H.M. Khoo:** None. **N. Tani:** None. **K. Isohashi:** None. **S. Nabatame:** None. **T. Yanagisawa:** None. **S. Oshino:** None. **H. Kato:** None. **H. Kishima:** None. **K. Kagitani-Shimono:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.09/E10

Topic: B.08. Epilepsy

Title: Utilizing singular value decomposition to identify resected intracranial eeg channels

Authors: *K. TYNER[1], M. MCCUMBER[2], C. KUGEL[1], S. V. GLISKE[1];
[1]Neurosurg., [2]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Approximately three million people in the United States suffer from epilepsy, a neurological disorder leading to recurrent and unpredictable seizures. Of those affected, around 30% have drug-resistant epilepsy, characterized as recurrent seizures despite treatment with two or more anti-seizure medications. For those individuals, resection to remove the epileptogenic zone (EZ), the theoretical region responsible for seizure generation, is the best treatment option. To identify the EZ, subjects may undergo invasive monitoring with intracranial EEG (iEEG) to capture ictal activity. However, identifying electrodes that should be resected can be a difficult and ambiguous task. In this study, we aim to identify whether singular value decomposition (SVD) could be a useful tool for identifying channels that may be candidates for resection. We preprocessed the iEEG data from 6 stereo-electroencephalography subjects (1 seizure per subject) from an online database (doi:10.18112/openneuro.ds004100.v1.1.1) who were diagnosed with medial temporal lobe epilepsy (MTLE) and had non-lesional MRIs. Bad channels were redacted based on the clinical notes, and data were preprocessed by filtering from 1 - 40 Hz, followed by common average referencing. We then performed a time-frequency analysis on each channel identified as the seizure onset zone (SOZ) and obtained the maximum

power of those channels in the first 10 seconds after the clinically defined seizure onset. We then identified a custom frequency range for seizure analysis based on the median identified frequency of all SOZ channels +/- 1 Hz and calculated the power in the identified frequency range for each channel in the recording in 1-sec epochs (no overlap). We calculated the mean and standard deviation of the power for each channel from 120 to 20 sec before marked seizure onset. We then identified epochs up to 10 seconds after marked seizure onset where the power in the epoch and the adjacent epoch were greater than the mean + 2SDs for that channel to identify electrographic changes associated with seizure onset. We then performed singular value decomposition (SVD) on 10 sec of data beginning at the electrographic seizure onset. The absolute value of the first left singular vector was thresholded at 0.2, and the status of channels above the threshold were identified. We find that a mean of 66% of channels identified by the algorithm were clinically identified as being in the SOZ and resected. Our results indicate that SVD may be a useful tool to identify channels that may be candidates for resection in MTLE.

Disclosures: K. Tyner: None. **M. McCumber:** None. **C. Kugel:** None. **S.V. Gliske:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.10/E11

Topic: B.08. Epilepsy

Support: We thank Abbey Becker, Thaddeus Brink, Jon Giftakis, David Linde and Robert Raike at Medtronic for their technical support in this study. NIH/NINDS UG3/UH3 NS112826

Title: Stimulation of the thalamus for arousal restoral in temporal lobe epilepsy (START) clinical trial: interim results

Authors: *H. BLUMENFELD[1], T. YADAV[2], Z. ZHANG[2], V. KREMEN[4], C. BENJAMIN[3], E. DAMISAH[3], L. H. HIRSCH[3], B. P. LITVINOV[3], D. SPENCER[3], V. SLADKY[4], J. HONG[5], K. A. BUJARSKI[5], R. M. ROTH[5], J. BAKER[6], E. Y. CHOI[7], J. HENDERSON[7], B. RUTT[7], J. GIACINO[8], B. H. BRINKMANN[4], G. W. CULLER[5], N. GREGG[4], B. LUNDSTROM[4], I. H. QURAISHI[3], J. P. ARONSON[5], J. GERRARD[3], J. VAN GOMPEL[4], C. R. BUTSON[9], N. D. SCHIFF[6], B. JOBST[5], G. WORRELL[4];
[1]Neurol., Yale Univ. Sch. of Med., New Haven, CT; [2]Neurol., [3]Yale Univ., New Haven, CT; [4]Mayo Clin., Rochester, MN; [5]Dartmouth-Hitchcock Med. Ctr., Manchester, NH; [6]Weill Cornell Med. Sch., New York city, NY; [7]Stanford Univ., Stanford, CA; [8]Harvard Med. Sch., Boston, MA; [9]Univ. of Florida, Gainesville, FL

Abstract: Impaired consciousness in seizures negatively impacts quality of life for people with temporal lobe epilepsy (TLE). Prior research suggests that deep brain stimulation of the thalamic intralaminar central lateral nucleus (CL) can be effective in improving conscious awareness. The

START clinical trial investigates the feasibility and safety of bilateral CL stimulation in restoring consciousness in patients with TLE. Five patients with medically refractory temporal lobe epilepsy were implanted with neurostimulator (investigational Medtronic Summit RC+STM). Parameters of CL stimulation were individually titrated initially to tolerance in the waking state, and then effectiveness for producing arousal was evaluated during natural sleep based on electrophysiological characteristics and video behavior. Patients then entered a randomized double-blind phase, where all seizures received 5sec of therapeutic responsive hippocampal stimulation but were randomly assigned to receive therapeutic or sham CL stimulation (125 Hz or 40 Hz). Behavior was scored (0-3) with an Automatic Response Testing in Epilepsy (ARTiE) Watch. Patients also completed an electronic seizure diary. CL stimulation during sleep decreased scalp EEG delta/theta (1-7 Hz) power in all patients, and produced behavioral arousal. Two patients exhibited robust arousal characterized by eye-opening, and natural spontaneous movements. Patients returned to sleep after stimulation was turned off. Behavioral responsiveness during seizures was significantly impaired compared to the baseline (2.19 vs. 2.97; $p < .05$, Wilcoxon ranksum test). Of 197 total seizure diary entries, patients reported impaired awareness in 52%; impaired recall of events in 62%; and impaired ability to respond in 55% of seizures. Among the two patients who exited the double-blind CL stimulation phase, one benefited from therapeutic CL stimulation, with significantly higher ARTiE scores in seizures with CL stim (n=16) vs. sham (n=12) ($p=0.021$). No significant differences were observed for the other patient . Findings from sleep titration and CL blinded phase support the effectiveness of thalamic CL stimulation to produce physiological and behavioral arousal from sleep and improved behavior during seizures in one patient to date. This suggests that thalamic CL may be an effective therapeutic target for impaired arousal in seizures and other disorders of consciousness. These results also highlight the potential benefits of automatic at-home behavioral assessment with wearable technology to evaluate impaired conscious awareness in the ictal and postictal periods.

Disclosures: **H. Blumenfeld:** None. **T. Yadav:** None. **Z. Zhang:** None. **V. Kremen:** None. **C. Benjamin:** None. **E. Damisah:** None. **L.H. Hirsch:** None. **B.P. Litvinov:** None. **D. Spencer:** None. **V. Sladky:** None. **J. Hong:** None. **K.A. Bujarski:** None. **R.M. Roth:** None. **J. Baker:** None. **E.Y. Choi:** None. **J. Henderson:** None. **B. Rutt:** None. **J. Giacino:** None. **B.H. Brinkmann:** None. **G.W. Culler:** None. **N. Gregg:** None. **B. Lundstrom:** None. **I.H. Quraishi:** None. **J.P. Aronson:** None. **J. Gerrard:** None. **J. Van Gompel:** None. **C.R. Butson:** None. **N.D. Schiff:** None. **B. Jobst:** None. **G. Worrell:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.11/E12

Topic: B.08. Epilepsy

Support: Germany´s Excellence Strategy EXC204989829218

Title: Spatial and temporal correlation structure in human brain dynamics

Authors: *P. M. MÜLLER[1,3,2], G. MIRON[1,4,5], M. HOLTKAMP[4,5], C. MEISEL[1,3,2,6,7]; [1]Computat. Neurology, Dept. of Neurol., [2]NeuroCure Cluster of Excellence, Charite Universtätsmedizin Berlin, Berlin, Germany; [3]Berlin Inst. of Hlth., Berlin, Germany; [4]Inst. for Diagnostics of Epilepsy, [5]Dept. of Neurol., Epilepsy-Center Berlin-Brandenburg, Berlin, Germany; [6]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [7]Ctr. for Stroke Res. Berlin, Berlin, Germany

Abstract: Understanding normal and aberrant cortical network function is crucial for improving diagnostics and treatments in patients with neurological disorders like epilepsy. The integration of inputs and information over space and time is believed an essential function of cortical networks for which spatial and temporal correlations (SC/TC) are regarded as respective measures. However, changes of SC/TC over multiple days under anti-epileptic drug (AED) action, between different states of vigilance, and various brain regions are still incompletely understood. To address this issue, we assessed SC/TC in multiday, intracranial EEG recordings of patients with epilepsy undergoing presurgical monitoring from two independent datasets (44/23 patients 17/12 female, mean age=30±7/28±13 years). We evaluated high gamma power fluctuations (56-96 Hz) as a proxy for neuronal firing. SC (only in the second dataset) were quantified as the area under the cross-correlation curve from 7 to 79mm, while TC were defined as the half-width at half maximum of the autocorrelation function. Additionally, we explored a neuronal network model with excitatory and inhibitory threshold neurons to investigate SC/TC characteristics. In the first dataset, we observed a significant decrease in TC from 0.5 to 0.4 seconds ($p<.01$) with increasing AED load. In the second dataset, TC decreased from 1.5 to 1.2 seconds with an increase in AED load ($p<.05$) and to 0.7 seconds during slow-wave sleep (SWS) ($p<.001$). SC were reduced by 9% under increased AED load ($p<.05$) and 20% during SWS compared to non-SWS ($p<.05$). Additionally, TC was up to two times higher in regions higher in the functional hierarchy of the visual pathway, indicating extended information integration capabilities in higher hierarchy regions. In the model, we found the longest SC/TC at a critical point, i.e., at a phase transition. The system was shifted away from the critical point by generally weakening the connections or by modifying the exhibition inhibition balance, similar to certain AEDs, leading to a decrease of SC/TC. Our findings suggest that the decline in SC/TC in human cortical dynamics under AED action could indicate a drift away from critical dynamics. Additionally, the extended TC observed in higher hierarchy regions may indicate their proximity to a critical point, which could facilitate complex computations. Our findings reveal systemic and functional links between measurable changes in cortical dynamics offering insights into the brain's information processing capabilities, which vary along a hierarchical cortical gradient, across different states of vigilance, and under AEDs.

Disclosures: P.M. Müller: None. **G. Miron:** None. **M. Holtkamp:** None. **C. Meisel:** 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.; NARSAD Young InvestigatorGrant.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.12/E13

Topic: B.08. Epilepsy

Support: Italian Ministry of healt grant PNRR-MAD-2022-12376068

Title: Compromised oligodendrocytes, myelination status and homeostasis in grey matter of type IIb focal cortical dysplasia: possible role in the epileptogenesis

Authors: *L. ROSSINI[1], E. MADERNA[1], L. TASSI[2], F. DELEO[1], L. UVA[1], M. M. DE CURTIS[1], R. GARBELLI[1];
[1]Inst. Nazionale Neurologico Carlo Besta, Milan, Italy; [2]Niguarda Hosp., Milan, Italy

Abstract: Loss of myelin and altered oligodendrocytes (ODC), the cells responsible for the myelin production, were reported in white matter in human tissues from different focal epilepsies. Due to the important role of ODC and proper myelin-coated axons in the extracellular potassium homeostasis, and the capability of high potassium accumulation to promote depolarization and seizures, it was hypothesized that demyelinated/less myelinated axons may contributed to hyperexcitability (de Curtis et al. 2021). Astrocytes are coupled through gap junctions, which is a prerequisite to redistribute elevated potassium from sites of excessive neuronal activity to sites of lower concentration. Spatial buffering depends on proper distribution and function of astrocytic potassium channels and gap junctions.
We aimed to investigate the grey matter myelination status, ODC density and the expression of Cx43 and Cx32, the main astrocytic and ODC connexin isoforms, in type IIb focal cortical dysplasia (FCDIIb), a developmental malformation and frequent cause of drug-resistant focal epilepsy, characterized by a well-known white matter pathology.
We studied post-surgical FCDIIb neocortical specimens (n=6) comparing lesional and perilesion area in the same tissue section, using electron microscopy to evaluate the number of myelinated axons and the presence of myelin sheaths abnormalities. Immunohistochemistry to evaluate mature and precursor ODC was performed (PDGFRalfa, Olig2, BCAS1, MBP, CNPase antibodies) and the expression of gap junctions (Cx32, Cx43) was investigated (n=18).
We demonstrated a reduction of grey matter myelin fibers in lesion compared to perilesion. Moreover, a proportion of myelin sheaths exhibit interruption points and myelin coating appear thinner. ODC were reduced in lesion in comparison with perilesion. We also found a peculiar pattern of gap junction expression in lesion with large aggregates of Cx43+ puncta clustered around balloons and astrocytes; Cx32 normally observed in ODC was found abnormally expressed in astrocytes and balloons.
These results indicate that: 1) in FCDIIb the myelination status is altered not only in white matter, as previously demonstrated, but also in grey matter; 2) abnormal pattern of gap junction expression is present suggesting an improper spatial buffering. These alterations, only present in the core of the lesion, seem to be part of the malformative spectrum observed in this dysplasia. We can speculate that the presence of less-myelinated axons and ineffective potassium buffering may amplified and sustained tissue hyperexcitability. Electrophysiological data will be necessary to validate this hypothesis.

Disclosures: **L. Rossini:** None. **E. Maderna:** None. **L. Tassi:** None. **F. Deleo:** None. **L. Uva:** None. **M.M. de Curtis:** None. **R. Garbelli:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.13/E14

Topic: B.08. Epilepsy

Support: NIH NINDS R01NS115868

Title: Children with Rolandic epilepsy have micro- and macrostructural abnormalities in white matter constituting networks necessary for language function

Authors: *L. OSTROWSKI[1,2], D. M. CHINAPPEN[2,4], S. M. STOYELL[2], D. Y. SONG[2], E. E. ROSS[2], M. A. KRAMER[4], B. C. EMERTON[3], C. J. CHU[2,5];
[1]UCSD Dept. of Neurosciences, La Jolla, CA; [2]Neurol., [3]Psychiatry, Massachusetts Gen. Hosp., Boston, MA; [4]Mathematics and Statistics, Boston Univ., Boston, MA; [5]Harvard Med. Sch., Boston, MA

Abstract: Introduction: Rolandic epilepsy (RE) is a transient developmental epilepsy syndrome affecting the centrotemporal cortex in which language function is often impaired. We compared language profiles and white matter (WM) features in a cohort of children with RE to gain insights into the relationship between anatomical findings and symptoms.
Methods: We enrolled children with active (n=13) and resolved (n=12) RE and age-matched controls (n=17). High-resolution MRIs including diffusion tensor imaging and standardized neuropsychological language assessments were conducted. We isolated superficial WM abutting the inferior rolandic cortex and superior temporal gyrus using a cortical parcellation atlas, and identified the arcuate fasciculus connecting these regions using probabilistic tractography. WM microstructural characteristics (axial, radial, and mean diffusivity, and fractional anisotropy) in each region were compared between groups and examined for relation to language scores.
Results: We found significant language deficits in children with RE compared to controls. Children with RE performed worse on assessments of phonological awareness (p=0.045) and verbal comprehension (p=0.050). Children with active RE performed worse compared to controls on phonological awareness (p=0.028), verbal comprehension (p=0.028), and verbal category fluency (p=0.031), and compared to children in remission on verbal category fluency (p=0.009), verbal letter fluency (p=0.006), and expressive one-word picture vocabulary (p=0.045). Abnormal superficial WM microstructure in centrotemporal regions was found in children with RE, characterized by increased diffusivity and fractional anisotropy compared (AD p=0.014, RD p=0.028, MD p=0.020, FA p=0.024). Structural connectivity of the arcuate fasciculus was lower (p=0.045) and all diffusivity metrics were higher (AD p=0.007, RD p=0.006, MD p=0.016) in children with RE, with no difference in fractional anisotropy (p=0.22). Although not passing correction for multiple comparisons, trends were seen between fractional

anisotropy in the arcuate fasciculus and verbal category fluency (p=0.047) and expressive one-word picture vocabulary (p=0.036).
Conclusion: We found impaired language development in children with RE, particularly those with active seizures, and abnormalities in WM adjacent to the cortical seizure onset zone. Although relationships between language scores and WM metrics did not withstand correction for multiple comparisons, these findings suggest atypical maturation in language-related WM fibers in this syndrome with concomitant language impairment.

Disclosures: L. Ostrowski: None. **D.M. Chinappen:** None. **S.M. Stoyell:** None. **D.Y. Song:** None. **E.E. Ross:** None. **M.A. Kramer:** None. **B.C. Emerton:** None. **C.J. Chu:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.14/E15

Topic: B.08. Epilepsy

Title: Analysis of Robustness of Epileptic Spike Cycles across different Modalities to forecast seizures and proactive intervention

Authors: *A. KASHYAP[1], P. MÜLLER[1], G. ARMIN[2], G. MIRON[2], C. MEISEL[3];
[1]Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Charite, Berlin, Germany; [3]Charité Berlin, Berlin, Germany

Abstract: Epileptic activity is marked by interictal spikes that are unique to the disease pathology as a result of large synchronous bursting of neurons. The frequency of these spikes occurs in cycles, both within individual days and over extended periods on the timeframe of a month. The phase in these monthly and daily cycles has been key in understanding and predicting the relative timing of seizure onset. Therefore, in order to monitor and forecast seizures it has become important in being able to characterize these cycles in individuals. Currently, various methods have been employed to examine these cycles in intracranial and surface electroencephalogram (EEG) recordings, but a definitive consensus on the most reliable approach for characterizing patient data remains elusive. This lack of agreement stems from the challenge of extrapolating the methodology to a diverse patient group, as the source and frequency of epileptic spike activity differ significantly among individuals. Additionally, each person is typically prescribed a distinct combination of medications that can impact spike activity. Further, robust identification of such cycles is crucial for application to subscalp EEG devices aimed at preventing seizures with adaptive stimulation approaches. Therefore, an improved understanding of how different methodologies are able to characterize the spike cycles and whether methods using fewer electrodes and subsampled data can recover the cyclical structure is crucial for further translation of these forecasting methods. In this study, we used a large-scale dataset containing long-term intercranial EEG recordings from 28 patients on different medications, and applied a suite of different methods to see how robust each one is at

characterizing spiking cycles. These methods include using an AI automated spike detection method (AEiD) as well as other techniques to characterize the interictal activity using faster and simpler measures, including signal variance. Our preliminary findings indicate that robust identification of seizure cycles is feasible even for short 10 min recordings from a single electrodes. In the future we aim to create a platform of these different methodologies and across recording devices. This may help to benchmark and identify the most efficient method application to forecasting seizures and initiating interventions using implantable subscalp EEG devices.

Disclosures: A. Kashyap: None. **P. Müller:** None. **G. Armin:** None. **G. Miron:** None. **C. Meisel:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.15/E16

Topic: B.08. Epilepsy

Support: EU ERC-Syn Grant (#855109)

Title: Multiscale neuro-inspired models for interpretation of EEG signals in epilepsy patients

Authors: *F. WENDLING[1], E. KOKSAL[2], M. AL HARRACH[1], F. BARTOLOMEI[3], P. BENQUET[4];
[1]Inserm, Rennes, France; [2]INSERM DR Grand Ouest, Inserm, Nantes, France; [3]INSERM & Inst. De Neurosciences Des Systè, INSERM & Inst. De Neurosciences Des Systè, Marseille, France; [4]LTSI-INSERM U1099, INSERM U1099 -LTSI, Rennes, France

Abstract: Title. Multiscale neuro-inspired models for interpretation of EEG signals in epilepsy patients
Context. For pre-surgical evaluation of epilepsy patients, electro-encephalographic (EEG) and stereo-EEG (SEEG, depth electrodes) signals are routinely recorded to improve the identification of epileptogenic networks and delineate at best the area to be resected to suppress seizures or significantly decrease their frequency. In this context, a key question is the neurophysiological interpretation of epileptiform events (interictal epileptic discharges -IEDs-, seizures) revealed by S/EEG recordings. **Methods**. We developed novel neuro-inspired computational models for neocortex at three different levels of description: i) microscale (detailed neuron models), ii) mesoscale (neural mass models) and macroscale (whole brain model). Although they are conceptually different, micro- and meso-scale models share similar features, like the typology of neurons (main pyramidal cells and various types of interneurons), the spatial distribution of these neurons in neocortical layers and their synaptic connectivity (excitatory and inhibitory). The brain model consists of a large-scale network of neural masses, the connectivity of which being based on the human connectome. **Results**. For the three levels of description, fine tuning of free

parameters and quantitative comparison with real data allowed us to reproduce IEDs with high degree of realism and make hypotheses about cell-related and network-related mechanisms underlying the generation of i) fast ripples, ii) interictal spike-and waves, iii) fast activity at the onset of seizures and iv) scalp-recorded epileptic spikes. Illustrative examples will be presented. **Conclusion**. Developed models have a high face, integrative and explanatory value. Future work will explore their ability to predict optimized therapeutic strategies based on neuro-stimulation/modulation.

Disclosures: F. Wendling: None. **E. Koksal:** None. **M. Al harrach:** None. **F. Bartolomei:** None. **P. Benquet:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.16/E17

Topic: B.08. Epilepsy

Support: NIH R25
R01MH120295
NSF 2034037
NHGRI T32HG012344

Title: Optogenetic modulation of seizure-like activity in human hippocampus

Authors: *J. P. ANDREWS[1], K. VOITIUK[4], M. ELLIOTT[5], D. SHIN[7], J. GENG[8], A. ROBBINS[6], A. WANG[10], M. KEEFE[2], J. SEVETSON[9], J. RIVERA-DE JESUS[12], K. DONOHUE[11], H. LARSON[10], D. ERLICH[6], K. I. AUGUSTE[3], S. SALAMA[13], E. F. CHANG[1], V. S. SOHAL[14], D. HAUSSLER[15], C. CADWELL[11], D. SCHAFFER[16], M. TEODORESCU[6], T. NOWAKOWSKI[1];
[1]Neurolog. Surgery, [3]Dept. of Neurosurg., [2]Univ. of California San Francisco, San Francisco, CA; [4]Univ. of California, Santa Cruz, CA; [5]UCSC, [6]UC Santa Cruz, Santa Cruz, CA; [7]Univ. of California, San Fransisco, San Francisco, CA; [8]Computer Sci. and Engin., [9]Univ. of California - Santa Cruz, Univ. of California, Santa Cruz, Santa Cruz, CA; [10]Neurolog. Surgery, [11]UCSF, San Francisco, CA; [12]Dept. of Bioengineering, Univ. of California Berkeley, Berkeley, CA; [13]Ctr. for Biomolecular Engin., of California, Santa Cruz and HHMI, Santa Cruz, CA; [14]Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA; [15]Genomics Institute, UCSC, Santa Cruz, CA; [16]Univ. of California, Berkeley, Berkeley, CA

Abstract: Temporal lobe epilepsy is a common seizure disorder, thought to emerge as a consequence of excitatory imbalance. Optogenetics is an experimental approach that involves exogenous expression of light-activated ion channels. While optogenetic control of neuronal networks has been ubiquitously employed across experimental models, direct application of these tools to control human network activity has not been reported. Here we use high-density

microelectrode array (HD-MEA) recordings of human hippocampus to show that a recently described, inactivating channelrhodopsin, expressed in human tissue, is effective at modulating seizure-like activity in human hippocampus. We use a custom hardware-software interface to illuminate the hippocampal slice while on the HD-MEA, which leads to immediate reduction in firing during spontaneous activity as well as during seizure-like activity induced with application of bicuculline. The model described here provides proof-of-principle for optogenetic gene therapy to directly modulate activity in human brain tissue. More broadly, with the advent of tools for cell-type specific gene expression, our strategy may be employed to target a range of therapeutically relevant targets and circuits.

Disclosures: **J.P. Andrews:** None. **K. Voitiuk:** None. **M. Elliott:** None. **D. Shin:** None. **J. Geng:** None. **A. Robbins:** None. **A. Wang:** None. **M. Keefe:** None. **J. Sevetson:** None. **J. Rivera-de Jesus:** None. **K. Donohue:** None. **H. Larson:** None. **D. Erlich:** None. **K.I. Auguste:** None. **S. Salama:** None. **E.F. Chang:** None. **V.S. Sohal:** None. **D. Haussler:** None. **C. Cadwell:** None. **D. Schaffer:** None. **M. Teodorescu:** None. **T. Nowakowski:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.17/Web Only

Topic: B.08. Epilepsy

Support: U01 NS090407

Title: Brain gray matter volume changes in children with epilepsy

Authors: B. ROY[1], J. A. OGREN[2], L. A. ALLEN[3], B. DIEHL[3], R. SANKAR[4], S. D. LHATOO[5], *R. KUMAR[6], R. M. HARPER[2];
[1]Dept. of Anesthesiol. and Perioperative Medicine, Univ. of California Los Angeles, Los Angeles, CA; [2]Dept. of Neurobiology, Univ. of California Los Angeles, Los Angeles, CA; [3]Dept. of Clin. and Exptl. Epilepsy, Univ. Col. London Inst. of Neurol., London, United Kingdom; [4]Dept. of Neurology, Univ. of California Los Angeles, Los Angeles, CA; [5]Dept. of Neurology, McGovern Med. School, Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [6]Univ. of California Los Angeles, Los Angeles, CA

Abstract: The risk for Sudden Unexpected Death in Epilepsy (SUDEP) in children with epilepsy, although lower than for adults, remains a concern. Whether potential brain sites mediating triggering of failure or collapse of vital functions in adults are shared in pediatric epilepsy is unknown, particularly since identification of dysfunctional sites is made difficult with normal developmental processes and possibly unique pathological changes in children. We examined regional brain volume changes in children with epilepsy at high risk for SUDEP using high-resolution T1-weighted imaging over healthy children, and related those findings to SUDEP risk scores. We collected high-resolution T1-weighted images from 21 children with epilepsy

[age, 14.1±4.1 years; male, 9; body mass index (BMI), 20.8±3.9 kg/m^2] and 62 control children (age, 16.0±3.8 years; male, 35; BMI, 21.5±4.4 kg/m^2) using MRI scanners. SUDEP risks were calculated based on partial seizure frequency (number of seizures per day). High-resolution T1-weighted images were partitioned into gray matter tissue type; gray matter maps were normalized to a common space, smoothed, compared between children with epilepsy and control subjects (SPM12; ANCOVA; False Discovery Rate; covariates, age, sex, and BMI, $p<0.05$). Significant correlations between regional brain volumes and partial seizure frequency indices in children with epilepsy were examined using partial correlations (SPM12, covariates, age, sex, and BMI, $p<0.005$; uncorrected). Of 21 children with epilepsy, 67% were at high risk (partial seizures ≥13 per year) for SUDEP. The cerebellar cortex, hippocampus, amygdala, putamen, cingulate, thalamus, and para-hippocampal gyrus showed increased gray matter volumes in pediatric epilepsy over healthy children. Decreased regional gray matter volumes emerged in the insula, temporal cortices, prefrontal cortices, lingual gyrus, and caudate compared to controls. Positive relationships with partial seizure frequency indices appeared with the frontal cortices, cingulate, insula, putamen, parietal cortices, and temporal cortical gray matter volumes in epilepsy patients. Children with epilepsy showed brain tissue volume changes in selected sites that differed in direction from those of adult patients at high risk for SUDEP, especially volume increases rather than decreases in cerebellar sites that are key structures for recovery from apnea or extreme hypotension. The volume increases may represent expansion by inflammatory or other processes that, with sustained repetitive seizure discharge, lead to later tissue volume declines described earlier in adults.

Disclosures: B. Roy: None. **J.A. Ogren:** None. **L.A. Allen:** None. **B. Diehl:** None. **R. Sankar:** None. **S.D. Lhatoo:** None. **R. Kumar:** None. **R.M. Harper:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.18/E18

Topic: B.08. Epilepsy

Support: NIH R01 NS085171

Title: Hippocampal ΔFosb expression is associated with cognitive impairment in patients with childhood epilepsies

Authors: *C.-H. FU[1], A. VIAENE[2], J. CHIN[1];
[1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Dept. of Pathology, The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and it is often comorbid with other neurological and neurodegenerative diseases, such as Alzheimer’s disease (AD). Patients with recurrent seizures often present with cognitive impairment. However,

it is unclear how seizures, even when infrequent, produce long-lasting deficits in cognition. One mechanism may be seizure-induced expression of ΔFosB, a long-lived transcription factor that can persistently regulate expression of plasticity-related genes and drive cognitive dysfunction. We previously found that, compared with cognitively-intact subjects, ΔFosB expression in the hippocampal dentate gyrus (DG) was increased in individuals with mild cognitive impairment (MCI) as well as individuals with AD. In MCI patients, higher ΔFosB expression corresponded to lower mini-mental state examination scores. Surgically resected DG tissue from patients with temporal lobe epilepsy also showed robust expression ΔFosB; however, it is unclear whether ΔFosB expression also corresponds to cognitive function in non-AD-related epilepsy. To test whether ΔFosB expression in the DG may also be indicative of cognitive function in epilepsies with different etiologies, we assessed ΔFosB expression in surgically resected hippocampal tissue from 33 patients with childhood epilepsies who had also undergone Wechsler Intelligence Scale for Children (WISC) testing prior to surgery. We found that ΔFosB expression is inversely correlated with Full Scale Intelligence Quotient (FSIQ) in patients with mild to severe intellectual disability (FSIQ < 85). Our data indicate that ΔFosB expression corresponds to cognitive impairment in epilepsies with different etiologies, and supports the hypothesis that ΔFosB may similarly epigenetically regulate gene expression and impair cognition in various epilepsies.

Disclosures: **C. Fu:** None. **A. Viaene:** None. **J. Chin:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.19/E19

Topic: B.08. Epilepsy

Title: Unmasking a potential role for HMGB1 protein complexes in patients with new onset refractory status epilepticus (NORSE)

Authors: *S. MUHAMMAD, B. D. S. CLARKSON, C. L. HOWE;
Translational Neuroimmunology Lab., Mayo Clin., Rochester, MN

Abstract: New onset refractory status epilepticus (NORSE) in both adults and children (in whom the disease is frequently referred to as febrile infection-related epilepsy syndrome, or FIRES) is a devastating seizure disorder of unknown etiology, involving unknown pathogenic mechanisms, and with limited treatment options. Recent studies have implicated broad inflammatory dyshomeostasis in these patients, resulting in ad hoc therapeutic manipulation of cytokines such as interleukin-1β (IL1β) and interleukin-6. Despite individual success with these therapies, most NORSE/FIRES patients are non-responsive and consequently suffer severe neurological impairment. Drug-resistant epilepsy is a common sequela in these patients, even in those individuals successfully treated with cytokine-targeting therapies. We and others have measured elevated levels of the endogenous alarmin high mobility group box 1 (HMGB1) in

serum from NORSE patients and in patients with drug-resistant epilepsy not directly linked to NORSE/FIRES. On this basis and given the known physical and functional interactions between IL1β and HMGB1, we have endeavored to identify potential pathogenic mechanisms mediated by HMGB1 in seizure disorders. We hypothesized that differential interactions between HMGB1 and binding partners would modulate subsequent downstream signaling responses mediated by toll-like receptor 4 (TLR4), TLR2, and the receptor for advanced glycation end-products (RAGE). We also hypothesized that such binding partners would differentially mask detection of HMGB1 in serum and cerebrospinal fluid (CSF) from NORSE/FIRES and DRE patients. Therefore, we used perchloric acid (PCA) to free HMGB1 from protein complexes in serum and CSF and compared the levels of unmasked HMGB1 with the levels measured in non-PCA-treated biofluids from the same subjects. Unexpectedly, we found that pediatric subjects with NORSE exhibited less masked HMGB1 relative to healthy controls while adult NORSE patients exhibited more complexed HMGB1. We also found that HMGB1 induced different responses in neural cellular targets depending upon complex formation. These findings suggest that altering HMGB1 association with other proteins may change the pathogenic outcomes induced by this factor, offering the tantalizing possibility that controlling HMGB1 complex formation may serve as a novel therapeutic intervention in patients with NORSE/FIRES.

Disclosures: S. Muhammad: None. **B.D.S. Clarkson:** None. **C.L. Howe:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.20/E20

Topic: B.08. Epilepsy

Support: NIH award R01-NS094399
NIH award K01-ES026839

Title: The impact of the state of vigilance on the detection of fast ripples as epilepsy biomarkers

Authors: *S. DAS[1], W. STACEY[2], S. GLISKE[1];
[1]Neurosurg., Univ. of Nebraska Med. Ctr. Dept. of Neurosurg., Omaha, NE; [2]Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: High frequency oscillations (HFOs) observed in intracranial EEG have emerged as promising biomarkers for identifying epileptogenic tissue. They can be further classified based on the frequencies: ripples (80-250 Hz) and fast ripples (FRs, 250- 500 Hz). While some studies have suggested a potential relationship between HFO rate and specific sleep stages, there is a lack of information regarding the association between HFOs, particularly FRs, and state of vigilance. The objective of this study was to assess the importance of state of vigilance in detection and interpretation of FRs. Subjects were selected from the intracranial EEG database of patients with drug-resistant epilepsy who underwent invasive EEG monitoring at the University

of Michigan. A total of 60 subjects with more than 5000 hours of multi-day, sleep-scored interictal data were included in the study. The rates of FRs and their association with epileptogenic tissue were compared across different states of vigilance (awake and the various stages of sleep). An automatic HFO detector was used for detecting HFOs (80-500 Hz) and FRs (250-500 Hz) while redacting muscle and fast transient artifacts and detections due to background fluctuations. The association of FRs with resected volume (RV) and seizure onset zone (SOZ) was quantified using an asymmetry measure. The overall FR rate and its asymmetry with respect to the SOZ were calculated for each subject within each state of vigilance. The median FR rate was the highest for the awake stage (0.69 counts/min) and was the least for the REM stage (0.25 counts/min) of sleep. The asymmetry in the SOZ was the lowest during the awake state ($p<0.001$, Wilcoxon Rank Sum). Moreover, the rate of FRs was statistically higher in the awake state and the overall recording ($p<0.001$, Wilcoxon Rank Sum). Thus, we can conclude that the FR rate and its association with epileptogenic tissue has some dependence on the state of vigilance, occurring maximally in the awake state, but the stage of sleep is not a determining factor. These findings help clarify the clinical relevance of state of vigilance for detecting and interpreting FRs.

Disclosures: S. Das: None. **W. Stacey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HFO detector (2016) licensed to Natus Neurology. **S. Gliske:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HFO detector (2016) licensed to Natus Neurology.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.21/E21

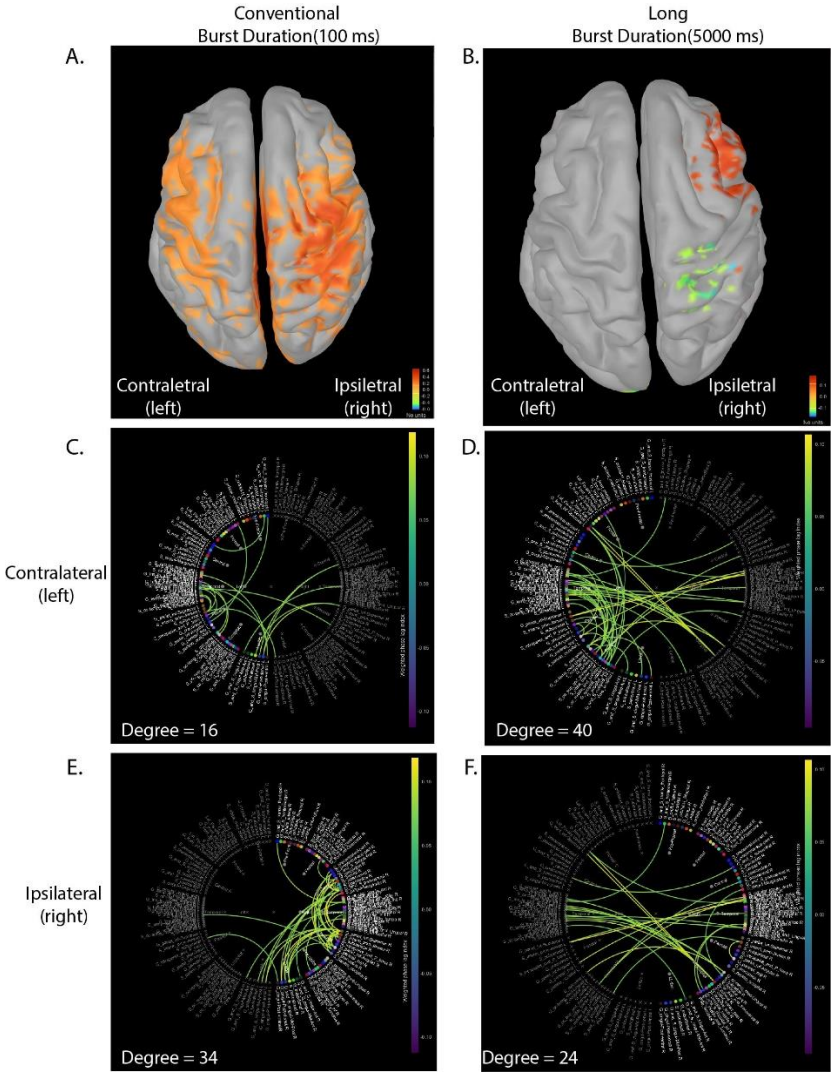
Topic: B.08. Epilepsy

Title: Cortical network effects of thalamic long bursting electrical stimulation in patients with drug resistant epilepsies: an electrical source imaging study

Authors: *Y. S. VAKILNA, R. T. MANJUNATHA, C. GANNE, J. C. MOSHER, S. D. LHATOO, J. GAVVALA, S. PATI;
Neurol., Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Closed-loop electrical stimulation using the NeuroPace Responsive Neurostimulator System (RNS) system is a promising treatment option for patients with drug-refractory epilepsies. Due to a lack of mechanistic understanding of the effects of electrical stimulation, the stimulation parameters are currently not tailored to patients. As such, we explored the effects of burst duration parameters via electrical source imaging using 128-channel EEG in 7 adult patients with RNS. We set constant stimulation amplitude (2.5 mA), frequency (100 Hz), and pulse width (100 ms), while the burst duration was set to either 5000 ms (long) or 100 ms

(conventional). Epochs of EEG with 5 seconds before and after unilateral RNS stimulation were averaged and source modeling was performed with dynamical statistical parametric mapping (dSPM) Minimum Norm Imaging. Cortical synchrony was estimated by computing the weighted phase lag index (wPLI) across the beta band (13-30 Hz) using the first principle component of source activity defined by the Destrieux atlas. An overall change in source power and synchrony due to stimulation was estimated by computing the trial-average of difference between pre- and post-stimulus broadband power and wPLI, respectively. When stimulated with a 5-s burst, an increase in broad-band source power was observed across the entire cortex, with maximum change on the ipsilateral parietal lobe (Fig 1A). In contrast, 100 ms burst stimulation decreased the broad-band power in the ipsilateral parietal lobe and increased the power in the ipsilateral frontal lobe (Fig 1B). 5000 ms burst stimulation increased synchrony on the ipsilateral side (Fig 1E) and 100 ms burst stimulation increased synchrony on the contralateral side (Fig 1D). Thalamic long-bursting stimulation shows significantly different effects on the cortex when compared to conventional stimulation. Further understanding the effects of stimulation parameters on cortical networks may lead to the development of patient-specific stimulation protocols, improving the overall efficacy of stimulation therapies.



Disclosures: **Y.S. Vakilna:** None. **R.T. Manjunatha:** None. **C. Ganne:** None. **J.C. Mosher:** None. **S.D. Lhatoo:** None. **J. Gavvala:** None. **S. Pati:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.22/E22

Topic: B.08. Epilepsy

Title: Focal epilepsy concentrates physiological brain pulsation frequencies

Authors: M. SUHONEN[1], M. JÄRVELÄ[3], V. O. KORHONEN[2], V. KIVINIEMI[3], ***J. KANANEN**[3];
[2]Oulu Univ. Hosp., [1]Oulu Univ. Hosp., Oulu, Finland; [3]Univ. of Oulu, Oulu, Finland

Abstract: Introduction: We have earlier shown in our fast functional magnetic resonance imaging (fMRI) studies, that patients with focal epilepsy (PWE) have altered brain pulsations power and respiratory pulsation synchrony. Our earlier research on sleep and epilepsy led us to hypothesize that the spread of the pulsation frequencies as reflected by spectral entropy is altered both in PWE and in non-medicated patients with suspected epilepsy (PSE) compared to healthy controls (HC).
Methods: The study group consisted of 198 HC (age:45.2+-16.2 years, 117 females), 33 PWE (age:35.4+-11.0, 15 females) and 31 PSE (40.5 +- 14.9 years, 9 females) that have had one seizure, but no actual diagnosis yet. Subjects were imaged with minimum of 5 min resting-state fast fMRI sequence (2861 frames, TR=100ms) during the day with normal vigilance using Siemens 3T MRI. The pre-processing was done using a standard FSL pipeline and statistical analysis with FSL randomise. Spectral entropy, which measures signal complexity, was calculated voxel-wise for the whole brain in the fullband frequency range (0-5Hz). Higher entropy indicates broader distribution of frequencies in the spectrum, whereas lower entropy suggests a concentration of frequencies with only a few peaks. Analysed voxel-wise brain maps were registered to MNI152 standard space. Statistical analyses were performed between HC vs. PWE, HC vs. PSE and PWE vs PSE.
Results: The HC group spectral entropy was significantly (family-wise error corrected, $p<0.05$) higher compared to PWE. Areas of increase were rather brain-wide, concentrating on posterior, and tempobasal regions of the brain. However, there were no significant differences between HC vs. PSE and PWE vs. PSE.
Conclusion: Our novel findings suggest that the physiological brain pulsations in focal epilepsy are more concentrated on specific frequencies rather than spread in a broader spectrum. In contrast, the brain physiological pulsations in the control population exhibit a more dynamic and complex pattern than a monotonous one. This may reflect ill functioning brain physiology that affects brain homeostasis, as it is possible that the pulsations are changed in PWE group due to a compensatory mechanism. In the future, it is important to investigate spectral entropy of different physiological pulsations independently.

Disclosures: **M. Suhonen:** None. **M. Järvelä:** None. **V.O. Korhonen:** None. **V. Kiviniemi:** None. **J. Kananen:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.23/E23

Topic: B.08. Epilepsy

Support: NS096234
Children's Brain Diseases Foundation

Title: Relation between immune signaling, cognitive ability, and epilepsy duration in human epilepsy

Authors: *Z. SADRI, Y. LI, P. NGUYEN, A. BREWSTER;
Southern Methodist Univ., Dallas, TX

Abstract: Memory problems are common in people with epilepsy, particularly those with medically refractory seizures. Evidence from experimental epilepsy supports that the activation of immune and inflammatory cascades contribute to seizure generation and memory deficits. However, in the context of human epilepsy, direct correlative evidence linking molecular immune changes in the brain to epilepsy pathophysiology is scarce in the literature. Therefore, the objective of this study was to compare the levels of brain immune signaling molecules to epilepsy duration and cognitive capabilities in patients with drug-resistant epilepsy. Here, we measured the levels of immune signaling molecules known to be altered by seizures and compared them to epilepsy duration and neurophysiological scores for cognitive ability assessed with the Wechsler Abbreviated Scale of Intelligence (WASI) test. Cortical tissues surgically resected from patients with refractory epilepsy were collected with informed consent under approved IRB protocols (n = 35). Brain biopsies were processed for western blotting and single or multiplex enzyme-linked immunosorbent assays to determine protein levels of immune signaling molecules. These included immune complement molecules (e.g., C1q, C3, C5), microglial receptors such as Trem2, and multiple cytokine and chemokine cell signaling molecules. Pearson correlation coefficient analysis was used to determine correlations between the levels of these proteins, epilepsy duration, and cognitive scores for full-scale intelligence quotient (FSIQ) obtained from WASI. We found significant correlations between epilepsy duration and higher levels of complement C3 ($p = 0.01$) and the soluble (s) form of Trem2 ($p = 0.04$). Although we did not find statistically significant correlations between the levels of membrane-associated Trem2 and the inflammatory cytokines TNF-α, IL-6, and IFN-γ with epilepsy duration or FSIQ scores, we observed moderate correlation coefficients trending towards significance. Specifically, we found that higher levels of membrane associated Trem2 and IL-6 paralleled lower cognitive scores and longer epilepsy durations. Our findings revealed significant associations between epilepsy duration and increased levels of C3 and sTrem2. C3

and sTrem2 can regulate microglial inflammatory and phagocytic responses, suggesting that microglial functions mediated by these molecules may help sustain epileptic networks. Thus, directing mechanistic studies and therapeutic interventions towards C3 and sTrem2 molecules could potentially guide the development of treatments aimed at reducing seizures in people with epilepsy.

Disclosures: **Z. Sadri:** None. **Y. Li:** None. **P. Nguyen:** None. **A. Brewster:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.24/E24

Topic: B.08. Epilepsy

Support: NS096234
Children's Brain Diseases Foundation

Title: Correlation between serum inflammatory cytokines and cognitive abilities in human drug-resistant epilepsy

Authors: *N. PINZON, Y. LI, A. BREWSTER;
Southern Methodist Univ., Dallas, TX

Abstract: Epilepsy is a common neurological disorder characterized by the occurrence of two or more unprovoked seizures. In addition, people with epilepsy have a higher risk of developing emotional and cognitive impairments. A growing body of clinical and experimental data strongly supports the idea that neuroinflammation plays a role in the generation of seizures as well as memory, learning, and psychiatric deficits. Interestingly, recent studies reported that high levels of inflammatory cytokines such as IL-1β, IL-6, and TNF-α in plasma correlate with seizure severity and reduced cognitive ability in both human and rodent models of epilepsy. These findings suggest the possibility that inflammation outside of the CNS may contribute to unprovoked seizures and cognitive comorbidities or may emerge as a result of disease progression. Thus, to further evaluate the association between peripheral blood inflammation and the epilepsy pathophysiology, we determined the correlation between cytokine levels in serum, epilepsy duration, and neurophysiological scores for cognitive ability assessed with the Wechsler Abbreviated Scale of Intelligence test in people with drug-resistant epilepsy. Blood samples were collected from patients with refractory epilepsy with informed consent under approved IRB protocols (n = 35). Multiplex enzyme-linked immunosorbent assays were used to measure the levels of 27 cytokine and chemokine cell signaling molecules in serum. Pearson correlation coefficient analysis was applied to determine correlations between levels of these proteins, epilepsy duration, and cognitive scores for the full-scale intelligence quotient (FSIQ). We found that epilepsy duration significantly correlated with higher levels of interleukin (IL) 17 ($p = 0.05$) and lower levels of TNF-α ($p = 0.05$). Lower cognitive ability scores significantly correlated

with higher levels of TNF-α (p = 0.02), IL-9 (p = 0.01), and CXCL10 (p = 0.02). Interestingly, we found that the serum levels of TNF-α were significantly higher in males than in females (p = 0.01). Our findings revealed that epilepsy duration positively correlated with IL-17 levels and negatively correlated with TNF-α levels, and that pronounced cognitive impairments correlated with higher levels of pro-inflammatory cytokines. It is possible that increases in inflammatory cytokines in the blood may leak into the brain through an unstable blood-brain barrier and contribute to disease severity and progression. Although future research is still required to determine whether inflammation is a cause or a consequence of seizures, our findings suggest that these cytokines may serve as biomarkers in epilepsy.

Disclosures: **N. Pinzon:** None. **Y. Li:** None. **A. Brewster:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.25/E25

Topic: B.08. Epilepsy

Support: NS096234
Children's Brain Diseases Foundation

Title: Assessment of tau phosphorylation andβ-amyloid pathology in human drug-resistant epilepsy

Authors: ***Y. LI**[1], P. NGUYEN[1], R. DAS[2], J. N. LUGO, Jr[3], A. BREWSTER[1];
[1]Southern Methodist Univ., Dallas, TX; [2]UT southwestern medical center, Dallas, TX;
[3]Psychology and Neurosci., Baylor Univ., Waco, TX

Abstract: Objective: Epilepsy can be comorbid with cognitive impairments. Recent evidence suggests the possibility that cognitive decline in epilepsy may be associated with mechanisms typical of Alzheimer's disease (AD). Neuropathological hallmarks of AD have been found in brain biopsies surgically resected from patients with drug-resistant epilepsies. These include hyperphosphorylation of the tau protein (p-tau) that aggregate into neuropil threads (NT) or neurofibrillary tangles (NFT), as well as presence of β-amyloid (Aβ) deposits. While recent studies agree on these AD neuropathological findings in epilepsy, some contrast in their correlation to cognitive decline. Thus, to further address this question we determined the abundance of p-tau and Aβ proteins along with their association to cognitive function in 12 cases of refractory epilepsy. Methods: Cortical biopsies surgically extracted from the temporal lobes of patients with refractory epilepsy were processed for immunohistology and enzyme-linked immunoassays to assess distribution and levels, respectively, of p-tau (Antibodies: Ser202/Thr205; Thr205; Thr181) and Aβ proteins. In parallel, we measured activation of mechanistic target of rapamycin (mTOR) via p-S6 (Antibodies: Ser240/244; Ser235/236). Pearson correlation coefficient analysis determined associations between these proteins and

neurophysiological scores for full scale intelligence quotient (FSIQ). Results: We found a robust presence of p-tau (Ser202/Thr205)-related NT and NFT pathology, as well as Aβ deposits, and p-S6 (Ser240/244; Ser235/236) in the epilepsy biopsies. We found no significant correlations between FSIQ and p-tau (Thr205; Thr181), Aβ or mTOR markers with FSIQ scores, although some correlation coefficients were modest to strong. Conclusions: These findings strongly support the existence of hyperphosphorylated tau protein and Aβ deposits in patients with human refractory epilepsy. However, their relation to cognitive decline is still unclear and requires further investigation.

Disclosures: **Y. Li:** None. **P. Nguyen:** None. **R. Das:** None. **J.N. Lugo:** None. **A. Brewster:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.26/E26

Topic: B.08. Epilepsy

Support: NS096234
Children's Brain Diseases Foundation

Title: Trem2 Signaling-Related Sex Differences in Human Drug Resistant Epilepsy

Authors: *S. KUMARI, P. NGUYEN, Y. LI, S. KHAN, A. BREWSTER;
Southern Methodist Univ., Dallas, TX

Abstract: A prominent pathological feature of epilepsy is the presence of reactive microglia, the brain's immune cells, and professional phagocytes. Although it is known that microglia mediate seizure-related inflammatory responses, considerably less is known about the contribution of microglial phagocytic properties to the pathophysiology of epilepsy. Recently, we and others found altered levels of phagocytosis-related signaling molecules in human and experimental epilepsy, including increases in complements C3b and C1q and decreases in the microglial receptor Trem2 (Triggering Receptor Expressed on Myeloid Cells 2). Trem2 signaling promotes microglial survival and proliferation, as well as phagocytosis and inflammatory signal regulation. However, the role of Trem2 signaling in epilepsy remains unknown. Therefore, to determine the extent to which the Trem2 signaling cascade is activated in drug-resistant epilepsy, we measured the protein levels of Trem2 as well as its downstream effectors in brain biopsies from epilepsy cases. Cortical tissues surgically resected from patients with refractory epilepsy were collected with informed consent under approved IRB protocols (n = 42; males, n = 21; females, n = 21). Immunoblots and enzyme-linked immunosorbent assays were used to determine protein levels of Trem2 and associated downstream effectors including Dap12, AKT/mTOR, ERK, SYK, and GSK3β (phosphorylated and total), as well as IBA1, cytokines, and complement proteins. The levels of these proteins were compared between males and females. We found sex differences in

the levels of Trem2, Dap12, SYK, the mTOR marker P-S6 (S235/236), the cytokines IL6 and IL8, and complement C3. Specifically, we found that males had lower levels of Trem2 ($p = 0.003$), Dap12 (*trend, $p = 0.09$), and SYK (*trend, $p = 0.06$) and higher levels of soluble Trem2 ($p = 0.003$), P-S6 (S235/236) ($p = 0.05$), IL6 ($p = 0.05$), and IL8 ($p = 0.02$) compared to females. There was no difference in the levels of AKT, ERK, GSK3β, PS6 (S240/244), TNFα, IFN-γ, IL4, IL10, and IBA1 between males and females ($p > 0.05$). To our knowledge, this is the first study to show that Trem2-related signaling differs in males and females with refractory epilepsy. We found that decreases in Trem2 signaling in males compared to females correlated with increases in the inflammatory cytokines IL8 and IL6, as well as complement C3, indicating a possible link between these molecular events. While mechanistic studies are needed to understand the role of Trem2 signaling in epilepsy, our findings suggest that sex differences in immune signaling may need to be taken into consideration for therapeutic interventions.

Disclosures: **S. Kumari:** None. **P. Nguyen:** None. **Y. Li:** None. **S. Khan:** None. **A. Brewster:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.27/E27

Topic: H.08. Learning and Memory

Support: ntramural Research Program of the National Institute of Neurological Disorders and Stroke

Title: Modeling and predicting dynamic neural responses to multisite direct electrical brain stimulation in humans

Authors: *U. MOHAN[1], J. WITTIG[3], O. FRUCHET[2], S. INATI[2], K. A. ZAGHLOUL[2]; [1]NIH, Washington, DC; [2]NINDS, NIH, Bethesda, MD; [3]iboss, San Diego, CA

Abstract: Direct electrical brain stimulation combined with intracranial electrophysiological recordings hold the potential to modulate and test the functional role of neural activity in the awake human brain. While clinicians have used direct electrical brain stimulation for functional mapping and treatment of neurological and psychiatric disorders, the effects of stimulation on neural activity are poorly understood. Changes in neural activity from stimulation in local and remote areas are often highly complex and variable. Stimulation has most often been delivered at locations individually, however, simultaneous or patterned stimulation at multiple locations holds the potential to modulate distributed networks more precisely. To better understand and precisely control the responses to stimulation in individual patients, we first took the approach of modelling the effects of stimulation on neural dynamics across the brain. We collected human electrocorticographic recordings from 8 neurosurgical epilepsy patients while systematically delivering cortical stimulation at different frequencies, amplitudes, durations, and locations while

patients were at rest. Using a dynamic linear state-space model framework, we fit input-output models to timecourses of neural activity, represented by high frequency activity, while patients received stimulation. Here, we first show that dynamic changes in brainwide neural activity following stimulation at individual locations can be accurately predicted using latent state space models. We further show the extent to which these dynamics are stable across different brain states. Lastly, we fit these models while patients were stimulated at individual locations to predict responses to simultaneous stimulation at multiple locations. Further characterizing and modeling of the neural responses to patterns of multisite stimulation could allow clinicians and researchers to design stimulation protocols for precise modulation of neural activity to more effectively probe functional brain networks and treat neurological disorders.

Disclosures: U. Mohan: None. **J. Wittig:** None. **O. Fruchet:** None. **S. Inati:** None. **K.A. Zaghloul:** None.

Poster

PSTR459. Neural Excitability and Pathology in Humans

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR459.28/E28

Topic: H.08. Learning and Memory

Support: NIH Grant UG3 MH125273
NIH Grant T32 5T32HL007901
McKnight Clinical Translational Research Scholarship in Cognitive Aging and Age-Related Memory Loss

Title: Hippocampal-dependent procedural memory consolidation is disrupted by pathological spikes in the hippocampus in humans with epilepsy

Authors: *B. S. BAXTER[1], W. SHI[2], M. THOMPSON[1], K. KWOK[1], B. DRISCOLL[1], R. PATEL[1], D. S. MANOACH[1], C. CHU[2];
[1]Psychiatry, [2]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: RATIONALE: Cortical slow oscillations, thalamocortical sleep spindles, and hippocampal ripples are implicated in sleep-dependent memory consolidation. Interictal epileptiform spikes may disrupt these oscillations and impair memory consolidation. The effect of hippocampal spike activity during sleep on memory consolidation is inconsistent and may depend on the memory modality. Here we examine the relationship between hippocampal spike rate and overnight procedural memory consolidation.
METHODS: 28 patients with epilepsy undergoing phase II presurgical evaluations, including simultaneous scalp and intracranial EEG (iEEG) with hippocampal contacts, were recruited from the Epilepsy Monitoring Unit. Archival data from 45 age-matched participants without epilepsy were used for the control group. All participants performed the finger-tapping Motor Sequence Task (MST) in the evening prior to sleep and were tested the following morning. The MST

requires participants to repeatedly type a 5-digit sequence as quickly and accurately as possible for twelve 30 s typing trials separated by 30 s rest periods. Overnight improvement is defined as the percent increase in correct sequences from the last three training trials to the first three test trials. Interictal spikes were detected during wake and sleep from the end of MST training to the start of testing across hippocampal iEEG contacts using clinical spike detection software.
RESULTS: MST learning during training was similar between the control and epilepsy participants. Participants with epilepsy showed a trend for reduced overnight sleep-dependent memory consolidation compared to controls (t = -1.5, p = .12). When stratifying participants with epilepsy based on their hippocampal spike rate, participants with a low spike rate (<= 0.5 spikes/min) had significantly better overnight consolidation compared to those with a high spike rate (> 4 spikes/min) (t = 2.4; p = .05).
CONCLUSIONS: Individuals with more hippocampal spikes had worse sleep-dependent memory consolidation. This supports our hypothesis that interictal spiking disrupts procedural memory consolidation. In ongoing work we will detect sleep oscillations in the cortex and hippocampus, evaluate their coordination, and investigate their relationship with memory consolidation. This work will indicate how these oscillations interact with pathological spikes to influence memory and provide targets for treatment development for individuals with impaired memory consolidation.

Disclosures: B.S. Baxter: None. **W. Shi:** None. **M. Thompson:** None. **K. Kwok:** None. **B. Driscoll:** None. **R. Patel:** None. **D.S. Manoach:** None. **C. Chu:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.01/E29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PRIN 2017A9MK4R_004

Title: Il-1β Triggers synaptic and memory deficits downregulating gene expression via the epigenetic MeCP2/HDAC4 complex

Authors: *D. D. LI PUMA[1,2], C. COLUSSI[3,2], R. PIACENTINI[1,2], B. BANDIERA[1], G. PULIATTI[1], M. RINAUDO[1,2], F. PACIELLO[1,2], C. RIPOLI[1,2], G. DE CHIARA[4], A. BERTOZZI[3,2], A. T. PALAMARA[5,6], C. GRASSI[1,2];
[1]Dept. of Neurosci., Univ. Cattolica Del Sacro Cuore, Rome, Italy; [2]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; [3]Dept. of Engineering, Inst. di Analisi dei Sistemi ed Informatica "Antonio Ruberti", Italian Natl. Res. Council, Rome, Italy; [4]Inst. of Translational Pharmacol., Natl. Res. Council, Rome, Italy; [5]Dept. of Infectious Dis., Inst. Superiore di Sanità, Rome, Italy; [6]Dept. of Publ. Hlth. and Infectious Dis., Sapienza Univ. of Rome, Rome, Italy

Abstract: Several evidence indicates that neuroinflammation plays a critical role in the onset and progression of many neurodegenerative diseases. However, the molecular mechanisms by which inflammatory mediators determine synaptic dysfunction and cognitive deficits have not been fully understood yet. Here we investigated the role the proinflammatory cytokine interleukin-1β (IL-1β), and the molecular cascade downstream the activation of its receptor, to the synaptic dysfunction by taking advantage of a mouse model of recurrent Herpes simplex virus type-1 (HSV-1) replication within the brain, induced by thermal stress (TS). After 2 TS-induced HSV-1 reactivations mice exhibited increased brain levels of IL-1β with respect to mock-infected ones (20.9±1.1 vs. 5.8±2.1 pg/mg, respectively; $p=7.9\times10^{-4}$) along with significant alterations of: i) cognitive functions; ii) synaptic plasticity at the hippocampal CA3-CA1 synapse, assessed by long-term potentiation paradigm; iii) expression profile of synaptic-related genes and pre- and post-synaptic proteins; iv) spine density at apical and basal dendrites and their morphology. These effects correlated with the upregulation of the epigenetic repressor MeCP2 in terms of both protein (+59% vs. mock, $p=2.8\times10^{-3}$) and mRNA expression (+4.8 folds vs. mock, $p=6.9\times10^{-4}$) that, in association with HDAC4, affected the expression of synaptic plasticity-related genes. Specifically, in HSV-1 infected mice, HDAC4 localized in the nucleus (+59%, $p=2.8\times10^{-3}$ vs. mock-infected ones) and promoted MeCP2 SUMOylation that is known to critically affect the repressive activity of MeCP2 (+45% of SUMO1, $p=7.9\times10^{-4}$). Finally, the ChIP assay revealed that HDAC4 was enriched on the promoters of the immediate early gene *c-fos* and the presynaptic gene *syn1* in HSV-1-infected mice compared with controls (+3.9 and +2.5 folds, respectively, $p=2.9\times10^{-3}$ for both) confirming that HDAC4 may function as co-repressor on MeCP2 target genes. The blockade of IL-1 receptors by the specific antagonist Anakinra prevented the MeCP2 increase and the consequent downregulation of gene expression along with rescuing structural and functional indices of neurodegeneration. Collectively, our findings provide novel evidence that increased levels of the proinflammatory cytokine IL-1β, occurring in the CNS following HSV-1 reactivation, negatively affects synaptic function and memory by downregulating the expression of synaptic plasticity-related genes via the epigenetic MeCP2/HDAC4 complex.

Disclosures: **D.D. Li Puma:** None. **C. Colussi:** None. **R. Piacentini:** None. **B. Bandiera:** None. **G. Puliatti:** None. **M. Rinaudo:** None. **F. Paciello:** None. **C. Ripoli:** None. **G. De Chiara:** None. **A. Bertozzi:** None. **A.T. Palamara:** None. **C. Grassi:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.02/E30

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of HDAC4 in protein function regulation at the pre- and post-synaptic microdomains in a mouse model of Alzheimer’s disease

Authors: *C. COLUSSI[1,2], A. BERTOZZI[3,1], G. ACETO[3,2], C. RIPOLI[3,2], R. PIACENTINI[3,2], D. LI PUMA[3,2], M. D'ASCENZO[3,2], C. GRASSI[3,2];
[1]Engin., Italian Natl. Res. Council, Roma, Italy; [2]Fondazione Policlinico Gemelli, Rome, Italy; [3]Univ. Cattolica del Sacro Cuore, Rome, Italy

Abstract: Early dysfunction in Alzheimer's disease (AD) is characterized by dysmorphic neurites, decreased spine density and cognitive deficits as a result of abnormal synapse structure and function. The class II member HDAC4, which has recently been recognized as a key player in synaptic plasticity and memory, was discovered to be affected in AD, albeit it is unclear how this may contribute to AD pathogenesis. HDAC4 localization and function was assessed in hippocampal tissue from adult (7-month-old) control (WT) and 3×Tg mice (AD) by confocal analyses, co-immunoprecipitation and biochemical fractioning. Cultured hippocampal neurons or organotypic brain slices from WT and AD mice were transduced to overexpress a cytoplasmic mutant form of HDAC4 ($HDAC^{SD}$) or the empty vector and their effects on synaptic protein localization and function, synaptic transmission and spine density were investigated. We found that in WT mice, HDAC4 was localized at synapses and interacted with synapsin I and several post-synaptic proteins, whereas in the AD mice it underwent nuclear import (synaptic HDAC4 in AD: 0.62±0.08 vs WT; nuclear HDAC4 in AD: 2.14±0.36 vs WT). Similar results were found in WT neuronal cultures treated with amyloid-β (Aβ) or tau (HDAC4 at synaptic fraction: Aβ 0.75±0.05; tau 0.54±0.09 vs control; HDAC4 at nuclear fraction: Aβ 1.43±0.11; tau 1.53±0.13 vs control). Loss of synaptic HDAC4 in AD was associated with decreased HDAC4-mediated SUMO2/3ylation of synapsin I and PSD95. Overexpression of $HDAC4^{SD}$ (5 fold increase vs AD) in AD hippocampal neurons recovered synapsin I SUMO2/3ylation and its clustering and interaction with actin favoring the formation of a reserve pool. At the post-synaptic domain $HDAC4^{SD}$ recovered PSD95 SUMO2/3ylation, dendritic length and expression of several synaptic proteins (fold change AD-$HDAC^{SD}$: NCAD 2.25±0.46, PSD95 2.19±0.18, GluA1 1.59±0.13, CaMKII 1.61±0.19 vs AD). Moreover, in AD organotypic hippocampal slices $HDAC4^{SD}$ rescued spine density and synaptic transmission. Overall these results highlight a new role of HDAC4 at both pre- and post-synaptic compartments, relying on post-translational modification (SUMOylation) of synaptic proteins and providing a scaffold for their membrane localization and proper function. Furthermore, our findings suggest that controlling HDAC4 localization may be a promising strategy to prevent and/or counteract synaptic dysfunction in AD.

Disclosures: C. Colussi: None. **A. Bertozzi:** None. **G. Aceto:** None. **C. Ripoli:** None. **R. Piacentini:** None. **D. Li Puma:** None. **M. D'Ascenzo:** None. **C. Grassi:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.03/E31

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The role of immediate early genes and complement system in synergistic pathology of epilepsy and Alzheimer's Disease: insights from a dual-pathology mouse model

Authors: *A. HARUTYUNYAN[1,3,4], A. ANDERSON[1], S. L. W. WARREN[2], N. C. JONES[1,4], P. KWAN[1,4];
[1]Dept. of Neurosci., [2]Monash MicroImaging, Monash Univ., Melbourne, Australia; [3]Dept. of Med., The Univ. of Melbourne, Parkville, Australia; [4]Dept. of Neurol., The Alfred Hosp., Melbourne, Australia

Abstract: There is increasing recognition that seizures and epilepsy frequently occur in Alzheimer's disease (AD) patients, and this co-occurrence is associated with accelerated cognitive decline. Previously, we developed an animal model of dual pathology (epilepsy and AD) by establishing a recurrent seizure phenotype in 5xFAD mice. Here, we aimed to investigate the molecular mechanisms driving the synergy between recurrent seizures and AD pathology by integrating data from multiple modalities. Female 5xFAD mice (N=20) and WT littermates (N=22) underwent electrical amygdala kindling or were treated as sham. In this experimental paradigm the kindled 5xFAD mice represent the subpopulation of AD patients who develop seizures and are at risk of accelerated cognitive deterioration, while WT and sham 5xFAD serve as respective controls. We employed immunohistochemistry (IHC) and behavioral testing (Y-maze) to evaluate the effect of recurrent seizures on amyloid load and cognitive performance. The hippocampal transcriptome was examined through RNA-sequencing. Correlation network analysis was employed to integrate electrophysiological, behavioural, histopathological and transcriptomic data into a multimodal network-based model. 5xFAD mice showed increased hyperexcitable phenotype ($p<0.001$) and impaired spatial memory ($p<0.05$) compared to WT group. The kindled 5xFAD mice showed enhanced Aβ deposition, evidenced by increased amyloid plaque area compared to shams ($p<0.01$) and vascular Aβ deposits in the leptomeningeal vessels. Differential expression analysis with nested comparisons identified a group of 326 genes that responded synergistically in the dual pathology group compared to all other groups (FDR<0.05, FC>2). Correlation network analysis identified modules of immediate early genes (IEG) and complement cascade proteins showing significant ($p<0.00001$) correlation with dual pathology. Notably, the regulatory hub gene of IEG module, Pcdh8 (Protocadherin 8) is involved in elimination of dendritic spines and was profoundly overexpressed in the dual pathology group. IHC labelling showed selective colocalization of Pcdh8+ puncta with PV-interneuron somas and dendrites. Our results suggest that IEGs and complement cascade have central roles in mediating the synergistic relationship between epilepsy and AD. We propose a mechanistic paradigm where seizure-induced increase in Aβ leads to complement-mediated synaptic pruning, while sustained overexpression of Pcdh8 leads to reduction in dendritic spines. This compounded loss of synapses likely drives the accelerated cognitive decline seen in seizure-prone subpopulation of AD patients.

Disclosures: A. Harutyunyan: None. **A. Anderson:** None. **S.L.W. Warren:** None. **N.C. Jones:** None. **P. Kwan:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.04/E32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Vanech family foundation
University of Missouri FastTrack Award
NIH R15GM119070

Title: Formation of Aβ42 fibrillar aggregates in synaptic endosomes precedes plaque formation in a mouse model of Alzheimer's like β-amyloidosis

Authors: *J. PACHECO-QUINTO[1], D. CLAUSEN[2,1], S. SOLÉ-DOMÈNECH[3], C. W. LEE[1], C. SINOBAS-PEREIRA[4], R. J. DOMALEWSKI[4], M. R. NICHOLS[4], E. ECKMAN[1]; [1]Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ; [2]Rutgers, The State Univ. of New Jersey, Cedar Knolls, NJ; [3]Dept. of Biochem., Weill Cornell Med. Col., New York, NY; [4]Dept. of Chem. & Biochem., Univ. of Missouri-St. Louis, Saint Louis, MO

Abstract: One defining trait of Alzheimer's disease (AD) brain is the abnormal accumulation of insoluble amyloid-β peptide (Aβ) fibrils. Fibrillar aggregates in a soluble conformation are also prominent in AD, can disrupt synaptic function, and are the preferential target of Lecanemab, a recently approved immunotherapy for AD. The origin of these soluble neurotoxic aggregates, however, remains elusive. Aβ originates largely in neuronal endosomes, where proteases such endothelin-converting enzyme-1 (ECE-1) digest the peptide, thus preventing Aβ accumulation. Based on this, we explored whether disrupted Aβ proteostasis in synaptic endosomes could lead to Aβ fibrillization in synapses. To investigate the nature of the synaptic compartments where Aβ accumulates in TgCRND8 mice, a model of Alzheimer's like β-amyloidosis, we isolated synaptosomal vesicles by density gradient centrifugation and characterized them by western blot analysis and electron microscopy. We also conducted a longitudinal analysis of the accumulation of multiple Aβ species in brain synaptosomes isolated from mice aged 1-4 months. Levels of soluble Aβ monomers and oligomers were measured by ELISA. Fibrillar aggregates were measured by 2 methods: by ELISA using antibodies specific for protofibrillar Aβ, and by dot blot using the anti-amyloid fibril antibody, OC. Learning and memory function were evaluated by contextual fear conditioning. To test the involvement of ECE-1 activity in endosomal Aβ aggregation in vitro, ECE-1 was knocked out in SH-SY5Y-APP cells using CRISPR-Cas9 technology. Results showed that well before the onset of amyloid deposition, mice developed deficits in learning, and synapses were burdened with detergent-soluble Aβ monomers, oligomers and fibrillar Aβ. Levels of all soluble Aβ species declined thereafter, as intravesicular Aβ42 turned predominantly insoluble and built up rapidly during the weeks preceding plaque formation. Insoluble Aβ42 in synapses accumulated in Aβ-producing endosomal vesicles with characteristics of multivesicular bodies. The detection of fibrillar Aβ in exosomes, plus super resolution microscopy analysis of OC-immunostaining *in vitro*, supported that insoluble intracellular Aβ represented fibrillar aggregates bound to membranes. Furthermore, findings from ECE-1 KO cells demonstrated that ECE-1 activity is a determinant factor controlling the fibrillization of nascent Aβ. Overall, our study supports that a loss of Aβ42 solubility in synapses is an early pathological finding, and that fibrillar Aβ accumulating at the synaptic level is

produced locally, within endosomal vesicles, and does not require the presence of amyloid plaques.

Disclosures: **J. Pacheco-Quinto:** None. **D. Clausen:** None. **S. Solé-Domènech:** None. **C.W. Lee:** None. **C. Sinobas-Pereira:** None. **R.J. Domalewski:** None. **M.R. Nichols:** None. **E. Eckman:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.05/E33

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tracing brain genotoxic stress and single-cell pathogenic analysis with a synthetic sensor-actuator circuit in Alzheimer’s Disease progression

Authors: ***C. E. HAACKER**[1], X. TIAN[2], M. B. EL-SAADI[3], M. GRAMES[4], X.-H. LU[5]; [1]Dept. of Pharmacology, Toxicology, And Neurosci., LSU Hlth. Sci. Ctr., Shreveport, LA; [2]Dept. of Pharmacology, Toxicology & Neurosci., Hlth. Sci. Ctr., Shreveport, LA; [3]Dept. of Pharmacology, Toxicology, And Neurosci., Louisiana State Univ. Hlth. Shreveport, Shreveport, LA; [4]Dept. of Pharmacology, Toxicology, And Neurosci., LSU Hlth. Sci. Ctr. - Shreveport, Shreveport, LA; [5]LSU Hlth. Shreveport, Shreveport, LA

Abstract: Alzheimer’s disease (AD) is a progressively debilitating disease that currently has no disease-modifying therapy. Current research suggests that genotoxic stress is implicated in neurodegenerative pathology. Human postmortem studies from our lab identified persistent brain genotoxic stress as a prominent feature of Parkinson's disease (PD). Age-dependent accumulation of neuronal somatic mutations has also been observed in AD. However, the causality and pathogenic mechanism of brain genotoxic stress in the progression of cognitive decline and neuropathology of AD is yet to be established. We hypothesize that brain genotoxic stress-mediated human neuron senescence and senescence-associated secretory phenotype (SASP) initiate and drive neurodegeneration to manifest the age-dependent progression of cognitive and neurodegenerative phenotypes in AD. Targeting genotoxic stress and consequent cell senescence can then be used as a disease-modifying therapy to slow the disease progression in AD. We have developed a viral sensor of genotoxic stress that employs viral transduction machinery and the instability of hypermutable mononucleotide repeat regions to trace cells in the presence of genotoxic stress. Membrane-tethered fluorescent proteins were used to characterize the subtle neurodegenerative changes. To validate our sensor, we developed a T7E1 assay for semi-quantitatively determination of genomic instability. Using the sensor in 3xTg mice at 6 months of age, we observed single-neurons labeled by the sensor in the hippocampus and cortex with minimal labeling in comparable regions of age and gender-matched control mice preceding Aβ deposits or Tau pathology at this stage. Immunostaining for Aβ and tau pathology, DNA damage, and senescence markers were conducted to be imaged with sensor labeled neurons and

quantified at single cell resolution. Using automatic behavioral tracking system, we also detected cognitive deficits in learning and memory in AD mice at 6 months, as revealed by the Barnes maze and T maze. Furthermore, we developed a novel synthetic sensor-actuator for precise genetic perturbation selectively in cells with accumulated genotoxic stress to ameliorate cognitive function and neuropathology in a mouse model of AD. Our study will shed light on the pathogenic role of brain genotoxic stress-mediated cell senescence in the manifestation of progressive cognitive and neurodegenerative outcomes in AD. Targeting genotoxic stress offers a novel disease-modifying strategy for halting the onset or progression of AD.

Disclosures: **C.E. Haacker:** None. **X. Tian:** None. **M.B. El-Saadi:** None. **M. Grames:** None. **X. Lu:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant GM122894 (M.S.W.)
NIH Grant AG66986 (M.S.W.)
NIH Grant AG79569 (M.S.W.)
National Natural Science Foundation of China Project 81920108015 (Y.S.)
Ministry of Science and Technology of China Project 2020YFA0509300 (Y.S.)
Key R&D Program of Zhejiang Province Project 2020C04001 (Y.S.)
NSF Grant 2121063 (Y.M.)
University of Kansas Alzheimer's Disease Research Center NIH Grant P30 AG072973

Title: Familial Alzheimer mutations stabilize synaptotoxic γ-secretase-substrate complexes

Authors: *M. WOLFE[1], S. DEVKOTA[1], R. ZHOU[3], V. NAGARAJAN[1], M. MAESAKO[4], H. DO[1], A. SARAF[1], Y. MIAO[1], B. D. ACKLEY[2], Y. SHI[3];
[2]The Univ. of Kansas Dept. of Mol. Biosci., [1]Univ. of Kansas, Lawrence, KS; [3]Tsinghua Univ., Beijing, China; [4]Massachusetts Gen. Hosp., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Alzheimer’s disease is characterized pathologically by cerebral deposition of 42-residue amyloid β-peptide (Aβ42), proteolytically produced from amyloid precursor protein (APP) by β- and γ-secretases. Although mutations in APP and presenilin, the catalytic component of γ-secretase, cause familial Alzheimer’s disease (FAD), a role for Aβ42 as the primary disease driver has not been clearly established and remains controversial. Here we show through comprehensive analysis of the multi-step proteolysis of APP substrate C99 by γ-

secretase that FAD mutations are consistently deficient in early proteolytic events, not later events that produce secreted Aβ peptides. Cryo-electron microscopy revealed that a substrate mimetic traps γ-secretase at the transition state for intramembrane proteolysis, and this structure closely aligns with activated enzyme-substrate complex captured by molecular dynamics simulations. *In silico* simulations and fluorescence lifetime imaging microscopy in cultured cells support stabilization by FAD mutations of enzyme-substrate and/or enzyme-intermediate complexes. Neuronal expression of C99 and/or presenilin-1 in *Caenorabditis elegans* led to age-dependent synaptic loss only when one of the transgenes carried an FAD mutation. Designed mutations that stabilize the enzyme-substrate complex and block proteolysis likewise led to synaptic loss. Collectively, these findings implicate the stalled process—not the released products—of γ-secretase cleavage of substrates in FAD pathogenesis.

Disclosures: **M. Wolfe:** None. **S. Devkota:** None. **R. Zhou:** None. **V. Nagarajan:** None. **M. Maesako:** None. **H. Do:** None. **A. Saraf:** None. **Y. Miao:** None. **B.D. Ackley:** None. **Y. Shi:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.07/E34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fondecyt Grant 3210260
NIH Grant R01 AA025718

Title: Loss of glycine receptors in the nucleus accumbens and ethanol rewarding in an Alzheimer´s Disease mouse model

Authors: *L. ARMIJO WEINGART, L. SAN MARTÍN, M. KONAR, A. ARAYA, S. GALLEGOS, E. FERNÁNDEZ, L. AGUAYO;
Univ. of Concepcion, Concepcion, Chile

Abstract: It is well known that Alzheimer's disease (AD) is characterized by alterations in cognitive and non-cognitive cerebral functions, and recent reports have implicated limbic regions like the nucleus accumbens (nAc) in the disease course. Accumbal neurons express high levels of inhibitory glycine receptors (GlyRs) that are allosterically modulated by ethanol and appear to have a role in controlling its intake. A previous study using the APP/PS1 mice model found decreased GlyRs expression and function in the nAc. Therefore, the global working hypothesis of the present study was to test if GlyRs alterations in AD affect nAc functions and reward-related behavior. We used a 6-month-old C57BL/6J.APP/PS1 AD model and control aged-matched litter mates in this study. We examined intracellular calcium dynamics using the fluorescent calcium protein reporter GCaMP. To assay calcium-related signals, we electrically stimulated the nAc and measured calcium responses using slice photometry. Increases in

fluorescence above the basal response (600 and 1100%, respectively) were observed after adding antagonists for GlyR and $GABA_A$ receptor (strychnine 1-4 μM; bicuculline 10 μM). Interestingly, the enhancing effect of strychnine was significantly reduced (by 45%) in the APP/PS1 mice indicating a smaller role of GlyRs in AD neurons. However, no differences between WT and APP/PS1 mice were observed after bicuculline treatment. Significantly, the GlyRα2 subunit was decreased in AD mice (by 90%). Additionally, ethanol potentiation was significantly decreased by 50% in APP/PS1 mice. Finally, we performed drinking in the dark (DID) experiments finding that APP/PS1 mice consumed significantly less ethanol on the last day of DID, consistent with lower blood ethanol concentration (150 vs 70 mg/dl). APP/PS1 mice also had lower sucrose consumption indicating that overall food rewarding was altered. In conclusion, the data support the role of GlyRs in nAc neuron excitability and a decreased glycinergic activity in the APP/PS1 mice that might lead to impairment in reward processing.
Acknowledgements: Fondecyt 3210260, 1221080, NIH R01 AA025718

Disclosures: L. Armijo Weingart: None. **L. San Martín:** None. **M. Konar:** None. **A. Araya:** None. **S. Gallegos:** None. **E. Fernández:** None. **L. Aguayo:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.08/E35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 NS117372
NIH Grant R21 NS121284
Simons Foundation Autism Research Initiative (SFARI) BTI award 551354
Brain and Behavior Research Foundation Young investigator award 27792

Title: Glial Sphingosine-mediated Epigenetic Regulation Stabilizes Synaptic Function in Drosophila Models of Alzheimer’s Disease

Authors: *T. WANG[1], P. YIN[1], Y. CAI[1], T. CUI[1], A. BERG[1], T. WANG[1], D. MORENCY[1], P. PAGANELLI[1], C. LOK[2], Y. XUE[1], S. VICINI[1];
[1]Pharmacol. and Physiol., [2]Biol., Georgetown Univ., Washington, DC

Abstract: Destabilization of neural activity caused by failures of homeostatic regulation has been hypothesized to drive the progression of Alzheimer’s Disease (AD). However, the underpinning mechanisms that connect synaptic homeostasis and the disease etiology remain to be elucidated. Here, we found that neuronal overexpression of Amyloid β (Aβ) causes abnormal histone acetylation in peripheral glia and completely diminishes Presynaptic Homeostatic Potentiation (PHP) at the neuromuscular junction in *Drosophila*. The synaptic deficits caused by Aβ overexpression in motoneurons are associated with motor function impairment at the adult

stage. Moreover, we found that a Sphingosine analogue drug, Fingolimod, ameliorates synaptic homeostatic plasticity impairment, abnormal glial histone acetylation, and motor behavior defects in the Aβ models. We further demonstrated that perineurial glial Sphingosine kinase 2 (Sk2) is not only required for PHP, but also plays a beneficial role in modulating PHP in the Aβ models. Glial overexpression of *Sk2* rescues PHP, glial histone acetylation, and motor function deficits that are associated with Aβ in *Drosophila*. Finally, we found that glial overexpression of *Sk2* restores PHP and glial histone acetylation in a genetic loss-of-function mutant of the Spt-Ada-Gcn5 Acetyltransferase (SAGA) complex, strongly suggesting that *Sk2* modulates PHP through epigenetic regulation. Taken together, we provided genetic evidence demonstrating that abnormal glial epigenetic alterations in Aβ models in *Drosophila* are linked to the impairment of PHP and that the Sphingosine signaling pathway displays protective activities in stabilizing synaptic physiology.

Disclosures: **T. Wang:** None. **P. Yin:** None. **Y. Cai:** None. **T. Cui:** None. **A. Berg:** None. **T. Wang:** None. **D. Morency:** None. **P. Paganelli:** None. **C. Lok:** None. **Y. Xue:** None. **S. Vicini:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.09/E36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grants NS105005 and NS105005-03S1
DoD Grants W81XWH-14-1-0061 and W81XWH-19-1-0202
NSF Grants IOS 1026527, IOS 1355158, DBI 1306528, DBI 1103738, EES 2200474
Alzheimer's Association Grants AARF-19-614794 and AARF-19-614794-RAPID
NIH Grant T32AA007565, T32DA041349 and F31NS117096
FRAXA Grant
NIH Grant AG073823
NIH Grant AA026551
NIH Grant P30AG072947 and P30AG049638
NIH Grant P50AG005136

Title: Aberrant DJ-1 expression underlies L-type calcium channel hypoactivity in dendrites in tuberous sclerosis complex and Alzheimer’s disease

Authors: *F. NIERE[1], A. UNERI[2], C. J. MCARDLE[2], H. EGIDO-BETANCOURT[2], L. P. CACHEAUX[2], S. V. NAMJOSHI[2], W. C. TAYLOR[2], X. WANG[3], S. H. BARTH[2], C. REYNOLDSON[2], J. PENARANDA[2], M. P. STIRRER[4], C. F. HEANEY[2], P. J. LAURIENTI[4], S. CRAFT[3], C. D. KEENE[5], T. MA[3], K. F. RAAB-GRAHAM[2];

[1]North Carolina Agr. and Tech. State Univ., Greensboro, NC; [2]Physiol. and Pharmacol., [3]Wake Forest Alzheimer's Dis. Res. Ctr. and Dept. of Gerontology and Geriatric Med., [4]Radiology, Wake Forest Sch. of Med., Winston Salem, NC; [5]Univ. of Washington, Univ. of Washington, Seattle, WA

Abstract: L-type voltage-dependent Ca^{2+} channels (L-VDCC) dysfunction is implicated in several neurological and psychiatric diseases. While a popular therapeutic target, it is unknown if molecular mechanisms leading to disrupted L-VDCC across neurodegenerative disorders is conserved. Importantly, L-VDCC integrate synaptic signals to facilitate a plethora of cellular mechanisms; however, mechanisms that regulate L-VDCC channel density and subcellular compartmentalization are understudied. Herein, we report that in disease models with overactive mammalian target of rapamycin complex 1 (mTORC1) signaling (or mTORopathies), that deficits in dendritic L-VDCC activity are associated with increased expression of the mTORC1-regulated RNA-binding protein DJ-1. DJ-1 binds the mRNA coding for the alpha and auxiliary Ca^{2+} channel subunits CaV1.2 and α2δ2, and represses their mRNA translation, only in the disease states, specifically preclinical models of tuberous sclerosis complex (TSC) and Alzheimer's disease (AD). In agreement, DJ-1 mediated repression of CaV1.2/α2δ2 protein synthesis in dendrites is exaggerated in mouse models of AD and TSC, resulting in deficits in dendritic L-VDCC calcium activity. Discovery of DJ-1 regulated L-VDCC activity in dendrites in TSC and AD provides a new signaling pathway that can be targeted in clinical mTORopathies.

Disclosures: F. Niere: None. **A. Uneri:** None. **C.J. McArdle:** None. **H. Egido-Betancourt:** None. **L.P. Cacheaux:** None. **S.V. Namjoshi:** None. **W.C. Taylor:** None. **X. Wang:** None. **S.H. Barth:** None. **C. Reynoldson:** None. **J. Penaranda:** None. **M.P. Stirrer:** None. **C.F. Heaney:** None. **P.J. Laurienti:** None. **S. Craft:** None. **C.D. Keene:** None. **T. Ma:** None. **K.F. Raab-Graham:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.10/E37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant/Other Support: : K99AG065645
Grant/Other Support: : R00AG065645

Title: Over-expression of microRNA-502-3p suppressed the GABAergic synapse function in Alzheimer's disease

Authors: *B. SHARMA, M. TORRES, S. KUMAR;
Dept. of Mol. and Translational Medicine,, Texas Tech. Univ., EL Paso, TX

Abstract: Alzheimer's disease (AD) is the most common dementia in the aged individuals. Synapse dysfunction is an early event in the brain that initiate AD progression. GABAergic

neurons, the most abundant inhibitory neurons in the human brain, were found to be reduced in AD. Current study investigated a new molecular relation between miR-502-3p and GABAergic synapse function in AD. First, we studied the status of GABA receptor proteins and miR-502-3p in AD postmortem brains. We found the reduced levels of GABA receptor subunits (α, β and γ) in the AD postmortem brains compared to the cognitively normal control brains. The levels of GABA α, β and γ subunits were reduced with AD severity in terms of patient's braak stages. On the other side, miR-502-3p were found to be upregulated in AD postmortem brains with their braak stages. Further, *in vitro* studies were performed using mouse hippocampal (HT-22) neurons, gamma-aminobutyric acid type A receptor subunit alpha1 (GABRα1) overexpression plasmid, miR-502-3p overexpression and miR-502-3p-sponge suppression plasmids. *In-silico* analysis and luciferase assay showed that miR-502-3p targets and binds to several locations at the GABRα1 mRNA. Further, qRT-PCR, immunoblotting and immunostaining analysis of GABRα1 confirmed that miR-502-3p suppressed the GABRα1 mRNA and protein levels. Therefore, overexpression of miR-502-3p decreased cell survival, GABRα1 levels, GABA current and overall GABA function. Our study found an inverse correlation between miR-502-3p levels and GABAergic synapse function in AD. Taken together, miR-502-3p could be a potential therapeutic target to enhance GABAergic synapses function in AD.

Disclosures: B. Sharma: None. **M. Torres:** None. **S. Kumar:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.11/E38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FONDECYT 1200908
ANID Doctorado nacional 21211001

Title: P2x2 receptor overexpression increases mitochondria-associated endoplasmic reticulum membranes through the activation of p2x2r-ampk axis in cellular models of alzheimer's disease

Authors: O. RAMIREZ-MOLINA, J. PANES-FERNANDEZ, P. A. GODOY, J. GAVILAN, R. DURAN, M. E. MEZA, P. CASTRO, G. YEVENES, G. MORAGA-CID, *J. FUENTEALBA;
Fisiologia, Univ. de Concepción, Concepcion, Chile

Abstract: One of the main toxic agents in Alzheimer's Disease (AD) are the soluble oligomers of the Aβ peptide (AβOs), as their size allows them to diffuse in the parenchyma and affect synaptic structure, neuronal function, and cell survival. In our laboratory, we have demonstrated that AβOs induce a calcium (Ca2+) increase into the intracellular medium, and ATP leakage, reducing the intracellular concentration of ATP, resulting in the modulation of ionotropic purinergic receptors (P2XR). We have also shown that AβOs increase the expression of P2X2,

further reinforcing the Ca2+ overload in the cytoplasm that modulate several proteins and pathways, including the AMP-activated protein kinase (AMPK), which plays diverse roles in protein, energy, and mitochondrial metabolism. AMPK is regulated by changes in Ca2+ levels and the intracellular AMP/ATP ratio. Two targets of this kinase are MFN2 and VDAC1, both proteins involved in the mitochondria-associated endoplasmic reticulum membranes (MAMs). MFN2 participates in the anchoring of the endoplasmic reticulum and mitochondria membranes, while VDAC1 facilitates calcium signaling communication from the endoplasmic reticulum to the mitochondria. In this work we used different cell lines to study the effect of farmalogical modulation of AMPK and its effect over MAMs proteins in AD. PC12 cells overexpressing P2X2R or treated with AβOs (0,5 μM, 24 h), and CAD cells expressing APP and APPswe, showed an increase in AMPK activitated levels compared to control cells. AMPK activity in hippocampal neurons exposed to AβOs (0,5 μM, 1 h) increased, while chronic exposure (0,5 μM, 24 h) led to a reduction in AMPK activity (20%). Regarding MFN2, both acute and chronic exposure to AβOs resulted in decreased protein levels, consistent with existing literature. Interestingly, treating these cells with AICAR (1 mM) or AICAR + AβOs significantly increases MFN2 protein levels over the control. On the contrary, treatments with AβOs at acute and chronic times showed an increase in VDAC1 protein levels (20%). Finally, electrophysiology assays on neurons treated with AβOs (0,5 μM), Metformin/AICAR (1 mM), and Compound C (5 μM) for 1 h demonstrated that Metformin greatly decreased the frequency (50%) and amplitude (90%) of spontaneous synaptic currents, whereas no such effect was observed with Compound C (5μM), an inhibitor of AMPK. These results suggest that the P2X2 - Intracellular Calcium - AMPK axis could represent a novel and unexplored pathway in Alzheimer's disease, opening new opportunities for the development of pharmacological strategies for the treatment of AD.

Disclosures: **O. Ramirez-molina:** None. **J. Panes-fernandez:** None. **P.A. Godoy:** None. **J. Gavilan:** None. **R. Duran:** None. **M.E. Meza:** None. **P. Castro:** None. **G. Yevenes:** None. **G. Moraga-cid:** None. **J. Fuentealba:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.12/E39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01 AG032969 (NIH)
R56 AG061869 (NIH)
R56 AG072599 (NIH)
R01 AG067598 (NIH)
P01 AG014449 (NIH)
P01 AG017617 (NIH)
R01 AG074004 (NIH)
RF1 AG077103 (NIH)
Coins for Alzheimer’s Research

ADDF
the Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research and the Experimental Therapeutics Center of the Memorial Sloan Kettering Cancer Center
P30 CA008748

Title: Alzheimer's Disease Stressors Induce Epichaperome Formation within Glutamatergic Neurons

Authors: *S. BAY[1], A. SANTHASEELA[1], C. S. DIGWAL[1], T. ROYCHOWDHURY[1], S. SHARMA[1], A. RODINA[1], P. PANCHAL[1], H. ZHANG[4], K. MANOVA-TODOROVA[2], O. ARANCIO[5,4,6], S. GINGSBERG[7,8], G. CHIOSIS[1,3];
[1]Chem. Biol., [2]Mol. Cytology, Sloan-Kettering Inst., NEW YORK, NY; [3]Dept. of Med., Sloan-Kettering Inst., New york, NY; [4]Taub Inst. for Res. on Alzheimer's Dis. and the Aging Brain, Columbia Univ., New york, NY; [5]Dept. of Pathology and Cell Biol., Columbia Univ., NEW YORK, NY; [6]Dept. of Med., Columbia Univ., New york, NY; [7]Ctr. for Dementia Res., Nathan Kline Inst., NEW YORK, NY; [8]Departments of Psychiatry, Neurosci. & Physiology, and the NYU Neurosci. Inst., New York Univ. Grossman Sch. of Med., New york, NY

Abstract: Stressors associated with disease remodel protein-protein interaction networks (PPIs) through the switch of chaperones into epichaperomes, long-lived assemblies and disease-associated pathologic scaffolds composed of tightly bound chaperones, co-chaperones, and other factors. Not to be confused with chaperones, ubiquitous proteins which fold and act through one-on-one dynamic complexes, epichaperomes act as pathologic scaffolds that form under disease conditions, notably neurodegenerative disorders including Alzheimer’s disease (AD). Epichaperome formation causes thousands of proteins to improperly interact and organize inside cells. This process of PPI rewiring is detrimental to neuronal function, such as synaptic plasticity, cell-to-cell communication, protein translation, cell cycle re-entry, axon guidance, metabolic processes, and inflammation, among others, leading to network-wide dysfunction and cognitive decline. We showed synaptic protein network connectivity, and in turn cognitive function, revert to normal levels upon treatment with small molecules, including PU-AD, that dismantle epichaperomes into individual, normal, folding chaperones, providing a therapeutic channel not optimally exploited to date. We discovered both epichaperome dismantling drugs, as well as diagnostics for precision application of epichaperome targeting drugs, and are in the process of translating them to human studies in AD and other CNS disorders. To advance our understanding of epichaperomes in AD and derive mechanistic insights into context-dependent epichaperome composition, structure, and function, chemical probes for use in confocal and single-molecule, super-resolution imaging approaches, are needed. Here we present the synthesis and characterization of a clickable epichaperome probe. To demonstrate proof-of-principle on the utility of our chemical probes to interrogate AD pathobiology we provide applications of the probe in brain resident cells. Specifically, we stressed glutamatergic neurons in culture with a well-established AD-relevant proteotoxic stressor, soluble Abeta oligomers. We tested the hypothesis that this well-known AD-related stressor results in epichaperome formation which can be rigorously and reproducibly visualized using this new chemical probe for understanding AD biology in the context of presynaptic and postsynaptic neural markers. We posit the newly developed probe will be an indispensable tool to understand epichaperome formation,

composition, and localization in different biological scenarios, including the pathogenesis of AD, with mechanistic and therapeutic implications.

Disclosures: **S. Bay:** None. **A. SanthaSeela:** None. **C.S. Digwal:** None. **T. Roychowdhury:** None. **S. Sharma:** None. **A. Rodina:** None. **P. Panchal:** None. **H. Zhang:** None. **K. Manova-Todorova:** None. **O. Arancio:** None. **S. Gingsberg:** None. **G. Chiosis:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.13/E40

Topic: C.02. Alzheimer"s Disease and Other Dementias

Title: Exploring the neuroprotective properties of $GABA_B$ receptors in Alzheimer’s disease

Authors: *C. MCARDLE[1], F. NIERE[2], A. UNERI[1], K. RAAB-GRAHAM[1];
[1]Wake Forest Univ. Sch. Of Med., Winston Salem, NC; [2]North Carolina Agr. and Tech. State Univ., Greensboro, NC

Abstract: Hyperexcitability and glutamate excitotoxicity are two hypothesized contributors towards synapse loss in Alzheimer’s disease (AD). Previous research has additionally highlighted parallel increases in inhibitory GABAergic signaling as a potential compensatory mechanism in AD models. This data, however, does not address the molecular mechanisms underlying changes in inhibitory signaling, but importantly, whether they hinder synaptic degeneration in AD. Therefore, the goal of this project is to examine the role of metabotropic $GABA_B$ receptors ($GABA_B$Rs) towards hippocampal synapse loss in AD. Utilizing 3-week-old primary hippocampal dissociated neurons from transgenic APP/PS1 mice, our data shows elevated cFOS expression and reduced synapse number, granting *in vitro* APP/PS1 neuronal cultures to serve as a valid model to investigate hyperexcitability and synapse loss in AD. Concurrently, our results indicate that APP/PS1 hippocampal neurons display elevated dendritic protein expression of both principal $GABA_B$R subunits: $GABA_{B1}$R and $GABA_{B2}$R. To examine changes in $GABA_B$R function, a calcium imaging protocol was utilized to quantify changes in calcium florescence following $GABA_B$R activation with baclofen. Wildtype hippocampal neurons treated with 50uM baclofen resulted in an approximate 10% decrease in calcium florescence, while APP/PS1 hippocampal neurons treated with baclofen resulted in an approximate 40% decrease. To next determine if the upregulated expression and function of $GABA_B$Rs in APP/PS1 neurons is neuroprotective against synapse loss, APP/PS1 neurons were treated with baclofen ($GABA_B$R agonist), CGP35348 ($GABA_B$R antagonist), or vehicle. APP/PS1 neurons treated with 50um baclofen increased synapse number as compared to vehicle treated neurons, while APP/PS1 neurons treated with 100uM CGP35348 did not increase synapse number compared to vehicle treated neurons. Finally, our goal was to determine how $GABA_B$R expression changes throughout the AD disease progression. In contradiction to elevated $GABA_{B1}$R and $GABA_{B2}$R levels in 3-week-old APP/PS1 neurons, our data shows

comparable and reduced expression of both subunits at 2 and 4-months of age, respectively, compared to wildtypes. Furthermore, under pathological conditions, activation of $GABA_BRs$ serves as a potential mechanism to combat against hyperexcitability, therefore preventing hippocampal synaptic loss in AD. However, prolonged excitability throughout the disease progression is hypothesized to contribute to downregulation in $GABA_BR$ expression.

Disclosures: C. McArdle: None. **F. Niere:** None. **A. Uneri:** None. **K. Raab-Graham:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.14/E41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant F31AG076289
NIH Grant R56AG072473
Emory ADRC grant 00100569

Title: Cortical region confers neuron-type-specific vulnerability to human APP expression

Authors: *A. M. GOETTEMOELLER[1], E. BANKS[1], M. J. M. ROWAN[2];
[1]Emory Univ. Sch. of Med. Neurosci. Grad. Program, Atlanta, GA; [2]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Alzheimer’s disease (AD) is a devastating and irreversible neurodegenerative disease, afflicting 50 million individuals worldwide, and is expected to escalate three-fold over the next 25 years. Preventative treatment is of dire importance, yet cellular mechanisms underlying the emergence of circuit dysfunction in early AD remain unclear. In humans with AD, circuit hyperexcitability is one of the earliest observed pathophysiological correlates to severe cognitive decline. In genetic AD models, early increases in circuit activity emerge in the entorhinal cortex, a region that is also highly vulnerable to cell death in human AD. The cellular and molecular underpinnings of this regional vulnerability remain unclear. Here we combined regional- and cell-type-specific proteomics and patch-clamp electrophysiology with adult-onset APP expression to evaluate this issue systematically. In entorhinal cortex, we found that AP firing of ‘fast-spiking’ inhibitory interneurons was strongly reduced after only 2-3 weeks of AAV-directed human APP expression. Surprisingly, this adult-onset human APP expression did not affect the biophysics of surrounding excitatory neurons. Together these cellular changes resulted in overall circuit hyperexcitability in entorhinal cortex. We also found that these vulnerabilities were region-specific because human APP expression did not affect neuronal function or overall circuit activity in the somatosensory cortex. To evaluate whether the deleterious effect of human APP expression on entorhinal PV interneurons was due to overexpression, we next expressed mouse APP in the same manner. Interestingly, mouse APP overexpression had no effect on PV interneuron function in the entorhinal cortex, suggesting that human-mouse sequence differences

were responsible for the observed functional alterations. Finally, we observed the relationship of human APP expression on early tau pathology in the entorhinal cortex. Together, this study suggests that disease interventions targeting inhibition may protect cortical regions with vulnerability in early AD.

Disclosures: **A.M. Goettemoeller:** None. **E. Banks:** None. **M.J.M. Rowan:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.15/F1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R21AG073610

Title: Role of Proline-rich 7 (PRR7) in amyloid beta-induced excitatory synapse loss

Authors: *D. NIEVES TORRES[1], S. SKAAR[1], K. HEASTER[1], D. GRAU[1], S. H. LEE[1,2]; [1]Pharmacol. and Toxicology, [2]Neurosci. Res. Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Excitatory synapse loss is one of the key pathologies within Alzheimer's Disease (AD) that is correlated most strongly to the degree of cognitive decline of the disease. Several molecular markers such as amyloid beta (Aβ) and neurofibrillary tangles also promote the progression of neurodegeneration by eliminating synapses. Previous studies suggest an Wnt inhibitor, Dickkopf-1 (Dkk-1), acts as a downstream mediator in soluble Aβ-induced synapse loss. However, molecular mechanisms underlying the Aβ- and Dkk-1-induced synapse loss are still poorly understood. We previously showed that a novel postsynaptic neuronal protein called Proline-rich 7 (PRR7) is released on exosomes to inhibit synaptogenic Wnt signaling and trigger excitatory synapse loss in hippocampal neurons. In this study, we aim to determine the potential role of PRR7 as a downstream effector of Aβ in promoting excitatory synapse loss. We have used immunocytochemistry of presynaptic marker vesicular glutamate transporter-1 (vGLUT-1) and the postsynaptic marker post-synaptic density protein-95 (PSD-95), soluble Aβ oligomers (ADDLs), and Western blotting analyses, combined with dissociated rat hippocampal culture neurons and a mouse model of AD (3xTg) to address the possibility. We found that ADDLs treatment drastically increases the exosomal secretion of PRR7 by hippocampal neurons > 5-fold and Dkk-1 produces similar effects. Moreover, we also found that PRR7 protein levels in exosomes purified from the brains of 3XTg mice are substantially elevated compared to WT control. Since exosomes containing high amounts of PRR7 causes synapse loss in recipient neurons, these data suggest that PRR7 acts as a downstream effector of Aβ and Dkk1 in synapse degeneration. Consistent with this notion, excitingly, CRISPR/Cas9-mediated knockout of PRR7 abolished synapse loss induced by ADDLs in cultured hippocampal neurons. Currently, studies are ongoing to examine the critical function of exosomal PRR7 in Aβ-induced synapse loss.

Taken altogether, our studies suggest the pathogenic function of PRR7 in AD-related synapse degeneration and exosome-mediated spread of AD pathology in the brain.

Disclosures: **D. Nieves Torres:** None. **S. Skaar:** None. **K. Heaster:** None. **D. Grau:** None. **S.H. Lee:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.16/F2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIGMS Grant 20GM109098-06A

Title: Dendritic spine nano-architecture underlies selective vulnerability of synapses to Amyloid-beta synaptotoxicity

Authors: *J. SCRIPTER, G. JONES, M. HRUSKA;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Alzheimer’s disease (AD) is characterized by a progressive synaptic loss that highly correlates with cognitive decline and memory loss. Neuropathological hallmarks of AD, including Amyloid-beta (A-Beta) and Tau aggregation, have been proposed to induce synaptic dysfunction before cognitive symptoms become apparent. Despite their central role in AD, how synapses of diverse shapes and sizes respond to A-Beta toxicity is unknown. Remarkably, in patients and mouse models of AD, the remaining spines are bigger with large post-synaptic densities (PSDs). This begs the question of whether small spines might be uniquely vulnerable to AD pathology and whether the molecular composition of synapses might regulate the degree of vulnerability to A-Beta synaptotoxicity.Previous studies using STimulated Emission Depletion (STED) nanoscopy have demonstrated that aligned nanomodules of pre-synaptic (Bassoon) and post-synaptic (PSD-95) proteins form the building blocks of dendritic spine synapses, where the number of aligned nanomodules scale with spine size. Using STED imaging of endogenous synaptic proteins combined with fluorescent labeling of neuronal morphology, we investigated how A-Beta impacts dendritic spines with diverse nanoarchitectures. We conducted experiments in days *in vitro* (DIV) 21 rat cortical neurons infected with lentiviruses for either amyloidogenic (CT100) or control**,** non-amyloidogenic (CT84) APP fragments. The 24-hour exposure to A-Beta led to a significant increase in the density of spines *without* PSD-95 nanomodules while simultaneously leading to a reduced number of spines with only *one* PSD-95 nanomodule compared to the control neurons. The density of spines with *two* or *more* nanomodules was not affected at this time point. Remarkably, spines with 1 and 2 nanomodules were larger in CT100 neurons compared to the control. We validated these findings using the 5xFAD mice that rapidly accumulate A-Beta in the brain. To gain additional insight into acute A-Beta actions, we monitored changes in the endogenous PSD-95 nanomodules labeled by PSD-95_FingR_EYFP in

individual spines using STED imaging of living neurons for 24 hours. Our results show that single nanomodule spines in A-Beta treated neurons lost nanomodules at a faster rate than the control spines or spines with two or more PSD-95 nanomodules. Remarkably, single nanomodule spines in which A-Beta caused PSD-95 loss were significantly smaller than the neighboring single and multiple nanomodule spines that did not lose PSD-95. Our results reveal novel actions of acute and chronic A-Beta toxicity on nanoscale disruptions of different synaptic populations.

Disclosures: **J. Scripter:** None. **G. Jones:** None. **M. Hruska:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.17/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PAPIIT- IN-206322

Title: Amylovis rescues inhibition of nicotinic currents by A-beta in hippocampus of 3xTg AD model

Authors: *P. RODRÍGUEZ ARELLANO[1], E. ORTA SALAZAR[1], J. J. GARCÍA COLUNGA[1], H. MARTÍNEZ CORIA[2], S. Y. DÍAZ CINTRA[1];
[1]Inst. de Neurobiología, Querétaro, Mexico; [2]Fac. of Med., Natl. Autonomous Univ. of Mexico, Mexico, Mexico

Abstract: In Alzheimer's disease (AD), β-amyloid oligomers (oAβ) are more toxic than neuritic plaques due to their interactions with other molecules. At pathological concentrations, oAβ binds to and inhibits nicotinic receptors. Amylovis, a naphthalene derivative, binds to Aβ and inhibits its oligomerization, aggregation, and its binding with other molecules (Rivera-Marrero et al., 2020). This raises the question of whether Amylovis could prevent the binding of Aβ1-42 to nicotinic receptors (nAChRs) and subsequently restore their function. The aim of this study was to analyze nicotinic modulation in the triple transgenic model (3xTg-AD) and the effect of Amylovis on currents elicited by these receptors. Female mice of the 3xTg-AD model from 6 months old were used. We recorded currents of interneurons in CA1 *stratum radiatum* of the hippocampus that were elicited by puffs of nicotine at 10 mM. To assess the effect of Amylovis, we pre-incubated the slices for 2 hours or added Amylovis at 1 µM to the bath. The average currents of WT animals were 156.06±42.76 pA (n=7). Although they were 52% larger than the currents of 3xTg-AD (81.10±40.94 pA, n=9), they did not reach statistical significance. However, currents elicited by cells pre-incubated with Amylovis (267.4±93.01 pA, n=7) were significantly larger than those elicited by 3xTg control. When we bath-applied Amylovis during the recording, the compound increased the currents after 10 minutes of application (n=3). The increase persisted during the application of Amylovis and slightly decreased afterward. This data

demonstrates that Amylovis influences currents produced by nAChRs, which may be due to its ability to prevent the interaction of Aβ with nicotinic receptors. We thank to A. Aguilar Vázquez, A. Castillo León, A. González, and A. Hernández-Abrego A for their excellent technical support. The present work was supported by PAPIIT- IN-206322.Rivera-Marrero, S., et al. (2020). A new naphthalene derivative with anti-amyloidogenic activity as potential therapeutic agent for Alzheimer's disease. *Bioorganic and Medicinal Chemistry, 28*(20). Doi:10.1016/j.bmc.2020.115700

Disclosures: P. Rodríguez Arellano: None. **E. Orta Salazar:** None. **J.J. García Colunga:** None. **H. Martínez Coria:** None. **S.Y. Díaz Cintra:** None.

Poster

PSTR460. Alzheimer's Disease: Molecular and Cellular Mechanisms II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR460.18/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI Grant-in-Aid for Exploratory Research JP 20K21466
JSPS KAKENHI Grant-in-Aid for Exploratory Research JP16K21268
Nagahisa Science Foundation
Yokohama Foundation for Advancement of Medical Science

Title: Lotus suppresses amyloid β-induced dendritic spine elimination through the blockade of amyloid β binding to pirb.

Authors: *Y. KAWAGUCH[1], J. MATSUBAYASHI[1], Y. KAWAKAMI[1], R. NISHIDA[2], Y. KURIHARA[2], K. TAKEI[1];
[1]Dept. of Regenerative Med., Yokohama City Univ., Suehiro-cho 1-7-29, Tsurumi Ward Yokoham, Japan; [2]Dept. of Med. Life Sci., Mol. Med. Biosci. Laboratory, Yokohama City Univ. Grad. Sch. of Med. Life Sci., Yokohama, Kanagawa, Japan

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide but has little effective treatment. Amyloid beta (Aβ) protein, a primary risk factor for AD, accumulates and aggregates in the brain of patients with AD. Paired immunoglobulin-like receptor B (PirB) has been identified as a receptor of Aβ and Aβ-PirB molecular interactions that cause synapse elimination and synaptic dysfunction. PirB deletion has been shown to suppress Aβ-induced synaptic dysfunction and behavioral deficits in AD model mice, implying that PirB mediates Aβ-induced AD pathology(Kim et al., 2013). Therefore, inhibiting the Aβ-PirB molecular interaction could be a successful approach to combating AD pathology. We previously showed that lateral olfactory tract usher substance (LOTUS) is an endogenous antagonist of PirB and that LOTUS counteracts Nogo-induced neurite outgrowth inhibition and growth cone collapse (Kurihara et al., 2020). Therefore, in this study, we investigated whether LOTUS inhibits Aβ-PirB interaction and Aβ-induced dendritic spine elimination. The inhibitory role of

LOTUS against Aβ-PirB binding was assessed using a ligand-receptor binding assay in Cos7 cells overexpressing PirB and/or LOTUS. We found that LOTUS suppressed the binding of Aβ to PirB overexpressed in Cos7 cells. Next, we assessed whether LOTUS inhibits Aβ-induced intracellular alterations and synaptotoxicity using immunoblots and spine imaging in a primary cultured hippocampal neuron. We found that Aβ-induced dephosphorylation of cofilin and Aβ-induced decrease in post-synaptic density-95 (PSD95) expression were suppressed in cultured hippocampal neurons from LOTUS-overexpressing transgenic (LOTUS-tg) mice compared with that in wild-type mice. Moreover, primary cultured hippocampal neurons from LOTUS-tg mice inhibited the Aβ-induced decrease in dendritic spine density. Finally, we studied whether human LOTUS protein suppressed Aβ binding to leukocyte immunoglobulin-like receptor subfamily B member 2 (LilrB2), a human homolog of PirB, and found that human LOTUS inhibited the binding of Aβ to LilrB2 in a similar manner. These findings show that LOTUS reduces Aβ-induced synapse elimination by suppressing Aβ-PirB interaction in rodents and also inhibits Aβ-LilrB2 interaction in humans. Thus, the data suggest that LOTUS may be a promising therapeutic agent in counteracting Aβ-induced AD pathologies. Future research will examine how LOTUS is involved in cognitive mechanisms in AD mice, and how LOTUS fluctuates in human AD patients.

Disclosures: Y. Kawaguch: None. **J. Matsubayashi:** None. **Y. Kawakami:** None. **R. Nishida:** None. **Y. Kurihara:** None. **K. Takei:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.01/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIDCD grant R01 DC014723
NSF BRAIN 1555880
NSF BRAIN 1555862
NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program (Award #2014217)

Title: Temporary hyper-responsive neurovascular coupling in middle-aged APOE4-TREM2 mice as early marker for LOAD.

Authors: *M. OUMOV[1,2], B. SANGANAHALLI[3,4,5], P. HERMAN[3,4,5], F. HYDER[3,4,5,6], J. VERHAGEN[1,2];
[1]John B. Pierce Lab., New Haven, CT; [2]Dept. of Neurosci., Yale Sch. of Med., New Haven, CT; [3]Magnetic Resonance Res. Ctr. (MRRC), [4]Quantitative Neurosci. with Magnetic Resonance (QNMR) Core Ctr., [5]Dept. of Radiology and Biomed. Imaging, [6]Dept. of Biomed. Engin., Yale Univ., New Haven, CT

Abstract: Alzheimer's disease (AD) is an incurable degenerative disease that affects millions worldwide, making early detection crucial for treatment. Many genes have been identified as risk factors for the predominant form, Late Onset AD (LOAD), two of the most impactful being Apoe4 and Trem2 (A4T2). These genes are involved with lipid trafficking, vascular function and inflammation and show age and sex-dependent effects. We have previously behaviorally phenotyped these mice finding evidence of AD-relevant deficits. Here we investigated the neurovascular coupling in the dorsal olfactory bulb (dOB) of aged A4T2 and WT mice. We hypothesize that in early stages of AD, neurovascular hyper-responsiveness will be detected due to compensatory efforts sustaining the degenerating tissues. In later stages of AD we expect neuro-vascular hypo-responsiveness, caused by vascular dysfunction, neuronal inflammation, and overall brain tissue degeneration. Anesthetized A4T2 mice (JAX #028709 created by MODEL-AD) and WT (C57/B6) were optically intrinsically imaged for blood volume changes during odor stimulation presented by an olfactometer utilizing ethyl butyrate (EB) and methyl valerate (MV) at 0.3, 1, 3% vapor pressure. These preliminary data are based on a "middle aged" group that consisted of 9-12 mo mice, 3 WT (1 male, 2 females avg age 10.6 mo), 4 A4T2 (1 male, 3 females avg age 10.7 mo). The "old" group (12+ mo) consisted of 5 WT (3 males, 2 females avg age 21.9 mo) and 5 A4T2 mice (1 female, 4 males avg age 25.4 mo). Headplate and optical window surgeries were performed under isoflurane with a 5-8 day recovery period before imaging under ketamine and dexmedetomidine (0.05 mg/Kg B.W). We quantified the response maximum (i.e. peak of blood volume increase) and area under the curve (dF/F% * 10s) of these preliminary data, which revealed consistent responses in the micro vessels and surrounding glomerular tissue. In middle aged A4T2 mice, neurovascular hyper-responsiveness was observed when compared to middle aged WT and old A4T2. Aged WT mice were found to have hyper-responsiveness compared to younger WT. Neurovascular hypo-responsiveness was not consistently detected in these subjects. These results suggest neurovascular dysfunction is detectable as a transient hyper-responsiveness in the early stages of these LOAD mice. We are continuing to expand this dataset. These preliminary data support vascular hyper-responsiveness as an early marker to LOAD detection.

Disclosures: M. Oumov: None. **B. Sanganahalli:** None. **P. Herman:** None. **F. Hyder:** None. **J. Verhagen:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.02/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG058748
NIH Grant AG072328
NIH Grant AG034570
NIH Grant AG062542

NIH Grant AG044292
Alzheimer's Association Award AARF-17–530186

Title: Higher amygdala tau is linked to hyperconnectivity of a locus coeruleus network in Alzheimer's pathological change

Authors: *J. H. PARENT[1], K. CASSADY[2], C. GORDON[1], W. J. JAGUST[2], A. S. BERRY[1]; [1]Brandeis Univ., Waltham, MA; [2]Univ. of California, Berkeley, Univ. of California, Berkeley, CA

Abstract: Recent advances in early detection of pathological changes in Alzheimer's disease (AD) have focused on relationships between AD-related tau and amyloid-beta pathology and the locus coeruleus (LC) norepinephrine system, one of the earliest regions impacted by pathological tau. AD-related disruption in this system has been associated with downstream cognitive decline. The LC-NE system is recruited in stress and affective regulation which are also disrupted in AD. Previously, we published associations between a novel in vivo measure of LC catecholamine function, AD-related tau pathology, and affective risk factors for AD in cognitively normal older adults, where lower LC catecholamine synthesis capacity ([^{18}F]Fluoro-meta-tyrosine (FMT) PET), was associated with greater amygdala tau ([^{18}F]Flortaucipir (FTP) PET) and higher trait neuroticism and depression symptoms. However, widespread functional characteristics associated with alterations in this system and AD-related neuropathology are unclear. Here, we used resting-state functional magnetic resonance imaging in cognitively normal older adults (n = 44, mean age = 77, range = 62-85 years; mean education = 16; 27 females) to test relationships between functional connectivity of a network of regions that are functionally connected to the LC, amygdala tau pathology, LC FMT, and affective risk factors. We found that in amyloid-positive individuals ([^{11}C]Pittsburgh Compound B PET), higher amygdala FTP and lower LC FMT were independently associated with greater LC network functional connectivity ($\beta_{\text{amyloid-status x amygdala FTP}}$ =0.07, t(36)= 2.96, p = .006; $\beta_{\text{amyloid-status x LC FMT}}$ =-4.9, t(38)= -2.36, p =.02). Further, we found that also in individuals with higher neuroticism and more depressive symptoms, higher amygdala FTP was associated with greater LC network functional connectivity ($\beta_{\text{depression symptoms x amygdala FTP}}$ =0.009, t(35)= 2.43 p = .02; $\beta_{\text{neuroticism x amygdala FTP}}$ = .008, t(35)= 2.47, p =.02). Lastly, we examined associations of LC network functional connectivity with reward-related memory performance. We found that those with higher amygdala FTP and less LC-network functional connectivity had worse memory performance for stimuli in the loss condition of the reward-memory task ($\beta_{\text{LC-network connectivity x amygdala FTP}}$ = -74.7, t(35) = -2.14, p = .04). These findings allude to potential hyperactivation of an LC functional network in individuals with AD-related pathological change, which may be intensified in individuals with affect-related AD risk factors.

Disclosures: J.H. Parent: None. **K. Cassady:** None. **C. Gordon:** None. **W.J. Jagust:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Optoceutics. F. Consulting Fees (e.g., advisory boards); Biogen and Bioclinica. **A.S. Berry:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.03/F7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG060610

Title: Testing generalizability of 3D CNN models for Brain Age Prediction from Diffusion MRI in North American and Indian Cohorts

Authors: *T. CHATTOPADHYAY[1], Y. FENG[3], J. VILLALON REINA[2], H. JOSHI[5], G. VENKATASUBRAMANIAN[6], J. P. JOHN[5], P. M. THOMPSON[4];
[2]Mark and Mary Stevens Neuroimaging and Informatics Inst., [1]USC, Los Angeles, CA; [3]Mark and Mary Stevens Neuroimaging and Informatics Inst., Univ. of Southern California (USC), Los Angeles, CA; [4]Stevens Inst. for Neuroimaging & Informatics, Univ. of Southern California (USC), Marina del Rey, CA; [5]Multimodal Brain Image Analysis Lab., [6]Translational Psychiatry Lab., Natl. Inst. of Mental Hlth. and Neuro Sci., Bangalore, India

Abstract: Diffusion-weighted brain MRI (dMRI) is sensitive to subtle alterations in the brain's microstructure, and can yield metrics that are correlated with age, dementia severity, and even levels of brain amyloid, a key cause of Alzheimer's pathology that is not directly measurable on MRI. Although a person's age is known and would not be useful to predict in clinical practice, brain age prediction in healthy control subjects is a common benchmarking task, as the ground truth is known with high accuracy. In addition, when such a trained model is applied to patients with different stages of dementia, the difference between the predicted age (the person's "BrainAge") and their true chronological age has been linked with future clinical decline, dementia, and mortality. We tested the generalizability of 3D CNN architecture trained on dMRI from 636 healthy control subjects (CN, mean age: 73.48+/-7.24 yrs., 375 F/ 261 M) from the (North American) ADNI dataset on an "out of distribution" 32 subjects from the NIMHANS dataset, collected in Bangalore, India (CN, mean age: 63.53+/-6.11 yrs, 16 F/ 16 M). The dMRI preprocessing pipeline follows the ENIGMA Processing Pipeline.
Results: The MAEs for trained models on the ADNI dataset were much lower than those on corresponding dMRI modalities from NIMHANS dataset. This shows a requirement to either harmonize the datasets using ComBat, ComBat-GAM, and CovBat, or even VAE-GAN architectures, or train the model on more diverse data to improve generalizability of the model.

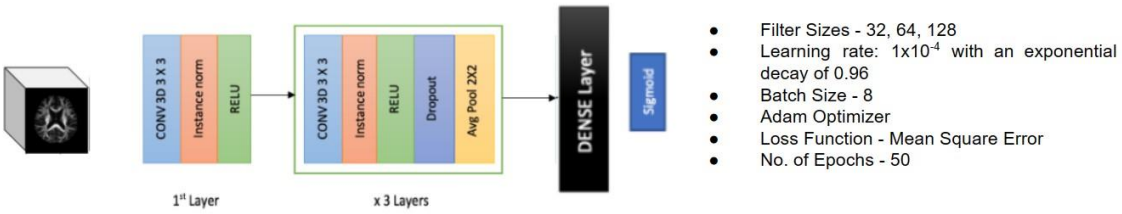


Fig 1. 3D CNN Architecture

	DWI-FA	DWI-AD	DWI-MD	DWI-RD
ADNI MAE	3.19	4.34	4.11	4.19
NIMHANS MAE	6.51	7.74	10.24	7.25

Table 1.. MAE of model on Test Datasets

Disclosures: T. Chattopadhyay: None. **Y. Feng:** None. **J. Villalon Reina:** None. **H. Joshi:** None. **G. Venkatasubramanian:** None. **J.P. John:** None. **P.M. Thompson:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.04/F8

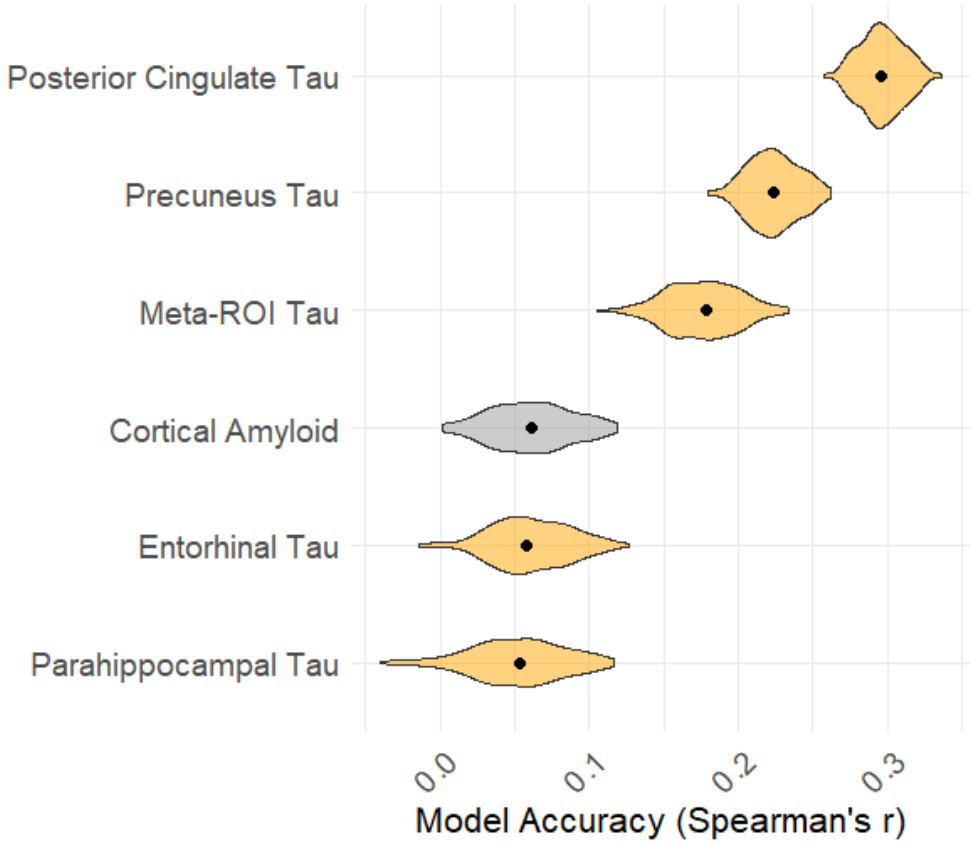
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1P30AG066508-01; 5K23AG059919-04
Alzheimer's Association 2019-AACSF-644153

Title: Characterizing the relationship between the functional connectome and tau PET in an asymptomatic preclinical population

Authors: *H. ABUWARDA[1], A. TRAINER[2], S. JU[2], R. T. CONSTABLE[3], C. A. FREDERICKS[2];
[1]Interdepartmental Neurosci. Program, Yale Med. Sch., New Haven, CT; [2]Dept. of Neurol., [3]Dept. of Radiology and Biomed. Imaging, Yale Sch. of Med., New Haven, CT

Abstract: Amnestic Alzheimer's Disease (AD) is characterized by stereotyped progression of tau pathology as well as characteristic early disruptions in functional connectivity, especially in the Default Mode Network (DMN). Yet, the relationship between the functional connectome and tau pathology is not fully understood. To unpack this, we sought to use the functional connectome to predict tau deposition in regions implicated in early-stage AD.
Methods. We use connectome-based predictive modeling (CPM) to identify the circuitry which predicts focal tau pathology in an at-risk, asymptomatic cohort. We used tau PET (AV-1451) and resting-state fMRI (rsfMRI) from the Anti-Amyloid in Asymptomatic Alzheimer's disease (A4) study, a clinical trial of cognitively unimpaired adults (aged 65-85) with elevated amyloid PET. Using the rsfMRI (t = 6.5 min) from 394 subjects, we sought to predict contemporaneous AV-1451 binding (standardized uptake value ratio (SUVR)) in individual temporal and parietal regions implicated in early-stage AD, and a meta-ROI (comprised of entorhinal, parahippocampal, amygdala, fusiform, middle temporal, and inferior temporal regions) previously described in the literature.
Results. Model performance was evaluated by Spearman's correlation between predicted and observed AV-1451 SUVR, and model significance was assessed against permuted models (n=1000 iterations). Models of posterior cingulate (r = 0.30) and precuneus (r = 0.23) tau performed best, while models of parahippocampal (r = 0.05), cortical amyloid (r = 0.06) and entorhinal (r = 0.06) tau demonstrated minimal predictive power. All models were significant $p<0.01$ against permuted models (Bonferroni-corrected).
Conclusions. Using the functional connectome, we can predict a portion of the variance in tau PET across parietal and temporal regions implicated in early-stage AD. Models were most predictive of tau in DMN parietal regions, but only weakly predictive of tau in temporal regions that show very early tau deposition. Future work will assess the networks that underpin our most successful models.



Disclosures: **H. Abuwarda:** None. **A. Trainer:** None. **S. Ju:** None. **R.T. Constable:** None. **C.A. Fredericks:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.05/G1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HABS-HD U19AG078109
NIH Grant 2U19AG024904-16

Title: Investigating the associations between race and ethnicity, Ab positivity and medial temporal lobe tau PET

Authors: ***K. WHEELER**[1], V. TENNANT[1], N. LEE[1], M. TUBI[1], J. TERNER[1], B. HALL[1], M. DAVISON[1], A. W. TOGA[2], S. O'BRYANT[3], A. L. CLARK[4], K. YAFFE[5], M. N. BRASKIE[1]; [1]Mark & Mary Stevens Neuroimaging & Informatics Inst., USC, Marina del Rey, CA; [2]Lab. of Neuro Imaging, USC, Los Angeles, CA; [3]Ctr. for Alzheimer's & Neurodegenerative Dis. Res.,

Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [4]Dept. of Psychology, Univ. of Texas at Austin, Austin, TX; [5]Weill Inst. for Neurosciences, Univ. of California at San Francisco, San Francisco, CA

Abstract: Alzheimer's Disease (AD) prevalence is higher in African American and Hispanic adults versus non-Hispanic white (NHW) adults and etiology may vary across ethnic/racial groups. Neurofibrillary tau is a pathological hallmark of AD, but studies of racial/ethnic differences in tau neuroimaging markers are limited. We evaluated (1) associations between race/ethnicity, Aβ positivity (Aβ+), and tau positron emission tomography (PET) of the medial temporal lobe (MTL) where tau deposits early, and (2) MTL tau associations with cognition. We acquired 18F-PI-2620 tau-PET and florbetaben amyloid-PET scans in 561 cognitively normal (CN) and 169 mild cognitively impaired (MCI) adults aged 50 to 90 from the Health and Aging Brain Study- Health Disparities cohort (**Table 1**). Aβ+ or Aβ- was determined by a global amyloid PET standardized uptake value ratio cutoff of 1.08. Race/ethnicity was self-reported. Multiple regressions evaluated associations between 1) race/ethnicity and MTL tau, 2) race/ethnicity interactions with Aβ on MTL tau and 3) MTL tau and cognition using the Spanish English Verbal Learning Test (SEVLT) Trials 1-5. Age, sex, and education were covariates. In CN adults, a trend level race/ethnicity by Aβ interaction was found on MTL tau (β=-0.09, p= 0.07). Compared to CN NHW adults, Aβ- was associated with higher MTL tau in CN Hispanic (β=0.27, p<0.001) and African American (β=0.26, p<0.001) adults. Aβ+ was associated with high MTL tau in CN NHW adults (β=0.25, p<0.001). CN African American adults had greater MTL tau than CN (β=0.19, p<0.001) and MCI NHW adults (β=0.18, p= 0.02). Across cognitive diagnoses, there was no association between SEVLT and MTL tau in African American adults, but trend level associations were in NHW (β=-0.10, p=0.06) and Hispanic adults (β=-0.11, p=0.09). MTL tau by race/ethnicity interactions existed between African American and NHW (β=-0.74, p=0.03) and Hispanic adults (β=-0.66, p=0.048). CN African American adults have high MTL tau suggesting cognitive resilience to tau. MTL tau may not be an early driver of cognitive impairment in African American adults.

Table 1. Sample Demographics of Cognitively Normal and Mild Cognitively Impaired Adults

	CN Non-Hispanic white (N=248)	CN Hispanic (N=153)	CN African American (N=160)	MCI Non-Hispanic white (N=40)	MCI Hispanic (N=58)	MCI African American (N=71)
Amyloid positivity (N)	191 Aβ- / 57 Aβ+	129 Aβ- / 24 Aβ+	140 Aβ- / 20 Aβ+	28 Aβ- / 12 Aβ+	47 Aβ- / 11 Aβ+	57 Aβ- / 14 Aβ+
Mean Age (years)	69 (+/-8) years old	64 (+/- 8) years old	62 (+/- 7) years old	71 (+/-9) years old	67 (+/-9) years old	62 (+/-7) years old
Sex (N)	98 males / 150 females	55 males / 99 females	50 males / 110 females	26 males / 14 females	22 males / 36 females	36 males / 35 females
Mean Education +/- SD (years)	16 (+/- 3) years	11 (+/- 4) years	15 (+/-3) years	16 (+/- 3) years	11 (+/- 4) years	14 (+/- 2) years

Disclosures: **K. Wheeler:** None. **V. Tennant:** None. **N. Lee:** None. **M. Tubi:** None. **J. Terner:** None. **B. Hall:** None. **M. Davison:** None. **A.W. Toga:** None. **S. O'Bryant:** None. **A.L. Clark:** None. **K. Yaffe:** None. **M.N. Braskie:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.06/G2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG061325
1R01AG059861

Title: Resting-state functional connectivity (RSFC) in pain-related brain regions in an ADNI cohort

Authors: M. D. FAILLA[1], A. JAHN[2], ***R. COWAN**[3,1,2], T. B. MONROE[1];
[1]Col. of Nursing, Ohio State Univ., Columbus, OH; [2]Psychology, Univ. of Michigan, Ann Arbor, MI; [3]Psychiatry, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Compared to healthy controls, people with Alzheimer’s disease (AD) receive fewer analgesic medications, verbally report pain less frequently, exhibit differences in neural responses to acute experimental pain, and have altered RSFC in pain-related brain regions. Our work showed that people with AD who were free from chronic pain had reduced RSFC between the posterior insula and the anterior cingulate gyrus (ACG). In this study, we aimed to expand our previous work using an independent sample from the Alzheimer's Disease Neuroimaging Initiative (ADNI) with adults, many who had chronic pain, extending our work by controlling for analgesic medication. Between groups analyses in RSFC were conducted among regions of interest implicated in both sensory and affective-related pain processing. We hypothesized that people with AD would have reduced RSFC between brain regions related to the sensory, cognitive, and emotional processing of pain. Participants included 90 adults, aged 56-95, with 34 (18 Female, 16 Male) adults living with AD and 56 (31 Female, 27 Male) cognitively normal (CN) older adults who had resting-state fMRI data collected as part of ADNI Wave 2 cohort. Using the CONN toolbox (SPM12), we conducted a seed region to whole-brain functional connectivity analyses with bilateral seed regions for: dorsolateral prefrontal cortex (DLPFC), sensorimotor cortex (SMC), anterior insula (aINS), and ACG. Group contrasts were calculated in the GLM with covariates known to impact the pain experience (age, anxiety levels, depression, and pain medication), and cluster-threshold p=0.001 uncorrected; FWE cluster-corrected p=0.05. A higher percentage of the AD group (82.3%, compared to 60.7% of the CN group, X-squared = 6.3912, df = 2, p-value = 0.041) were taking analgesics at the time of scanning. The AD group had increased connectivity between the left aINS (L-aINS) and the bilateral SFG, decreased connectivity between the bilateral ACG and L-SMC regions, and decreased connectivity between the L-DLPFC to R-precuneus and bilateral frontal pole. Increased RSFC between L-aINS and

SFG (regions involved in working memory and cognitive appraisal of pain) in AD may reflect either stronger feedforward synchrony from aINS to SFG, SFG to aINS, or a combination of the two. Decreased RSFC between ACG and L-SMC and between L-DLPFC and precuneus/frontal pole in AD may result in a reduced ability to modulate the cognitive, sensory, and affective components of pain. These findings of altered functional connectivity in pain regions suggest that people with AD may have altered neural circuit function that might explain in part the observed findings of altered pain perception in AD.

Disclosures: M.D. Failla: None. **A. Jahn:** None. **R. Cowan:** None. **T.B. Monroe:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.07/G3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Measures of cortical thickness in Alzheimer's disease with and without history of traumatic brain injury

Authors: *G. M. D'SOUZA[1,2], N. W. CHURCHILL[2,3], D. X. GUAN[4], M. A. KHOURY[1,2], S. J. GRAHAM[1,5], S. KUMAR[1,6], C. E. FISCHER[1,2], T. A. SCHWEIZER[1,2];
[1]Univ. of Toronto, Toronto, ON, Canada; [2]St. Michael's Hosp., Toronto, ON, Canada; [3]Toronto Metropolitan Univ., Toronto, ON, Canada; [4]Univ. of Calgary, Calgary, AB, Canada; [5]Sunnybrook Res. Inst., Toronto, ON, Canada; [6]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: There is evidence that traumatic brain injury (TBI) has long-term consequences for brain health, including an increased risk for dementia and progressive brain atrophy. To date, however, there has been little direct examination of how TBI affects the rate of neurodegeneration for individuals with Alzheimer’s disease (AD). The present study examined this issue using a mixed design approach, applied to a cohort of 1124 participants from the National Alzheimer’s Coordinating Center (NACC) including 343 with AD, 127 with AD with TBI, 266 cognitively normal adults with TBI, and 388 cognitively normal adults without TBI. For these groups, cortical thickness measures were obtained from T1-weighted magnetic resonance imaging (MRI) data using FreeSurfer and in-house software. An initial cross-sectional analysis of this group at baseline used multiple linear regression to determine the interaction effects of AD and TBI on measures of cortical thickness. Among those with AD, TBI was associated with an earlier age of AD onset but, counter-intuitively, less cortical thinning in fronto-temporal regions, relative to non-AD controls. The results suggest that AD with TBI represents a physiologically distinct group from AD at baseline assessment. A second longitudinal analysis of this group used a partial least squares approach to measure group differences in the longitudinal change of cortical thickness values, for a subset of 154 participants with follow-up scans, assessed over an average time span of 33 months. The AD

groups with and without TBI history more strongly expressed patterns of longitudinal frontal and temporal atrophy, while the cognitively normal control group displayed an intermediate pattern of atrophy, and the cognitively normal TBI group expressed the least atrophy. Further, comparison of AD and AD+TBI to their respective control groups showed a more pronounced effect of AD for the TBI groups. These results provide preliminary evidence of a relationship between TBI history and risk of accelerated cortical thinning in frontal and temporal regions. An improved understanding of the long-term outcomes of TBI has the potential to aid in the diagnosis and treatment of individuals with AD combined with history of TBI.

Disclosures: **G.M. D'Souza:** None. **N.W. Churchill:** None. **D.X. Guan:** None. **M.A. Khoury:** None. **S.J. Graham:** None. **S. Kumar:** None. **C.E. Fischer:** None. **T.A. Schweizer:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.08/G4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Changes in autocorrelation of resting-state functional MRI in Alzheimer's disease and mild cognitive impairment

Authors: *C. HUMPHRIES[1], J. J. BERO[1], Y. LI[1], A. KUMAR[1], S. SARKAR[1], H. LEE[1], D. LEE[1,2];
[1]Neurogazer USA, Baltimore, MD; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder in older adults that is characterized by progressive cognitive decline. Over the course of the disease, increasing levels of brain atrophy result in widespread loss of neuronal function and disruption of coordinated neural activity across the brain. One expected outcome of this degeneration is a change in the spatial pattern and temporal dynamics of global brain activity. AD-related changes in the spatial pattern of brain activity have been well documented by measuring differences in functional connectivity using functional magnetic resonance imaging (MRI). However, less is known about how the temporal dynamics of individual brain systems change over the course of the disease. In the current study, we investigated the temporal properties of neural activity in subjects with AD, mild cognitive impairment (MCI), and age-matched healthy controls using resting-state functional MRI. Structural and functional MRI scans were downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. A total of 691 subjects were used in the analysis, which included 77 AD, 225 MCI, and 389 Controls. MRI processing was conducted using AFNI and included motion correction, registration, normalization, artifact removal using CompCor, and estimation of regions of interest (ROI) time courses using the Schaefer 400 parcellation. To measure changes in temporal dynamics, we calculated the autocorrelation function and power spectrum of the functional MRI time course. Statistical analyses were used to test for differences between groups using age as a covariate. The results showed a widespread

decrease across the brain between controls and AD subjects in both the first lag of the autocorrelation function and low-frequency (0.01 to 0.1 Hz) spectral energy, most notably in the posterior cingulate cortex, inferior parietal lobule, and prefrontal cortex. This pattern of results could arise from two possible mechanisms caused by neural degeneration that are not mutually exclusive. First, reduced neural activity from cortical atrophy might lead to decreased blood flow, resulting in reduced power in the low-frequency range of the hemodynamic response and a reduction in autocorrelation. Second, impaired neuronal function might affect global processing at longer timescales resulting in a general reduction in autocorrelation and low-frequency spectral energy.

Disclosures: C. Humphries: A. Employment/Salary (full or part-time):; Neurogazer Inc. **J.J. Bero:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **Y. Li:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **A. Kumar:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **S. Sarkar:** A. Employment/Salary (full or part-time):; Neurogazer Inc. **H. Lee:** A. Employment/Salary (full or part-time):; Neurogazer Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer Inc. **D. Lee:** A. Employment/Salary (full or part-time):; Neurogazer Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurogazer Inc.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.09/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant MOP-84480
SynAD grant

Title: Diagnostic prospects of labelled native-PLGA nanoparticles in Alzheimer's Disease (AD) pathology

Authors: *K. GOVINDARAJAN, S. KAR;
Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Diagnostic prospects of labelled native-PLGA nanoparticles in Alzheimer's Disease (AD) pathology

Govindarajan Karthivashan[1,2] and Satyabrata Kar[1,2,3]
Departments of Medicine (Neurology)[1], Centre for Prions and Protein Folding Diseases[2], Neuroscience and Mental Health Institute[3], University of Alberta, Edmonton, Alberta, Canada T6G 2M8.

ABSTRACTAlzheimer's disease (AD) is the most common cause of dementia among the elderly. Evidence suggests that elevated β-amyloid (Aβ) peptide levels/aggregation and increased

phosphorylation of tau protein play crucial role in the development of AD. Presently, AD is clinically diagnosed using cognitive tests, neuroimaging and detecting abnormal Aβ and tau levels/deposition. While measuring Aβ and tau levels can suggest disease state, neuroimaging of aggregated Aβ and tau protein in the brain using positron emission tomography (PET) allows for monitoring pathological changes in AD patients. We recently reported that FDA-approved native PLGA nanoparticles without conjugation with any drug/agent can interact with Aβ to reduce its aggregation/toxicity in cellular and animal models of AD. As a follow up, we evaluated if labelled native PLGA can interact with Aβ-containing neuritic plaques in 5xFAD mouse model of AD. 5xFAD-Tg and wild-type (WT) mice were injected with a single intracerebral dose of labelled native-PLGA and then animals were fixed with Paraformaldehyde at 1h, 3h, 12h, 24h, 72h and 1week post-treatment. The brain tissues were then processed for Aβ-immunostaining or Congo red dye to evaluate their co-localization with labelled PLGA using the Pearson coefficient values from the merged-channel images. Our results revealed that fluorescence labelled native PLGA following single acute intracerebellar injection can identify majority of the immunostained Aβ as well as Congo red labelled neuritic plaques in the cortical regions of 5xFAD mouse brains. PLGA-labelled plaques are apparent at 1hr, peak around 3hr and then start declining by 24hr after injection. No fluorescent labelled PLGA was detected in the control cerebellum of 5xFAD mice or in any brain regions of wild-type control mice. These results provide the very first evidence that native PLGA nanoparticles can be developed as a novel agent for the diagnosis and tracking pathological changes in AD.

Disclosures: **K. Govindarajan:** A. Employment/Salary (full or part-time):; University of Alberta. **S. Kar:** A. Employment/Salary (full or part-time):; University of Alberta.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.10/G5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG053555
NIA U19 AG024904
AG024904

Title: Assessing Alzheimer’s disease progression in the medial temporal lobes using diffusion imaging based NODDI metrics

Authors: *D. M. PARKER[1], J. N. ADAMS[3], S. KIM[3], J. JANECEK[3], L. MCMILLAN[3], M. A. YASSA[2];
[2]Univ. of California Irvine, [1]Univ. of California Irvine, Irvine, CA; [3]UC Irvine, Irvine, CA

Abstract: Damage to white matter (WM) within the medial temporal lobe (MTL) may reflect disease progression from healthy aging to Alzheimer’s disease (AD). We measured MTL WM

tract integrity using diffusion tensor imaging and Neurite Orientation Dispersion and Density Imaging (NODDI). We tested if WM integrity was associated with clinical impairment, episodic memory, Alzheimer's pathology (amyloid-β and tau), and hippocampal neurodegeneration.
We analyzed 199 participants from the Alzheimer's Disease Neuroimaging Initiative (ADNI) 3.0 study with multishell diffusion MRI. Participants were divided into cognitively normal and cognitively impaired which included participants diagnosed with mild cognitive impairment and dementia. Standard diffusion metrics of fractional anisotropy (FA) and mean diffusivity (MD) were calculated. NODDI Microstructure Diffusion Toolbox was used to generate the neurite density index (NDI), representing number of voxels with unhindered diffusion, and the orientation dispersion index (ODI), representing the variability of neurite orientation. We calculated these measures for the following MTL tracts from the JHU WM Atlas: cingulum-hippocampus, fornix column/body, and fornix/stria terminalis. A subset of participants received 18F-florbetapir (FBP) to measure Aβ pathology (n = 146), 18F-flortaucipir to measure tau pathology (n=135), neuropsychological testing to measure clinical impairment (Clinical Dementia Rating Sum of Boxes score; CDR-SB) and episodic memory (Rey Auditory Verbal Learning Test; RAVLT) and genetic testing for APOE4 (n=149).
NDI was more strongly related to Aβ status, entorhinal tau pathology, RAVLT score, CDR-SB than ODI and FA ($p<0.01$). NDI associations were strongest with integrity of the hippocampal cingulum, but occurred across all MTL tracts. Random forest analyses were conducted to determine area under the receiver operating characteristics curves (AUROC) for classifying CDR-SB level (CDR>0), RAVLT immediate performance (median split), and Aβ status. These results indicated that a combination of NODDI and traditional tensor metrics are the strongest predictors of these factors.
NODDI measures showed strong associations with MTL WM integrity, suggesting it might have increased sensitivity over traditional tensor metrics to detect WM degeneration in aging and AD. Further, our results suggest that using a combination of NODDI as well as diffusion tensor metrics provides a more robust understanding of how WM degeneration may contribute to clinical outcomes in AD.

Disclosures: **D.M. Parker:** None. **J.N. Adams:** None. **S. Kim:** None. **J. Janecek:** None. **L. McMillan:** None. **M.A. Yassa:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.11/G7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG048076
R01AG074339
K99AG075184
Stanford Center for Precision Health and Integrated Diagnostics (PHIND)
5K23AG059919

Title: Anteroventral and mediodorsal thalamic volume and hippocampal-dependent memory in cognitively unimpaired older adults

Authors: *J. S. LI[1], W. XU[1], A. N. TRELLE[2], E. CHOI[2], Y. ZHAO[1], E. C. MORMINO[2], B. K. RUTT[2], A. D. WAGNER[2], C. FREDERICKS[1];
[1]Yale Sch. of Med., New Haven, CT; [2]Stanford Univ., Stanford, CA

Abstract: The anteroventral (AV) and mediodorsal (MD) thalamus play roles in memory and may be affected in Alzheimer's Disease (AD). While MD is typically spared of AD pathology until late AD, AV accumulates tau early in AD and is anatomically linked to the hippocampus and posterior cingulate. Performance on memory tasks that tax the hippocampus varies as a function of CSF biomarkers of AD in cognitively unimpaired (CU) older adults. We therefore assessed the relationship between AV and MD volume and performance on two hippocampal-dependent memory tasks (mnemonic discrimination; associative memory), standard neuropsychological tests of delayed recall, and CSF AD biomarkers in a cohort of CU individuals (n=143; age 60-88 years) from the Stanford Aging and Memory Study. We used THOMAS, a thalamic segmentation tool, on structural images to extract MD and AV nuclei volumes. CSF biomarkers were measured using the automated Lumipulse G system. The hippocampal-dependent tasks were a paired word-picture cued recall and a mnemonic discrimination task where participants distinguished between studied objects, novel objects, and perceptually similar "lure" objects. Analyses examined cross-sectional associations between memory performance and a) thalamic nuclei volume and b) CSF proteins, controlling for age, sex, education, and intracranial volume (ICV). The 95% confidence intervals (CI) from 1000 bootstraps are reported. We observed a negative association between left AV (CI: -0.352 to -0.012) and lure-new d' in the mnemonic discrimination task, while right MD (CI: 0.003 to 0.403) was positively correlated. Left and right MD significantly interacted with AB42:40 (CI: 0 to 0.364; CI: -0.531 to -0.092 respectively) on lure-new d'. On the associative memory task, only left MD thalamic volume demonstrated a significant positive relationship with associative d' (CI: 0.004 to 0.476). No predictors of interest had a relationship with delayed recall composite from standard neuropsychological testing.Our results suggest a complex relationship between AV and MD volume, AD biomarkers, and hippocampal-dependent memory task performance in this CU cohort. Contrary to our expectations, we found that both AV and MD significantly predicted hippocampal-dependent memory, despite only the AV nucleus being anatomically connected to the hippocampus. These results support the idea that specific thalamic nuclei play a complex role in supporting memory performance in CU older adults and affirm the importance for further analysis into the role of thalamic nuclei in AD.

Disclosures: J.S. Li: None. **W. Xu:** None. **A.N. Trelle:** None. **E. Choi:** None. **Y. Zhao:** None. **E.C. Mormino:** None. **B.K. Rutt:** None. **A.D. Wagner:** None. **C. Fredericks:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.12/G8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant/Other Support: Mt. Jade Young Scholarship Award from Ministry of Education, Taiwan
Grant/Other Support: Ministry of Science and Technology (MOST), Taiwan Grant MOST111-2321-B-A49-011
Grant/Other Support: Ministry of Science and Technology (MOST), Taiwan Grant MOST111-2628-B-A49-012

Title: Unveiling Intrinsic Brain Network Distinctions in Alzheimer's Disease and Mild Cognitive Impairment

Authors: *W.-X. TSAI, A.-C. YANG;
Natl. Yang Ming Chiao Tung Univ., Taipei City, Taiwan

Abstract: Background: As a widespread neurodegenerative disorder, Alzheimer's disease (AD) is a significant public health concern. Early detection of AD and its prodromal stage, mild cognitive impairment (MCI), is vital for timely intervention and effective disease management. A number of previous studies have examined the difference between AD and MCI through the comparison of functional brain networks derived from the BOLD signal of fMRI. Nevertheless, few studies have adequately investigated the network characteristics of AD and MCI based on different time-scale intrinsic components of the BOLD signal. We aimed to determine the difference between AD and MCI based on traditional functional brain networks as well as the networks constructed on the basis of intrinsic BOLD signals. **Methods:** A total of 78 participants (including 26 AD and 52 MCI) from the ADNI database were enrolled in this study. The ensemble empirical mode decomposition (ensemble EMD) was utilized to decompose the BOLD signal into multiple intrinsic mode functions (IMFs). Subsequently, various time-specific functional brain networks were constructed in response to the BOLD signal, the IMF1, IMF2, and IMF3 signals. After that, the difference between the two groups was determined by the estimation of several network features. **Results:** The IMF3 network clearly distinguished AD from MCI in both global and local network features but was rarely observed in traditional BOLD signal networks. Moreover, we pinpointed numerous brain regions that reflect the severity of AD progression. These regions include the left postcentral gyrus in both BOLD signals and the IMF1 networks, along with the bilateral precentral gyri, bilateral Rolandic gyri, the left insula, left parahippocampal gyrus, and numerous brain regions in the IMF3 network. **Conclusions:** These results highlighted the necessity of further investigating the intrinsic BOLD signal functional brain networks. This method may help us better understand the underlying mechanisms and spot the abnormalities in the AD progression earlier.

Disclosures: W. Tsai: None. **A. Yang:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.13/G9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG068563
FRQS Postdoctoral Fellowship

Title: Isodendritic core integrity is associated with global and discrete patterns of white matter microstructure, tauopathy, and APOE status

Authors: *A. WEARN[1], C. L. TARDIF[1], I. R. LEPPERT[1], C. J. GAUTHIER[2], S. TREMBLAY[2], G. BARACCHINI[1], J. TREMBLAY-MERCIER[3], J. POIRIER[3], S. VILLENEUVE[1], T. W. SCHMITZ[4], G. R. TURNER[5], N. SPRENG[1];
[1]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada; [2]Concordia Univ., Montreal, QC, Canada; [3]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [4]Univ. of Western Ontario, London, ON, Canada; [5]York Univ., Toronto, ON, Canada

Abstract: Introduction
Characterization of early brain changes due to Alzheimer's disease (AD) is essential to develop effective therapies. The earliest sites of tauopathy in AD are the diffusely-projecting neuromodulatory nuclei of the isodendritic core (IC): locus coeruleus, dorsal raphe, ventral tegmental area, and the Nucleus basalis of Meynert. We hypothesized that variation in IC microstructural integrity would explain variation in whole-brain white matter (WM) microstructure in a cohort of older adults at increased risk for AD.
Methods
We acquired 3T MRI scans from 133 participants with familial history of AD (from the PResymptomatic EValuation of Experimental or Novel Treatments for AD (PREVENT-AD) cohort (mean age 67.9y, 72% female).
IC integrity was assessed using a multiparametric mapping sequence to quantify R1 and R2* relaxation, magnetization transfer saturation and proton density. WM microstructure was assessed using Neurite Orientation Dispersion and Density Imaging (NODDI), to estimate neurite density (NDI), orientation dispersion (ODI) and free water fraction.
Results
Using partial least squares, we identified two patterns characterizing multivariate associations between IC and WM microstructure (Fig 1):
Pattern 1 (p &lt .0001) represented a pattern of IC microstructural covariance with global NDI.
Pattern 2 (p &lt .002) highlighted associations of IC microstructure with ODI across limbic areas of WM and tracts directly connected to the brainstem. This pattern was associated with p-Tau load in the CSF, indicating specificity for AD.
Both patterns were largely specific to people with at least one APOE4 allele.
Conclusions
Our study highlights patterns of microstructural covariance between neuromodulatory nuclei of the IC and whole-brain WM. IC microstructure covaried with two spatial patterns across WM, which respectively represent 1) general brain health and 2) early AD pathology. With a better understanding of the early neuropathology of these systems we will be better placed to develop disease-modifying therapies targeted to specific early brain changes.

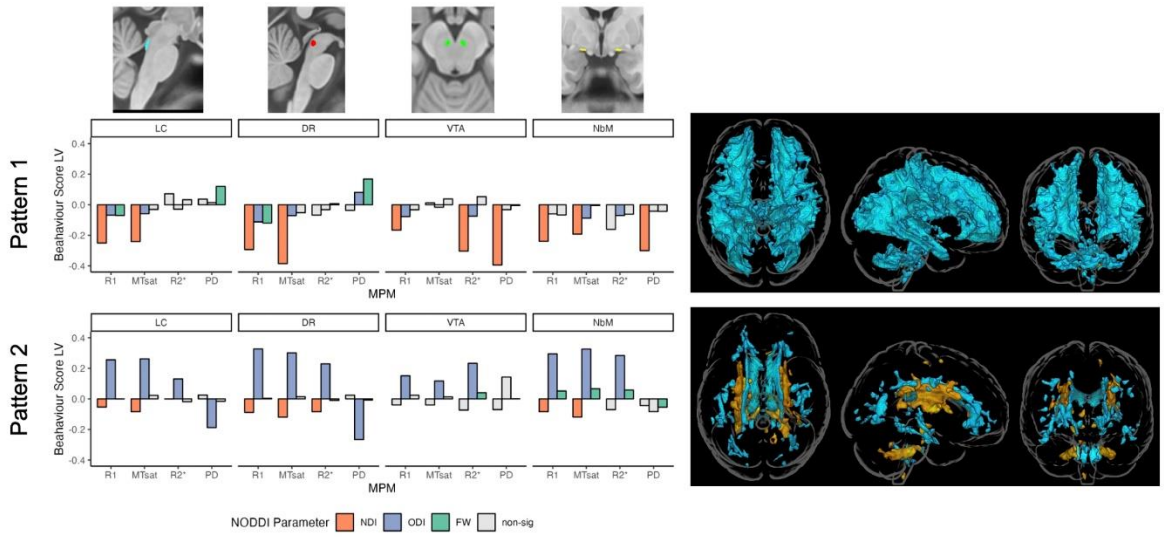


Figure 1 | PLS correlations between isodendritic core MPM parameters and whole-brain white matter NODDI metrics*. The left bar charts show the strength and direction of the relationship that each measure of Isodendritic core microstructure (R1, MTsat, R2*, PD) has with each measure of white matter microstructure (NDI, ODI or FW) in the voxels highlighted in the corresponding brain maps to the right. Grey bars in the left plots represent non-significant correlations. The yellow voxels in the brain maps indicate a positive relationship with the pattern shown in the top panel. The blue voxels indicate a negative relationship. Isodendritic core regions-of-interest are shown at the top above each corresponding panel. PLS = Partial Least Squares, MPM = Multiparametric mapping, NODDI = Neurite Orientation Dispersion and Density Imaging, LC = Locus Coeruleus, DR = Dorsal Raphe, VTA = Ventral Tegmental Area, NbM = Nucleus Basalis of Meynert, ODI = Orientation Dispersion Index, NDI = Neurite Density Index, FW = Free-water fraction, MTsat = Magnetization Transfer Saturation, PD = Proton Density.*

Disclosures: A. Wearn: None. **C.L. Tardif:** None. **I.R. Leppert:** None. **C.J. Gauthier:** None. **S. Tremblay:** None. **G. Baracchini:** None. **J. Tremblay-Mercier:** None. **J. Poirier:** None. **S. Villeneuve:** None. **T.W. Schmitz:** None. **G.R. Turner:** None. **N. Spreng:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.14/G10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG056573

Title: Locus Coeruleus neuroimaging validation voxel to voxel- Postmortem novel MRI sequences to postmortem human tissue reconstructions

Authors: *T. LAMORE[1], S. HUANG[3], Y. CHEN[4], S. LI[5], W. LEE[5], L. CARREIRA[6], H. HEINSEN[7], M. OTADUY[8], Y. SHI[3], L. T. GRINBERG[2];
[2]Neurol., [1]Univ. of California San Francisco, San Francisco, CA; [3]USC, Los Angeles, CA; [5]Neurol., [4]Univ. of California, San Francisco, San Francisco, CA; [6]Radiology, Faculdade de Medicina da Univ. de São Paulo, Sao Paulo, Brazil; [7]Univ. Wuerzburg, Univ. Wuerzburg,

Wuerzburg, Germany; [8]Dept. and Inst. of Radiology, Med. Sch. of the Univ. of Sao Paulo, Brazil, Sao Paulo, Brazil

Abstract: The locus coeruleus(LC) volume remains stable in normal aging but loses about 8% of its volume each stage of AD pathology, starting at the first stage (Braak). Therefore, LC volumetry is an attractive potential biomarker for monitoring progression of AD pathology even at asymptomatic stages. However, it is critical to validate the sensitivity/specificity of LC volume based on MRI to enable its use in the clinical setting. This project aims to investigate the accuracy of MRI sequences developed to segment LC in humans by comparing MRI-based LC volumes to the gold standard ie. histology volumes. As a proof of principle, we analyzed two cases: a 66 -year old, Braak 1, male and a 94-year old, Braak 5, male. Immediately following death, whole brain (0.7mm) 3D-T1 images and 2D-2mm thick NM-sensitive axial gradient recalled echo(GRE) images were obtained (in situ, ex vivo) with a 7T MRI(Siemens Magnetom). The brainstems were extracted and embedded in celloidin, cut at 300 um thickness and 3D reconstructed into a digital volume. We used affine and spline registration and calculated Dice coefficients to measure registration accuracy. From there, we performed an overlap analysis to investigate GRE detection of LC borders compared to histology. Using 3d slicer, we examined the voxel amount in each mask. The number of voxels overlapping in both LC masks were divided by the total number of voxels in the respective LC mask, histology or MRI, to reach an overlap percentage relative to each mask. Results unveiled in the Braak 1 case, the percentage of overlapping voxels relative to the histology mask was 6.26% and 19.57% for the GRE LC mask. For the Braak 5 case, the percentage of overlapping voxels relative to the histology was 23.20% and 39.73% for the GRE. Notably upon dividing the LC into thirds, the Braak 1's most caudal third displayed the lowest degree of overlap (8.34%) followed by rostral (38.89%) and then middle third (52.78%) with the greatest overlap. In the Braak 5 case however, the rostral region had the least amount of overlap (17.24%), followed by caudal (34.48%), and finally middle (48.28%) with the highest overlap percentage. The dispersed and relatively less dense nature of the caudal neurons in the LC, along with degeneration caused by advanced stages of AD pathology in the rostral region, could explain these observations. Based on these initial findings, future investigations into MRI signal specificity and sensitivity should focus on the middle region of the LC. Data on histology to MRI correlations can be used to train algorithms to improve LC recognition in MRIs, leading to more accurate masking of the entire LC and thus supporting in vivo investigation in humans of this important area.

Disclosures: T. LaMore: None. **S. Huang:** None. **Y. Chen:** None. **S. Li:** None. **W. Lee:** None. **L. Carreira:** None. **H. Heinsen:** None. **M. Otaduy:** None. **Y. Shi:** None. **L.T. Grinberg:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.15/H1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5U19AG073585-02

Title: Resilience to pathology in "oldest-old" individuals with varying levels of white matter lesion burden in the Human Connectome Project in Aging/Aging Adult Brain Connectome cohort

Authors: *C. MICHEL[1], M. JUTTUKONDA[1], B. RASHID[1], S. YADAV[1,2], J. JACOBY[1], C. ACCORSI[1], S. BOOKHEIMER[3], M. TERPSTRA[4], K. UGURBIL[4], E. YACOUB[4], X. LI[4], B. ANCES[5], R. BUCKNER[6], R. WOODS[3], D. SALAT[1,7];
[1]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [2]Brigham and Women's Hosp., Boston, MA; [3]UCLA, Los Angeles, CA; [4]Univ. of Minnesota, Minneapolis, MN; [5]Washington Univ. in St Louis, St Louis, MO; [6]Harvard Univ., Cambridge, MA; [7]Neuroimaging for Veterans Center, VA Boston Healthcare Syst., Boston, MA

Abstract: White matter lesions (WMLs) are common in older adults and have been linked to cerebrovascular dysfunction, including hypoperfusion. A growing body of work has indicated a role for WMLs in cognitive impairment and the conversion of mild cognitive impairment to dementia due to Alzheimer's disease. However, the impact of WMLs on cognition is heterogeneous, with some individuals exhibiting substantial lesion load yet minimal impairment. In this exploratory study, "oldest-old" adults with varying WML burden and cognitive ability were examined for differences in brain perfusion as a possible mechanism of cognitive resilience in late life. This study examined typically aging adults aged 80-90+ years from the Lifespan Human Connectome Project in Aging (HCP-A)/Adult Aging Brain Connectome (AABC) cohort (n=102; 54 females, age=85.78 ± 4.53 years). Global cognitive function was assessed using a cognitive composite index (CCI) score derived from summed z-scaled, multi-domain cognitive measures encompassing indices of general cognition, memory, processing speed, and executive functioning from the HCP-A neuropsychological battery. WML load was quantified as log-normalized volume of white matter signal abnormalities generated from T1-weighted images using FreeSurfer after correction for estimated total intracranial volume. Individuals were classified into groups of relatively high or low WML load and relatively high or low cognition based on their residual sign from sex-stratified age regression lines. Resting-state cerebral blood flow (CBF) measurements were derived from pseudo-continuous arterial spin labeling data (resolution = 2.5 mm^3; label duration = 1500 ms; post-labeling delays = 200, 700, 1200, 1700, and 2200 ms). CBF was calculated using a two-compartment model and mapped to the cortical midthickness surface using the ribbon-mapping method. Surface-based general linear models were generated to compare CBF in high (n=18; 8 female) and low (n=27; 14 female) cognitive performers within the oldest-old cohort with high WML load. In this preliminary study, WML load was modestly inversely associated with mean cortical CBF (R^2 = -0.14, p=0.15). Adults with high cognition in late life despite high WML load exhibit higher CBF in regions across cerebral cortex compared to those with high WML damage and low cognitive ability (p = 0.02). These preliminary findings suggest that individuals with elevated cortical CBF may be cognitively resilient to the effects of high subcortical WML burden. These results require confirmation through analyses using alternate WML and cognitive classifications, as well as longitudinal analyses from the HCP-A/AABC cohort.

Disclosures: C. Michel: None. **M. Juttukonda:** None. **B. Rashid:** None. **S. Yadav:** None. **J. Jacoby:** None. **C. Accorsi:** None. **S. Bookheimer:** None. **M. Terpstra:** None. **K. Ugurbil:**

None. **E. Yacoub:** None. **X. Li:** None. **B. Ances:** None. **R. Buckner:** None. **R. Woods:** None. **D. Salat:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.16/H2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC grant MR/X003418/1
Wellcome Trust grant 105586/Z/14/Z
Batelle Foundation PhD studentship
BHF grant PG/20/10010

Title: Neurovascular coupling in multi-morbid mouse models of Alzheimers Disease and Atherosclerosis.

Authors: B. EYRE[1], D. DREW[2], O. SHABIR[2], L. LEE[1], S. FRANCIS[2], C. HOWARTH[1], ***J. BERWICK**[1];
[1]Univ. of Sheffield, Sheffield, United Kingdom; [2]Dept. of Infection, Immunity and cardiovascular disease, Sheffield Univ., Sheffield, United Kingdom

Abstract: Alzheimers disease is an increasing issue within society, with vascular changes occurring early in disease. However, Alzheimers disease does not occur in isolation and there are many comorbidities associated with the disease especially diseases of the vasculature. Atherosclerosis is a known risk factor for the subsequent development of Alzheimers disease; therefore understanding how both diseases interact will provide a greater understanding of co-morbid disease progression and aid the development of potential new treatments. The current study used APPPS1 Alzheimer's mice, atherosclerosis mice (induced using a viral injection of a gain-of-function mutation of PCSK9 and a western diet) and a mixed disease group (APPPS1 plus atherosclerosis). Recognition memory was assessed using the novel object recognition test. Vascular function was assessed in awake mice using 2D-Optical imaging spectroscopy to assess whisker-evoked haemodynamic responses. Immunohistochemistry was used to assess amyloid pathology. We found no effect of disease on non-spatial recognition memory. We found no effect of disease on evoked-haemodynamic responses when locomotion was ignored, or ranked across the whole trial. However, we did find that when locomotion was ranked during the whisker stimulation (5 to10 seconds) during the least locomotion trials, the atherosclerosis group had the most reduced haemodynamic responses compared to wild-type mice. We also observed preserved vascular responses in Alzheimers and mixed disease mice and found no difference in amyloid pathology between Alzheimers and mixed disease mice. These findings suggest that systemic atherosclerosis may impact vascular function. Our results also reveal that there may be potentially different mechanisms that are impaired in atherosclerosis, as locomotion-induced coupling was preserved whereas whisker-evoked coupling was impaired.

Disclosures: **B. Eyre:** None. **D. Drew:** None. **O. Shabir:** None. **L. Lee:** None. **S. Francis:** None. **C. Howarth:** None. **J. Berwick:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.17/H3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RS-2023-00208884

Title: Classification of Alzheimer’s Disease Using Region-specific Anomaly Detection with Generative Adversarial Network

Authors: *H. KIM, J. LEE;
Kumoh Natl. Inst. of Technol., Gumi, Korea, Republic of

Abstract: Alzheimer's disease (AD) is the leading cause of dementia, and effective early diagnosis plays an important role in alleviating the progression of the disease. Among the approaches for the diagnosis, especially the method of diagnosing using MRI data has recently shown breakthrough diagnostic performance by using deep learning techniques. However, deep learning technique for classification has low explanatory power for anatomical characteristics by brain region, and the results are difficult to explain in connection with clinical characteristics. This study aims to perform classification by extracting region-specific information of mild cognitive impairment (MCI) and AD patients through the deep learning-based generative model, not a classification model. In this study, the Alzheimer's Disease Neuroimaging Initiative (ADNI) dataset was used. Cognitive normal (CN) data (809 scans) were used for learning the generative adversarial network (GAN). MCI and AD data (MCI, 1832 scans; AD, 602 scans) were used as data for generating virtual slice images from the generative model that completed the learning. When three consecutive axial slice images are entered into the generative model, the model generates the next virtual slice images. L2 loss was used as an anomaly value between the virtual and real slice images. The anomaly values were constructed into a three-dimensional map, divided by 115 brain regions, and used as features for classification. The support vector machine was used as a classifier, and 20% of MCI and AD data was used as test data for performance measurement. The classification accuracy was 0.808 by using an average value of all voxels in each brain region. The accuracy was additionally measured by using an average value of the top N% voxels with high anomaly values in each region because meaningful anomaly values were partially distributed in each region. N was chosen through k-fold cross-validation using a sub-training dataset, and it showed the best performance when it was 5. The final accuracy was 0.833 by using an average value of the top 5% voxels with high anomaly values in each region. We performed MRI-based AD detection by using anomaly values of brain regions from the generative model. In the future, we will propose a method to extract various MRI-based AD features through research to investigate region characteristics that contribute to

AD diagnosis and research to investigate the relationship between region-specific characteristics and clinical data.

Disclosures: H. Kim: None. **J. Lee:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.18/H4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1 AG071805 (National Institutes of Health)
U01 AG032969 (National Institutes of Health)
R56 AG061869 (National Institutes of Health)
R56 AG072599 (National Institutes of Health)
R01 AG067598 (National Institutes of Health)
R01 AG074004 (National Institutes of Health)
R01 AG067598 (National Institutes of Health)
Coins for Alzheimer's Research
Mr. William H. Goodwin and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research
Experimental Therapeutics Center of the Memorial Sloan Kettering Cancer Center
MSKCC Brain Tumor Center
P30 CA008748 (NCI Core Facility Grant)
U54 OD020355
NIH Small-Animal Imaging Research Program Grant No. R24 CA83084
BrightFocus Foundation (Award ID: A2022020F)

Title: Development of epichaperome imaging probes for precision medicine in Alzheimer's disease

Authors: *S. SHARMA[1], V. JALLINOJA[2], C. S. DIGWAL[1], A. RODINA[1], P. PANCHAL[1], S. BAY[1], T. ROYCHOWDHURY[1], S. D. GINSBERG[4,5], M. ISHII[6,7], N. PILLARSETTY[2,8], G. CHIOSIS[1,3];
[1]Program in Chem. Biol., [2]Dept. of Radiology, [3]Dept. of Med., Mem. Sloan Kettering Cancer Ctr., New York, NY; [4]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; [5]Departments of Psychiatry, Neurosci. & Physiology, and the NYU Neurosci. Inst., New York Univ. Grossman Sch. of Med., New York, NY; [6]Feil Family Brain and Mind Res. Inst., [7]Dept. of Neurol., [8]Dept. of Radiology, Weill Cornell Med., New York, NY

Abstract: Alzheimer's disease (AD) is influenced by genetic, epigenetic, and environmental factors, resulting in individualized brain circuitry changes and cognitive decline. Identifying

therapeutic targets for AD is challenging due to multiple pathological pathways. Our team found that AD-related stressors affect protein-protein interaction (PPI) networks through epichaperomes, pathologic scaffolds composed of chaperones and other factors, providing a unifying AD mechanism. Epichaperomes, found in diseased cells, alter protein interactions, impacting neuronal and brain function. Higher epichaperomes lead to more severe perturbations in affected cells, affecting functions declining in AD. Targeting epichaperomes with small molecules restores protein networks and cognitive function in preclinical studies. Epichaperomes thus represent a promising target for detection and reversal of functional imbalances associated with AD. Developing effective small molecule probes for epichaperomes presents a challenge. These probes should selectively target pathologic chaperone assemblies (epichaperomes) over physiologic chaperones. Considering the abundance and ubiquity of chaperones compared to epichaperomes, as well as their structural similarities, achieving selectivity is not straightforward. In this study, we present our work on the design, synthesis, and characterization of small molecule epichaperome probes. Through various biochemical and functional assays, both in vitro and in vivo, including studies on mice and humans, we demonstrate how small molecule HSP90 binders can kinetically select and distinguish the small fraction of HSP90 in epichaperomes from the abundant HSP90 pools found in the same cell and throughout the body. We provide proof-of-principle evidence from mouse models, showing how an epichaperome imaging probe can reveal the region- and age-dependent formation of epichaperomes in disease-relevant areas. Additionally, we present the results of a pilot feasibility clinical study demonstrating that epichaperomes can be imaged and quantified in human patients using PET scans. In conclusion, epichaperome imaging probes have significant diagnostic applications in AD. When combined with other neuroimaging techniques and plasma biomarkers, they can be used to diagnose and quantify molecular changes underlying functional decline in the AD brain before the onset of tau and amyloid pathology. They can also serve as companion diagnostics in the development of epichaperome drugs, aiding in patient selection, real-time monitoring of target engagement, and quantitative evaluations to optimize dose and treatment schedules.

Disclosures: **S. Sharma:** None. **V. Jallinoja:** None. **C.S. Digwal:** None. **A. Rodina:** None. **P. Panchal:** None. **S. Bay:** None. **T. Roychowdhury:** None. **S.D. Ginsberg:** None. **M. Ishii:** None. **N. Pillarsetty:** None. **G. Chiosis:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.19/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PA Department of Health SAP #4100083102
National Institute on Aging Grant R24AG073190
Intramural Grants from IDOR

Title: Alzheimer's disease affects the heterotopicity of cortical regions controlling linguistic, emotional, and motor processing

Authors: L. LJUNGQVIST BRINSON[1], D. SZCZUPAK[1], F. G. M. FERREIRA[3], F.-C. YEH[2], X. TIAN[1], S.-H. CHOI[1], F. TOVAR-MOLL[3], *A. C. SILVA[1];
[1]Dept. of Neurobio., [2]Dept. of Neurolog. Surgery, Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept. of Neuroimaging, D'Or Inst. for Res. and Educ., Rio de Janeiro, Brazil

Abstract: Early detection of Alzheimer's Disease (AD) allows families and patient care teams to craft plans to manage and prevent disease progression. Previous studies have demonstrated significant structural and functional brain connectivity changes in AD patients. Here, we investigated whether AD explicitly affects interhemispheric callosal connections. We analyzed diffusion weighted imaging (DWI) and resting-state fMRI (rsfMRI) scans collected at 3T from an aged cohort of AD (n = 26) and healthy controls (DWI: n= 51, rsfMRI: n = 45) using DSI Studio and AFNI, respectively. We computed the callosal connections between the two cortical hemispheres and classified them as interhemispheric connections, homotopic (HomC) or heterotopic (HetC). Heterotopicity index (HetI) was quantified as HetC/(HetC + HomC) using MATLAB. We analyzed the whole-brain (WB) Network Based Statistics (NBS) using GRETNA toolbox. Full-Network (FN) connectivity was computed for DWI by tracking and mapping every connection across nodes, and creating a graph network. For rsfMRI the FN was calculated by computing each pair of brain regions' correlation coefficient for the functional connectivity. We computed the Non-heterotopic (NH) network by subtracting the heterotopic connections from the WB connectome FN. Delta NBS values were computed by subtracting the NH values from each subject's FN values, leaving only heterotopic contributions to the NBS. We further used the weighted brain connectome to compare the WB connections. We found that cortical regions controlling linguistic, emotional, and motor processing are affected heterotopically by Alzheimer's Disease structurally and functionally. Additionally, we found that these altered heterotopic connections play a significant role in the network properties of the AD brain, both structurally (Hierarchy, Efficiency, Assortativity) and functionally (Hierarchy, Small Worldliness, Efficiency, Assortativity). Lastly, investigated how these findings correlate to their clinical cognitive assessments and mapped the topography of the heterotopic connections to investigate their trajectory in the corpus callosum. This study shows that AD profoundly affects the structural and functional callosal connectivity; changing the heterotopicity index of brain regions controlling linguistic, emotional, and motor processing. The heterotopic connections altered via AD effect the NBS. These connectivity changes are also evidenced by the change in the functional comparative connectome. Future studies will investigate larger cohort's heterotopicity in AD populations.

Disclosures: L. LjungQvist Brinson: None. **D. Szczupak:** None. **F.G.M. Ferreira:** None. **F. Yeh:** None. **X. Tian:** None. **S. Choi:** None. **F. Tovar-Moll:** None. **A.C. Silva:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.20/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA-K99AG068602 (T.J.Z.)
The Harrison Gardener, Jr. Award (T.J.Z./B.T.H.)

Title: A library-screening approach to optimize antibody labeling for cleared human and mouse tissue

Authors: *J. BAILEY, B. WOOST, Z. HOGLUND, B. HYMAN, T. ZWANG;
Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Over the past few years, significant advancements have been made in the methodologies employed for visualizing the characteristics of brains, organs, and entire organisms within expansive, three-dimensional volumes. One promising method, tissue clearing, involves lipid removal and refractive index matching to significantly decrease light scattering. The inability to achieve consistent and uniform staining poses a significant obstacle that hinders the widespread adoption of tissue clearing techniques for imaging human tissue. We have designed an approach to overcome these difficulties by testing libraries of pre-processed tissue sections and quantitatively comparing staining quality with user-friendly machine learning to offer a simple, accessible, and fast approach for researchers to optimize immunohistochemistry with new antibodies in less than one week. Tissue libraries were prepared from mouse and human tissue slices with a thickness ranging from 0.5mm to 1.0mm. Each section underwent pre-treatment and clearing procedures with nearly identical protocols that only vary by a single condition from at least one other protocol to allow for a complete understanding of how each change influences staining. We tested antibodies that stained for tau (AT8), nuclei (DAPI), neurons (HuD &NeuN), microglia (DAKO), vasculature (Glut1), smooth muscle actin (SMA), and beta-amyloid plaques (Beta-amyloid), among others. Results with this method provide initial evidence that there is not a universal staining protocol ideal for all antibodies but indicate how our methodology could be used to select optimal conditions for multiplexed labeling experiments. Additionally, by comparing human and mouse tissue libraries, we found that optimal staining conditions for an individual antibody in mouse tissue correlated well with the optimal conditions for human tissue. This suggests that antibody optimization can be performed in more plentiful mouse tissues to preserve limited human samples for experimental investigation. Altogether, this work will enable new experiments and insight into the organization of individual cells and pathology in human brain tissue.

Disclosures: J. Bailey: None. **B. Woost:** None. **Z. Hoglund:** None. **B. Hyman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Contracted Research/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., His laboratory is supported by sponsored research agreements with AbbVie and F prime, His laboratory is supported by research grants form the national institutes of health, cure Alzheimers fund, tau consortium, and JPB foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

funds); Ownership interest(Sotck,stock options,royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds), He has a family member who works at novartis and owns stock in Novartis, He serves on the SAB of depoint and owns stock. F. Consulting Fees (e.g., advisory boards); Consulting fees (e.g., advisory boards), He serves on a scientific advisory board or is a consultant for AbbVie, Avrobio, Axon, Biogen, BMS cell Signaling, Genentech, ionis, Novartis, Seer, Takeda, the US dept of justice, vigil, voyager.. **T. Zwang:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.21/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG056014
2020 GRIN-28837

Title: Anatomy and MR imaging of the anterior collateral sulcus variability in relation with Brodmann's areas 35 and 36 (BA35) (BA36).

Authors: *R. INSAUSTI[1], E. ARTACHO-PÉRULA[2], M. MARCOS-RABAL[2], M. MUNOZ[2], C. DE LA ROSA PRIETO[3], A. M. INSAUSTI[4], N. VILLASECA-GONZÁLEZ[2], S. TAPIA-GONZÁLEZ[2], S. CEBADA-SÁNCHEZ[2], J. MONTÓN-ECHEVERRIA[2], J. C. DELGADO-GONZÁLEZ[2], S. RAVIKUMAR[5], N. SADEGHPOUR[5], A. DENNING[5], R. ITTYREAH[5], S. A. LIM[5], L. WISSE[6], P. A. YUSHKEVICH[5], M. ARROYO-JIMENEZ[7];
[1]Human Neuroanatomy Lab., Univ. of Castille-La Mancha, Albacete, Spain; [2]Human Neuroanatomy Lab., Univ. of Castilla-La Mancha, Albacete, Spain; [3]Human Neuroanatomy Lab., Univ. Castilla-La Mancha, Albacete, Spain; [4]Human Neuroanatomy Lab., Publ. Univ. of Navarra, Pamplona, Spain; [5]Penn Computing and Sci. Lab. (PICSL), Univ. of Pennsylvania, Philadelphia, PA; [6]Diagnos. Radiology, Univ. of Lund, Lund, Sweden; [7]Human Neuroanatomy Lab., Univ. of Castilla La Mancha, Albacete, Spain

Abstract: The anterior temporal lobe presents sulci that orient the assignment of cortical areas such as Brodmann’s areas (BA) 20, 21, and 22. However, such landmarks are not so easily distinguishable to identify the anterior most aspect of BAs 35 and 36 of the medial temporal Lobe (MTL) in histological sections or MRI. At more caudal levels in the MTIL, there is good correspondence between different subareas of the hippocampal formation (HF) such as the entorhinal cortex (ERC) and the uncus (uncinate gyrus or intralimbic gyrus). Medial to cs lies the parahippocampal gyrus. The occipitotemporal and the collateral sulci define the lateral and medial occipitotemporal gyri or fusiform gryus. While there is a very good correspondence between different portions of the MTL and different parts of the hippocampal formation, the anterior part of the MTL lack consistent landmarks other than the anterior part of the cs (***Acs)***, and therefore, BA35 and 36 are largely undetermined rostrally. Thereby, the anterior portions of

BA35 and 36 may be misplaced or ignored under other labels. We analyzed the ***Acs*** *i*n a series of more than 140 cases (49 cases from an earlier study, Insausti et al., 1998), histologically annotated for BA35 and 36. In 36 cases of the series 9.4T *ex-vivo* MRI were also studied. Additionally, 41 cases had also *in-vivo* and *ex-vivo* MRI. Four varieties of the ***Acs*** were observed and were classified as types I-IV. **Type I** comprised more than one-half of the cases and showed an ***Acs*** that turned dorsomedially to join the rhinal sulcus (not to be mistaken with the rhinal sulcus used by other authors) that consists of a dorsomedially located small notch at the round border of the MTL, not always conspicuous). The ***Acs*** depth tapers off caudally, and eventually disappears from the surface. BA35 and 36 follow a boundary at the medial bank of the ***Acs,*** according to the depth of the sulcus, previously described (Insausti et al., 1998). **Type II** comprised about one-third of cases. It consisted in a long, straight, little ramified sulcus as far as the beginning of the temporo-occipital transition. In those cases, the ***Acs*** remains independent of the rhinal sulcus, and BA35 and 36 extend into its lateral wall. **Type III** was found in about 10% of cases. The pattern of the ***Acs*** consisted in a series of small sulci and gyri that criss-crossed the surface of the rostral MTL. The extent of BA35 and 36 varied in relation to the ***Acs*** ramifications, although the lateral boundary could be set at the crest of the fusiform gyrus (BA20). Finally, **Type IV** was seldom found (< 1%) and corresponded sulci patterns that could not be included into types I-III. The location of BA35 and 36 was variable.

Disclosures: R. Insausti: None. **E. Artacho-Pérula:** None. **M. Marcos-Rabal:** None. **M. Munoz:** None. **C. De la Rosa Prieto:** None. **A.M. Insausti:** None. **N. Villaseca-González:** None. **S. Tapia-González:** None. **S. Cebada-Sánchez:** None. **J. Montón-Echeverria:** None. **J.C. Delgado-González:** None. **S. Ravikumar:** None. **N. Sadeghpour:** None. **A. Denning:** None. **R. Ittyreah:** None. **S.A. Lim:** None. **L. Wisse:** None. **P.A. Yushkevich:** None. **M. Arroyo-Jimenez:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.22/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1AG057892
R01 AG060610

Title: Alzheimer's Disease effects on white matter tracts mapped using 3D tractometry in an Indian cohort

Authors: Y. FENG[1], B. Q. CHANDIO[3], *J. VILLALON REINA[4], S. THOMOPOULOS[3], H. JOSHI[5], G. VENKATASUBRAMANIAN[6], J. P. JOHN[5], P. M. THOMPSON[2];
[1]Univ. of Southern California (USC), Marina Del Rey, CA; [2]Stevens Inst. for Neuroimaging & Informatics, Univ. of Southern California (USC), Marina del Rey, CA; [4]INI, [3]USC, Los Angeles,

CA; [5]Multimodal Brain Image Analysis Lab., [6]Translational Psychiatry Lab., Natl. Inst. of Mental Hlth. and Neuro Sci., Bengaluru, India

Abstract: Microstructural alterations in the white matter (WM) have been detected using diffusion tensor imaging (DTI) metrics in Alzheimer's disease (AD), but cohorts have primarily included people of European ancestry. Here, we replicate and extend these DTI findings in an independent cohort from India; we use a state-of-the-art tractometry method to localize WM alterations in AD with high spatial precision. We used Bundle Analytics (BUAN) to perform a novel along-tract analysis of WM microstructural metrics derived from diffusion MRI (dMRI). By contrast with standard region-of-interest methods, we extract, map, and visualize DTI abnormalities on 3D models of fiber tracts, yielding fine-scale maps of the effects of AD. We analyzed 3T dMRI data in a pilot sample of 66 participants from the National Institute of Mental Health and Neuro Sciences cohort (mean age: 67.1±7.4 years; 26F/40M). The scan protocol consisted of 64 diffusion-weighted (b=1000 s/mm^2), and 1 b=0 s/mm^2 volume, voxel size=2mm^3. 34 participants were diagnosed with AD and 32 were cognitively normal controls (CN). We generated whole-brain tractograms and applied the BUAN tractometry pipeline to evaluate the effects of AD on 4 DTI metrics along the length of the tracts. We ran Linear Mixed Models with group, age, and sex modeled as fixed effects and subject as a random effect term on 38 WM bundles. The response variable was each DTI metric being analyzed along-tract. We corrected for multiple comparisons using the false discovery rate method. We found significant group differences in 4 association and 1 projection tracts: the Right Cingulum (C_R), Left Fornix (F_L), Left Middle Longitudinal Fasciculus (MdLF_L), Left Uncinate fasciculus (UF_L), Left Medial Longitudinal fasciculus (MLF_L) (Figure 1). We found lower FA in C_R and F_L; higher RD in C_R, F_L, MdLF_L and MLF_L; higher MD in UF_L and MLF_L; and higher AxD in UF_L. These findings are consistent with prior studies on the effect of AD on DTI metrics in the WM and will guide our future work on characterizing microstructure in larger more diverse cohorts.

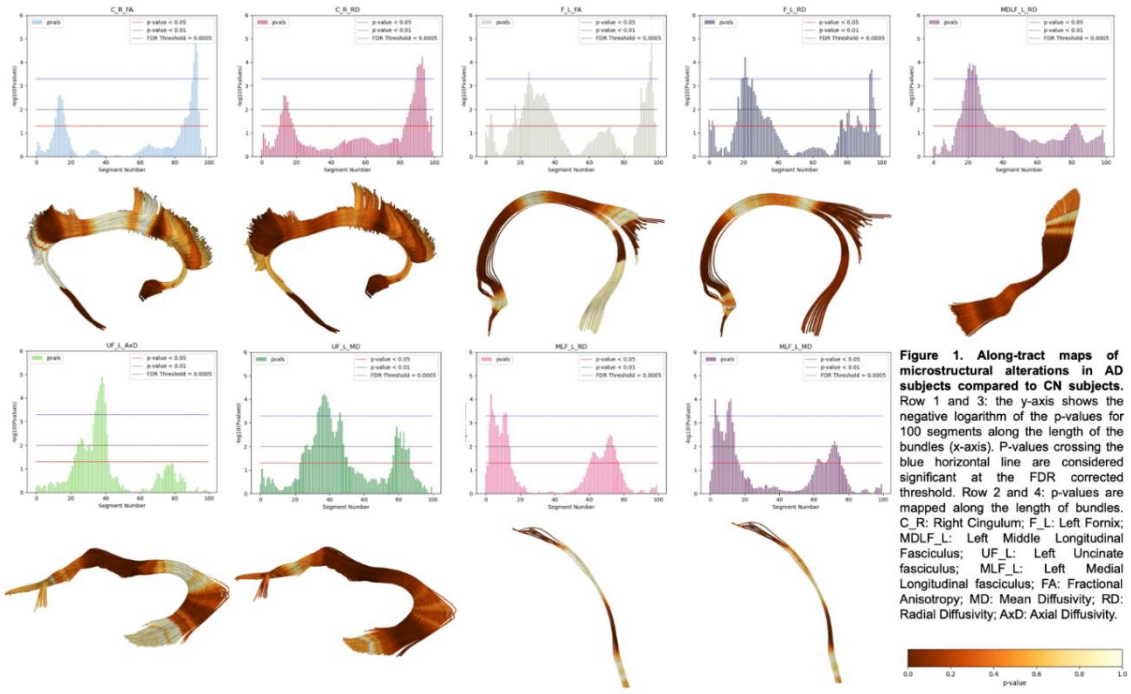


Figure 1. Along-tract maps of microstructural alterations in AD subjects compared to CN subjects. Row 1 and 3: the y-axis shows the negative logarithm of the p-values for 100 segments along the length of the bundles (x-axis). P-values crossing the blue horizontal line are considered significant at the FDR corrected threshold. Row 2 and 4: p-values are mapped along the length of bundles. C_R: Right Cingulum; F_L: Left Fornix; MDLF_L: Left Middle Longitudinal Fasciculus; UF_L: Left Uncinate fasciculus; MLF_L: Left Medial Longitudinal fasciculus; FA: Fractional Anisotropy; MD: Mean Diffusivity; RD: Radial Diffusivity; AxD: Axial Diffusivity.

Disclosures: **Y. Feng:** None. **B.Q. Chandio:** None. **J. Villalon Reina:** None. **S. Thomopoulos:** None. **H. Joshi:** None. **G. Venkatasubramanian:** None. **J.P. John:** None. **P.M. Thompson:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.23/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Developing Micro-CT Imaging for Cerebrospinal Fluid Flow Mapping in Peripheral Nerves

Authors: *A. LIGOCKI, A. VINSON, E. SCOTT;
Mol. Genet. and Microbiology, Univ. of Florida, Gainesville, FL

Abstract: The recent discovery of Cerebrospinal fluid (CSF) flow into and within peripheral nerves affords a potential new tool to assess peripheral nerve health by measuring alterations in CSF transport in peripheral nerves using Computed Tomography (CT) imaging. CSF is an aqueous fluid that bathes the brain and spinal cord, with important roles in waste clearance and nutrient delivery. Until recently, CSF flow was believed to be contained entirely in the central nervous system (CNS), established by vital dye studies, never seen entering into peripheral nerves. Although, the vital dyes were not seen in peripheral nerves, no explanation could be made for the origin of the CSF-like endoneurial fluid found in peripheral nerves. Due to nutrient sized nanotechnology, roughly the size of glucose, we identified the extension of the CSF flow into peripheral nerves, contributing down to the axonal level. The enhanced staining capability of the nanoprobe allows for more sensitive detection compared to previous approaches, as well as having diagnostic imaging capabilities. Disruptions of CSF flow within the CNS are related to neurological disorders such as Alzheimer’s disease (AD), often exhibiting peripheral neuropathies. Thus, our discovery has opened the possibility to study how aberrant CSF flow, such as seen in AD, results in disorders of the peripheral nervous system. To address this hypothesis, a method for live imaging of peripheral CSF flow was developed by leveraging the diagnostic imaging capabilities of the nanoprobe for CT imaging in C57BL/6 mice and mice modeling AD. For this, the nanoprobe was infused into the CSF of adult mice under physiologic pressure, then visualized by CT or micro-CT and corroborated though histology. We identified the ability of the nanoprobes to be visualized by CT within the lateral ventricles of the brain immediately following nanoprobe infusion. However, the small size of murine nervous system and the low resolution afforded to us by the machine required us to utilize micro-CT with it’s enhanced resolution. The high-resolution micro-CT imaging allows detection of CSF/nanoprobe flowing from the CNS into peripheral nerves, producing a method to study the peripheral nervous system and peripheral nerve disorders. Given the resolution afforded by micro-CT, we believe CSF leakage and altered flow patterns in peripheral nerves of mice modeling neurodegenerative disorders will be able to be visualized. Thus, this method for visualizing CSF

flow in peripheral nerves has the potential to be translated into a clinically relevant diagnostic imaging modality for peripheral nerve disorders associated with CNS disorders.

Disclosures: **A. Ligocki:** None. **A. Vinson:** None. **E. Scott:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.24/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2R01 DA029718
RF1DA048808
R01AG064798

Title: Impaired cerebral blood flow and hemodynamic reactivity in transgenic Alzheimer's disease mice: in vivo optical imaging studies

Authors: *H. JEONG, Y. PAN, D. ZHU, C. DU;
Biomed. Engin., Stony Brook Univ., STONY BROOK, NY

Abstract: Marked by accumulation of amyloid plaques and neurofibrillary tangles in the brain, Alzheimer’s disease (AD) presents a clinical threat to aging individuals without a well-defined etiology. Despite a wide range of pathological manifestations, vascular defects such as cerebral hypoperfusion and capillary rarefaction have been reported in epidemiological studies, thereby raising the possibility that neurovascular dysfunction in the brain may be associated with AD development. While *in vitro* and *in vivo* studies demonstrated regional vascular alterations in the brain of AD patients and animals, it has been challenging to access the functional integrity of neurovascular network in a living AD brain with a high spatiotemporal resolution, including detection of individual blood vessels in response to vasoactive stimuli, i.e., cerebrovascular reactivity (CVR), and quantitative imaging of cerebrovascular blood flow velocity (CBFv) with a single vessel resolution in a large field of view of cortex.
To tackle this problem, we applied two cutting-edge *in vivo* imaging technologies, i.e., the Multi-Wavelength Imaging (MWI) and Optical Coherence Tomography (OCT), that enabled us to examine CVR at high spatiotemporal resolution and 3D quantitative CBFv of neurovascular network in living transgenic AD mice compared to those of non-transgenic littermates (WT) at 7-10 months old (n=8/group). During MWI, cocaine (1mg/kg, i.v.) was acutely administered as vasoconstrictive stimulus, and the changes in total hemoglobin (Δ[HbT]) in individual veins, arteries, and tissue were recorded as a function of time. Our results showed that cocaine-induced Δ[HbT] (%) decrease in arteries of AD mice was significantly less (p=0.023) than those of WT mice (i.e., -12.12±1.30% vs -16.37±1.04%, respectively). Furthermore, the recovery time of Δ[HbT] to the baseline was shorter for AD mice than those observed from WT mice. The reduced CVR to cocaine indicated the reduction of vascular flexibility that might be associated

with the vascular stiffness observed in AD patients. In addition, our 3D CBFv images showed that arterial CBFv were significantly decreased ~ 25% ($p<0.001$) in AD mice compared to WT mice, indicating vascular impairment in the resting state as well in AD's brain.
These results demonstrate impairment in basal CBF and CVR in the neurovascular network, especially in arteries at an early stage of AD in the transgenic mice by using optical imaging, thereby adding new evidence of vascular alterations in the AD brain. These novel *in vivo* imaging might provide new insight into the causality between vascular dysfunction and disease development for future early diagnosis of AD disease.

Disclosures: H. Jeong: None. **Y. Pan:** None. **D. Zhu:** None. **C. Du:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.25/I1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 5T32AG071444

Title: Impaired integrity of white matters in an amyloid-B mouse model

Authors: *M. AL AMIN[1,2], B. KIM[1,2], M. D. TATE[2], S. K. JOHN[1,2], N. WANG[2,3], J. KIM[1,2,4]; [1]Med. and Mol. Genet., Indiana Univ., Indianapolis, IN; [2]Stark Neurosci. Res. Inst., Indianapolis, IN; [3]Radiology and Imaging Sci., [4]Med. Neurosci. Grad. Program, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Alzheimer's disease (AD) is one of the leading causes of cognitive decline and dementia. Patients carrying mutations in the amyloid precursor protein, presenilin-1, or presenilin-2 genes will develop familial AD. Previous studies have suggested that the reduction of hippocampal volume and impairment of white matter integrity are early indicators of developing AD. Neuroimaging studies also showed disrupted structural connectivity and reduced network topology in humans. In addition, a reduction in functional connectivity is reported in APP^{NL-G-F} mice. However, our knowledge of integrity of white matter, structural connectivity, and network topology in a mouse model of AD is limited.
To examine the integrity of white matter tracts, we tested $APP^{NL-G-F/NL-G-F}$ knock-in (APP-KI) mice. We assessed spatial memory formation in the C57Bl/6j (n = 16) and APP-KI (n = 18) mice using active place avoidance task at early (3 Months) and late (14 months) age. In addition, we acquired diffusion tensor imaging (DTI) at 14.5 months of age using a 9.4 T Bruker BioSpec MRI scanner. We performed immunohistochemistry to quantify the extent of myelination. Regardless of age, APP-KI mice consistently showed a significant reduction of latency to enter the shock zone while increased entry into the shock zone in the active place avoidance task, suggesting an impaired memory formation. DTI data exhibited a reduction of the fractional anisotropy of white matter tracts including anterior commissure, hippocampal commissure,

corpus callosum, external capsule, fimbria, and fornix in APP-KI mice. There was a significant reduction of axial diffusivity in the anterior commissure while an increase in radial diffusivity in the anterior commissure and fornix in APP-KI mice. APP-KI mice displayed a disrupted structural connectivity centered on a cortical hub region known as the Insular cortex. Moreover, APP-KI mice exhibited a lower network architecture and a reduced network efficiency compared to C57Bl/6j mice. Most importantly, there were significant correlations between the fractional anisotropy of each white matter tract and the latency of the active place avoidance task. Immunostaining with myelin basic protein antibody and staining with luxol fast blue revealed a significant reduction of myelin in the corpus callosum and hippocampus.
Taken together, we demonstrate that deficits in spatial memory start in APP-KI mice in an early stage of life and the reduced integrity of white matter is associated with spatial cognition. Therefore, investigating the white matter tracts of the APP-KI mice model may provide valuable clues to design new therapeutics to stop the progression of AD.

Disclosures: **M. Al amin:** None. **B. Kim:** None. **M.D. Tate:** None. **S.K. John:** None. **N. Wang:** None. **J. Kim:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.26/I2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG065819-01
McKnight Brain Institute Foundation
AMRIS DMR-1644779

Title: Age and sex differences in behavior and functional connectomic measures in the human ApoE4 transgenic mouse model

Authors: *Z. SIMON[1], P. CHAKRABARTY[1], M. FEBO[2];
[1]Neurosci., [2]Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Homozygosity of the Apolipoprotein-ε4 (ApoE4) gene in humans is the strongest genetic risk factor for Alzheimer's Disease (AD). The protein variant encoded by this gene has been linked to aberrant microglial activation, blood-brain barrier breakdown, and decreased protein clearance. These outcomes of the ApoE4 genotype suggest that it may affect functional network activity in brain regions involved in cognitive and affective behaviors. We tested the hypothesis that age alters behavior and functional network topology, particularly markers of network integration, in ApoE4 homozygous mice. Given that the effects of ApoE4 vary with age and there is a strong sex difference in AD risk, we imaged two age groups (1.5-2 mo and 8.5-11 mo) of male and female ApoE4 mice. Mice were scanned on an 11.1 Tesla scanner under combined dexmedetomidine/isoflurane sedation using the parameters: single shot echo planar

images with echo time of 15 ms and repetition time of 1.5 seconds (600 total repetitions; 0.3mm^2 in plane resolution, 0.7 mm slice thickness n 17 slices). Images were processed and analyzed using nodes created via independent component analysis on the common coordinate framework mouse atlas (version 3) and graph theory algorithms in the brain connectivity toolbox. After fMRI, mice underwent contextual fear conditioning (CFC) over three consecutive days. On day 1, mice received CFC training for 17 min over 4 trials in the presence of a foot shock (0.90mA, 1 second) applied at equal intervals. On day 2, mice were placed in the same chamber for the same time without the presence of a foot shock in a 'recall' session. On day 3 the recall session was repeated with visual and tactile contextual cues modified. Compared to young ApoE4 mice, adult ApoE4 mice showed decreased measures of network integration: specifically, assortativity and transitivity/clustering. Additionally, compared to age-matched males, female mice showed trends of increased integration measures. During CFC, all mice developed robust freezing behavior on day 1, with adult ApoE4 mice showing more robust fear conditioning than the young mice . Adult ApoE4 mice also showed greater freezing during both recall sessions and less of a decrease in freezing during modified context recall. Females exhibited a trend toward greater freezing during the same-context recall session. The data suggest that reduced network integration in adult ApoE4 mice is linked to increased fear behaviors and a decreased ability to recognize contextual changes. This suggests a link between network integration and contextual memory stability, Also, male and female mice of both age groups may have differential responses to the ApoE4 genotype.

Disclosures: Z. Simon: None. **P. Chakrabarty:** None. **M. Febo:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.27/I3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Key Research and Development Project of China Grant 2018YFC1315200)
National Science Fund for Distinguished Young Scholars Grant 81625025
Funds for International Cooperation and Exchange of the National Natural Science Foundation of China Grant 81820108034
National Natural Science Foundation of China Grant 31700997

Title: Amyloid spread characterization in the brain of Alzheimer's disease patients with depression

Authors: *M. DANG, D. WANG, S. ZHAO, Y. CHEN, Z. ZHANG;
Beijing Normal Univ., Beijing, China

Abstract: Depressive symptoms are common in patients with Alzheimer's disease (AD) and exacerbate their cognitive and functional impairments. Using stepwise connectivity approach, we aim to clarify the spread characteristics of depression-related amyloid-β (Aβ) in AD patients. Based on [18F]Florbetapir positron emission tomography, the spread characteristics of Aβ in 28 cognitively normal elderly, 38 AD patients without depressive symptoms, and 37 AD patients with depressive symptoms were analyzed. Our results showed that in the AD patients without depressive symptoms, Aβ in the medial temporal lobe followed a distant spread pattern, first directly to the prefrontal-occipital region and then indirectly to a broad neocortical region dominated by the prefrontal cortex; while in AD patients with depressive symptoms, Aβ in the medial temporal lobe follows a spread pattern associated with neuropsychiatric symptoms, spreading directly to the subcortical putamen region, and then indirectly to a broad neocortical region dominated by the frontal and prefrontal lobes. Unlike patients with AD, Aβ in the medial temporal lobe spread primarily locally in the surrounding neighborhood, directly and indirectly to the parietal-occipital lobe region in normal elderly. Our findings suggest that the AD-related neurodegenerative process is associated with a wider spread of Aβ and that the presence of depressive symptoms is associated with the spread of Aβ toward subcortical pathways.

Disclosures: **M. Dang:** None. **D. Wang:** None. **S. Zhao:** None. **Y. Chen:** None. **Z. Zhang:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.28/I4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: State Key Program of National Natural Science of China Grant 82130118
International Cooperation and Exchange of the National Natural Science Foundation of China Grant 81820108034
Natural Science Foundation of China Grant 32171085
Natural Science Foundation of China Grant 31971038

Title: The diagnostic role of episodic memory-related amyloid-β deposition in the Alzheimer's disease continuum

Authors: *D. WANG, M. DANG, S. LONG, X. LI, Z. ZHANG;
State Key Lab. of Cognitive Neurosci. and Learning, Beijing Normal Univ., Beijing, China

Abstract: Exploring the relationship between amyloid-beta (Aβ) deposition and cognition is of great value to understand the pathology of Alzheimer’s disease (AD). The present study would study the role of cognition-related regional Aβ in the AD diagnosis. 135 participants completed florbetapir PET, structural MRI scans, and an extensive cognitive battery covering six domains. Partial correlation was used to examine the relationship between global and regional Aβ

deposition and cognitive functions. Then, a support vector machine (SVM) was applied to determine whether specific cognition-related Aβ deposition regions can adequately distinguish the cognitively normal controls (HC, 76 participants) and mild cognitive impairment (MCI, 30 participants) groups or MCI and AD (29 participants) groups. The result showed that Aβ deposition regions were mainly located in the frontoparietal cortex, calcarine fissure and surrounding cortex and temporal pole regions. Among them, episodic memory-related regions included the frontoparietal cortices, executive function-related regions included the frontoparietal, temporal and occipital cortices, processing speed-related regions included the frontal and occipital cortices. The predictive analysis further showed that only episodic memory-related Aβ deposition regions had better classification performance in the AD continuum than global. Using key cognitive function and focusing on specific regional Aβ deposition is helpful for AD diagnosis.

Disclosures: D. Wang: None. **M. Dang:** None. **S. Long:** None. **X. Li:** None. **Z. Zhang:** None.

Poster

PSTR461. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR461.29/Web Only

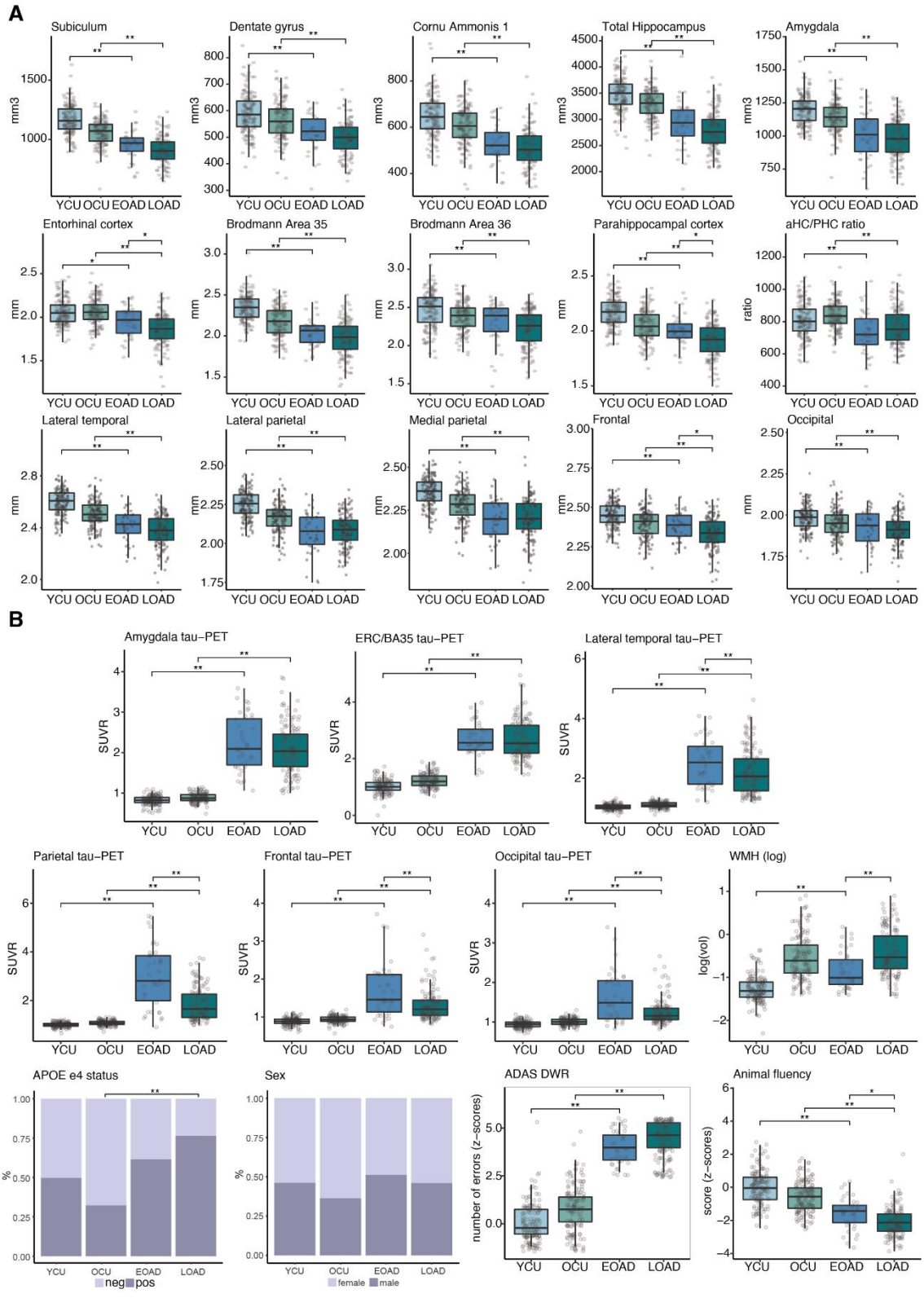
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Medial temporal lobe subregional atrophy patterns in early- and late-onset amnestic Alzheimer's disease

Authors: *A. WUESTEFELD[1], H. WUTT[2], H. BAUMEISTER[3], A. PICHET BINETTE[2], N. SPOTORNO[2], E. STOMRUD[2], N. MATTSSON-CARLGREN[2], O. STRANDBERG[2], R. SMITH[2], S. PLAMQVIST[2], D. VAN WESTEN[2], O. HANSSON[2], L. WISSE[2];
[1]Clin. Memory Res. Unit, [2]Lund Univ., Lund, Sweden; [3]German Ctr. for Neurodegenerative Diseases, Magdeburg, Magdeburg, Germany

Abstract: The medial temporal lobe (MTL) is hypothesized to be relatively spared in early-onset (EO) compared to late-onset (LO) Alzheimer’s disease (AD), while parietal regions are suggested to be more affected. Yet, detailed examination of MTL subfields and neocortical regions comparing biomarker characterized early- and late-onset AD (EOAD *vs.* LOAD) specially in amnestic cases is lacking. We compare these groups on atrophy patterns and brain pathology.
We included individuals with mild cognitive impairment or clinical AD dementia from the BioFINDER-2 study with objective memory impairment, and who exhibited Aβ (abnormal CSF Aβ42/40) and tau (abnormal tau-PET) pathology. EOAD individuals were >65 (n=39), LOAD +70 (n=137) years of age. MTL subfields were measured with Automated Segmentation for Hippocampal Subfields; neocortical composite region thickness and white matter hyperintensities (WMH) measured with FreeSurfer. Two age-matched Aβ negative cognitively unimpaired (CU) groups were included.

EO- and LOAD were similar for sex and *APOE* genotype. LOAD had thinner entorhinal and parahippocampal cortices and frontal regions compared to EOAD (Fig. 1A). LOAD showed lower tau-PET uptake across all neocortical regions (not in MTL) and higher WMH volumes, compared to EOAD (Fig. 1B). Compared to age-matched CU, EO- and LOAD showed atrophy and higher tau-PET uptake across all regions. Focusing on linking regional measures and pathologies, we investigated continuous associations between corresponding regions grouping the CI groups. Higher MTL tau uptake was related to smaller ERC thickness (β=-.23, $p<.05$) with similar β in both AD groups, while higher WMH volumes were associated with lower frontal thickness (β=-.24, $p<.05$) with larger β for EOAD. No other associations were found. In contrast to prior studies, we found overall similar levels of tau pathology and downstream neurodegeneration in EOAD and LOAD in the MTL. However, EOAD had greater tau pathology in the neocortex and EOAD had thinner frontal thickness, where the latter may be associated with WMH and related to their advanced age.



Disclosures: A. Wuestefeld: None. **H. Wutt:** None. **H. Baumeister:** None. **A. Pichet Binette:** None. **N. Spotorno:** None. **E. Stomrud:** None. **N. Mattsson-Carlgren:** None. **O. Strandberg:**

None. **R. Smith:** None. **S. Plamqvist:** None. **D. Van Westen:** None. **O. Hansson:** None. **L. Wisse:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.01/I5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Elucidation of molecular mechanisms of AD-specific vesicular traffic impairment and development of screening methods for multifunctional therapeutics targeting APP-βCTF

Authors: *N. TAKASUGI[1], N. KANESHIRO[2], M. KOMAI[1], A. IKEDA[1];
[1]Medicinal Pharmacology, Grad. Sch. of Medicine, Dent. and Pharmaceut. Sci., Okayama Univ., Okayama-ken, Japan; [2]Medicinal Pharmacology, Grad. Sch. of Medicine, Dent. and Pharmaceut. Sci., Okayama Univ., Okayama-shi, Japan

Abstract: Alzheimer's disease (AD) is characterized by the formation of senile plaques composed mainly of amyloid-β (Aβ) and neurofibrillary tangles composed of highly phosphorylated microtubule-binding protein tau. In particular, senile plaques are observed at an early stage, and the amyloid hypothesis proposes that the increase and aggregation of Aβ is a central molecular mechanism in the pathogenesis of AD. However, much work remains to be done to develop treatments based on the amyloid hypothesis, and there is a need to elucidate the pathophisiological mechanisms that complement the amyloid hypothesis. Recently, it has been suggested that disruption of vesicular trafficking occurs in the early stages of AD before Aβ accumulation, and the traffic jam hypothesis has been proposed as a trigger for the onset of AD. Strong correlations have been reported between the mechanism of Aβ production and the traffic jam hypothesis that underlies the amyloid hypothesis, and this has attracted attention as a promising therapeutic target. In such a research situation, the accumulation of βCTF, a precursor of Aβ, is attracted attentions as a trigger for transport defects. Targeting βCTFs was expected to enable the development of AD-specific drugs with fewer side effects, but on the other hand, the detailed mechanism of their toxicities remained unclear.We identified TMEM30A as a factor that specifically binds to βCTF, and its complex formation with βCTF is involved in the pathogenesis of AD by inducing the enlargement of transport vesicles. TMEM30A is a component of flippase, a membrane phospholipid transporter involved in the regulation of vesicular trafficking.In this study, we focused on the regulatory mechanism by βCTF to clarify the relationship between flippase activity and the pathogenesis of AD, and to develop novel preventive and therapeutic strategies to ameliorate vesicular trafficking defects in AD. We established a system to measure LF activity and vesicular trafficking defects and verified the effects of βCTF accumulation. We also identified peptide, T-RAP, that binds to βCTF and ameliorates AD-specific tranfficking deficiencies. We have also established a screening system for small molecule compounds that bind to βCTF using T-RAP.Drug targeting of βCTF is expected to have multifunctional properties such as inhibitiing Aβ production and aggregation, in

addition to amelioration of trafficking deficiencies, and will help advance the development of basic therapies for AD.

Disclosures: **N. Takasugi:** None. **N. Kaneshiro:** None. **M. Komai:** None. **A. Ikeda:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.02/I6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2022R1A2C209333612).

Title: E3 ligaseregulates ER stress-induced apoptosis by miRNA7/ciRS-7 pair in Brain of Alzheimer’s Disease Mouse

Authors: *S. KIM;
Bundang hospital in seoul national Univ., SEONGNAM CITY, Korea, Republic of

Abstract: Amyloid-beta (Aβ), one of the hallmarks of Alzheimer’ disease (AD), shows an aberrant distribution in AD patients. It is degraded from the C-terminal fragment of the amyloid precursor protein (APP) in neurons by β-secretase and γ-secretase in the cortex and hippocampus. Aβ deposits in AD is generated as a complex process of protein aggregation, involving misfolding of Aβ into soluble and insoluble aggregates. The accumulation of aggregated proteins in the endoplasmic reticulum (ER) of mouse brain occurs ER stress and triggers the UPR (unfolded protein response). Frustration to solve ER stress by oligomeric amyloid beta (oAβ) contributes to apoptosis via a yet unclear mechanism. Here, we confirm that RNF183, a membrane-spanning RING finger protein, places to the ER and possess classic E3 ligase activities by oAβ. Continuous ER stress enhanced by oAβ deposits increases RNF183 expression post-transcriptionally in an IRE1α-dependent manner. Reduced IRE1 increases the level of miRNA-7, which increases the stability of RNF183 transcripts and decreases the level of ciRS-7. In addition, overexpression of RNF183 leads to increased apoptosis and its acceleration aggravates ER stress-induced apoptosis. Furthermore, RNF183 correlates with polyubiquitinates Bcl-xL for degradation, an antiapoptotic member of the Bcl-2 family. Thus, RNF183 plays a pivotal role in enforcing programmed cell death upon prolonged ER stress by oAβ deposits, likely by inducing apoptosis through miRNA-7 and Bcl-xL.

Disclosures: **S. Kim:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.03/I7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS RF1NS116450

Title: Sex differences in brain creatine of Alzheimer's disease mouse model

Authors: *Y. ZHAO[1], T. JIN[2], N. F. FITZ[3], Y. LU[3], R. KOLDAMOVA[3], B. E. IORDANOVA[4];
[2]Radiology, [3]Environmental/Occupational Hlth., [4]Bioengineering, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: Alzheimer's disease (AD) begins with brain energy disruptions driven by age and sex that precede cognitive decline[1]. Previously, we reported sex-specific blood flow[2], vascular reactivity[3], and decoupling of glycolysis[4] in familial AD mouse model. When primary energy systems are disrupted, the brain can use high-energy phosphocreatine for ATP production with byproduct of intracellular acidification. Evidence shows cognition is affected by creatine-phosphocreatine brain energy system, but the mechanism is poorly understood. We quantify brain creatines and amide in AD model across lifespan to understand age and sex effects on the AD bioenergetic disruptions. **Methods:** We used 9.4T MRI of APPswe.PSEN1dE9 (28 females and 22 males, 4-24 months old) and control mice (n=21). We quantified brain metabolites with chemical exchange saturation transfer (CEST) MRI. Z-spectrum was acquired between 3.5-3.7ppm for amide; 2.5-2.7ppm for phosphocreatine and 1.9-2.1ppm for creatine, and the magnetization transfer (MT)-effect was normalized outside ± 6 ppm, and the CEST signals were normalized by T1 relaxation. We used single-cell RNA sequencing (scRNA-seq) in male and female AD mice at the age of 8mo to evaluate the gene expression sex-differences. **Results:** We found significant sex differences in AD mice, that we did not observe in controls. AD males had higher amide ($p=0.0469$) and total creatine (the summation of phosphocreatine and creatine, $p=0.0173$) levels in the cortex compared to AD females. The metabolite trajectories for amide and total creatine across the lifespan for both sexes showed rise in early life followed by drop in late life with males having higher concentrations than females. In astrocytes, AD males had higher expression of genes associated with cholesterol and fatty acids metabolism compared to females. In AD females upregulated were Apoe, Aldoc, Gfap and Clu. **Conclusions:** In the presence of declining blood flow, females use anaerobic glycolysis at the cost of lactic acidosis, while males do not[1,2,4]. The present work shows that males may utilize the brain phosphagen energy system using creatine as an energy source more efficiently than females. **References:** 1.Demetrius at al. (2021 Endoc Metab). 2. Zhao et al. (2021 AAIC). 3. Schweitzer et al. (2022 Brain).4. Zhao et al. (2022 AAIC)

Disclosures: Y. Zhao: None. **T. Jin:** None. **N.F. Fitz:** None. **Y. Lu:** None. **R. Koldamova:** None. **B.E. Iordanova:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.04/I8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R56AG077637

Title: Identification of the Nicastrin subunit of Gamma secretase as a cholesterol binding protein using chemoproteomics

Authors: *S. HOSSEINIBARKOOIE[1], K.-L. HSU[2], H. A. FERRIS[3];
[1]Neurosci. department, [2]Dept. of Chem., [3]Univ. of Virginia, Charlottesville, VA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative condition characterized by memory loss, cognitive decline, and behavioral changes. Research suggests that cholesterol plays a significant role in AD pathophysiology. Cholesterol, as an essential lipid molecule, is involved in various neuronal functions, including myelin production, synapse formation, and neuronal signaling. Amyloid beta (Aβ) plaques, which are formed from the cleavage of amyloid precursor protein (APP) in the amyloidogenic pathway, are considered a hallmark of AD. Cholesterol plays a pivotal role in Aβ peptide production and plaque deposition. Manipulating cholesterol levels in different apoE isoforms or altering cholesterol synthesis pathways in astrocytes can reduce Aβ peptide production and amyloid plaque formation in AD individuals and AD mouse models. However, the precise interaction between cholesterol and AD pathophysiology is still not fully understood. Here, we employed a chemoproteomic approach to understand the cholesterol interactome using a diazirine-based cholesterol molecule. By applying different mass spectrometry approaches, including SILAC, TMT, and Nano-LC MS/MS, we identified 606 proteins that bind to cholesterol in both C6 rat astroglioma cells and mouse-derived primary astrocytes. To gain insights into the functional significance of the cholesterol interactome, a pathway analysis was conducted using Enricher. Nicastrin, a subunit of the gamma-secretase complex, was identified as a cholesterol interactor with a direct connection to AD-related genes. To confirm our mass spectrometry data, we performed independent pull-down assays using mouse and human Nicastrin. We confirmed both proteins interact with cholesterol, and this interaction can take place in vitro using different methods for cholesterol delivery, including methyl-beta-cyclodextrin and ethanol. Furthermore, we demonstrated that the Nicastrin-cholesterol interaction takes place within the gamma-secretase complex using immunoprecipitation and click chemistry. We compared the binding of the cholesterol probe to mouse and human Nicastrin in the presence of excess cholesterol and observed a stronger probe interaction for mouse Nicastrin. To identify the residues responsible for cholesterol binding, we generated several Nicastrin deletion constructs. Our data showed that the removal of the transmembrane domain of human Nicastrin completely abolished cholesterol binding to Nicastrin. Further experiments are needed to elucidate the role of cholesterol and oxysterol interactions with Nicastrin on the function of the gamma-secretase complex and on Aβ production.

Disclosures: S. Hosseinibarkooie: None. **K. Hsu:** None. **H.A. Ferris:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.05/J1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Institute for Veterinary Science, Seoul National University
NRF-RS-2023-00208475
2021R1A5A103315713
BK21 Four Future Program for Creative Veterinary Science Research

Title: Urolithin A inhibits D-galactose-dysregulated amyloid precursor protein processing by blocking AhR-mediated MT1-MMP upregulation

Authors: *J. YOON, C. CHAE, J. CHO, H. HAN;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Aging is the greatest risk factor for Alzheimer's disease (AD), likely caused by defective amyloid precursor protein (APP) trafficking and processing which leads to amyloid β (Aβ) production. Urolithin A, a gut metabolite produced from ellagic acid, has gained attention as a potential candidate with neuroprotective effects in AD. However, the detailed mechanisms by which aging regulates APP trafficking and the protective effect of urolithin A on it remain unclear. Here, we have examined the effects and regulatory mechanism of urolithin A on neuronal amyloidogenesis in D-galactose-induced aging model. We used SH-SY5Ys treated with D-galactose, biotinylation assay and immunocytochemistry to identify the underlying mechanisms. We found that the effects of D-galactose on increased APP processing to early endosomes, followed by Aβ accumulation and cognitive impairment was reversed by urolithin A treatment. Based on these results, we identified the pathogenesis of Aβ production induced by D-galactose and the specific mechanism of urolithin A suppressing it. D-galactose decreased phosphorylation of APP which allowed the binding of APP to adaptin protein 2 (AP2) and promoted APP endocytosis. Furthermore, D-galactose increased the expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) through aryl hydrocarbon receptor (AhR) activation. MT1-MMP upregulation lead to tropomyosin receptor kinase B (TrkB) cleavage, decreasing phosphorylation of APP. Urolithin A suppressed conversion of tryptophan to kynurenine, which is an activator of AhR, to reduce nuclear translocation of AhR and downregulate D-galactose-induced MT1-MMP expression. Moreover, *MT1-MMP* silencing inhibited APP processing into early endosomes, preventing Aβ production. In conclusion, urolithin A has a protective effect against D-galactose-induced amyloidogenesis by downregulating AhR-mediated MT1-MMP which in turn inhibits APP endocytosis.

Disclosures: **J. Yoon:** None. **C. Chae:** None. **J. Cho:** None. **H. Han:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.06/J2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG063407
NIH RF1AG064821
NIH R01AG073182

Title: Impaired Long-Term Potentiation in 7-Month-Old App$^{s/h}$ Rats: Insights into App Function and the Role of JCasp

Authors: *M. YESILTEPE, L. D'ADAMIO;
Pharmacology, Physiol. and Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: The APP gene is linked to AD as a precursor of Aβ peptides, which accumulate in plaques and are widely regarded as the primary cause of AD. Furthermore, APP mutations cause familial AD. We generated three modified versions of the rat App allele to understand how APP contributes to cognitive decline. Firstly, we humanized the Aβ region of the *App* gene (*App*^{h} allele). Second, we generated a null *App* allele (*App*^{k}). Lastly, we created a humanized *App* allele carrying the Swedish mutation (*App*^{s}), which is one of the known pathogenic mutations. The humanization of APP allows us to study potential pathogenic effects of human Aβ. We previously demonstrated that the *App*^{s} mutation affects the function of APP at glutamatergic synaptic vesicles (SV) and leads to an increased release probability of SV (Pr). To gain further insights into the mechanisms underlying memory impairments in AD, we investigated the impact of the *App*^{s} mutation on long-term potentiation (LTP), which is considered a cellular correlate of memory and related to long-term plasticity. We observed a significant impairment in early-LTP (60 min) and late-LTP (120 min) in 7-month-old male and female *App*$^{s/h}$ rats similar to our previous publications (Two-way ANOVA, $p<0.0001$). To explore the relationship between Aβ levels and LTP impairment, we analyzed *App*$^{k/s}$. No significant differences were found between *App*$^{s/h}$ and *App*$^{k/s}$ rats, suggesting that Aβ levels do not explain the observed LTP impairment ($p=0.2786$). Next, we investigated the effects of JCasp, a segment of the cytosolic domain of APP, on LTP impairment in 7-month-old rats. We have previously shown JCasp reduces the Pr of SV by interfering with the APP interactome at pre-synaptic termini. Remarkably, JCasp (500μM) attenuated LTP impairment of *App*$^{s/h}$ rats, indicating that the functional role of APP, rather than human Aβ levels, contributes to the observed synaptic plasticity deficits ($p<0.0001$). Collectively, our findings support the hypothesis that APP function, particularly its impact on glutamatergic SV release, plays a crucial role in LTP impairment seen in *App*^{s} mutation. Furthermore, the partial restoration of LTP impairment by JCasp highlights the potential therapeutic implications of targeting APP function. In conclusion, these results provide further support for the BACE1 on APP-Dependent Glutamate release model (BAD-Glu), which suggests that cleavage of APP by BACE1 within SV promotes glutamate release through the facilitation of βCTF-SV cytosolic interactions. Understanding the intricate mechanisms underlying APP

function and its impact on synaptic plasticity may offer new avenues for therapeutic interventions in neurodegenerative disorders.

Disclosures: M. Yesiltepe: None. **L. D'Adamio:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.07/J3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Margaret "Peg" McLaughlin and Lydia A. Walker Opportunity Fund
NIH Grant P30AG035982
NIH Grant R00AG056600
NIH Grant R01AG078186-01

Title: The functional role of amyloid precursor protein at mitochondria

Authors: *T. STROPE[1], H. WILKINS[2], C. LYSAKER[2];
[1]Biochem. and Mol. Biol., [2]Neurosci., KUMC, Kansas City, KS

Abstract: The Functional Role of Amyloid Precursor Protein at Mitochondria Taylor A. Strope[1], Colton R. Lysaker[2], Heather M. Wilkins[1,2] [1] University of Kansas Medical Center Department of Molecular Biology and Biochemistry[2] University of Kansas Alzheimer's Disease Center and Department of Neurology
Background: Aβ is generated from amyloid precursor protein (APP) via sequential proteolytic processing. While APP processing and its localization are well understood, the functional role of APP is largely unknown. APP is expressed almost ubiquitously but is concentrated in neuronal synapses and localizes to mitochondria. The functional consequence of mitochondrial APP localization is not well understood. We leveraged a mutant APP construct which does not localize to mitochondria to interrogate the functional role of APP at mitochondria.
Methods: We used two mutant forms of APP (3M APP) which harbors mutations at amino acids +41, +44, and +52 (His to Asp), and D23A APP which harbors a single point mutation. SH-SY5Y cells were transfected for 24-48 hours with either WT or 3M APP plasmids. Following transfection, mitochondrial function was analyzed using Seahorse Technology or Vmax spectrophotometric assays of electron transport chain (ETC) function. Mitophagy was examined by qPCR against mitochondrial DNA content. A co-localization assay using an adenovirus expressing EGFP-Cox8 was used as a separate measure of mitophagy. To activate mitophagy, cells were either treated with deferiprone (DFP), starvation (glucose and amino acid deprivation), or PINK1 was overexpression. Mitochondrial biogenesis and turnover were measured using an adenoviral vector (MitoTimer). We further assessed the ability of APP to interact with known mitophagy proteins using immunoprecipitation.
Results: Compared to WT APP, cells expressing 3M APP showed a decrease in mitochondrial

localization and D23A showed an increased in mitochondrial localization, as expected. 3M and D23A APP led to a significant decrease in complexes I, II, and III flux of the ETC. 3M APP showed significantly lower mitochondrial mass and biogenesis. Finally, 3M APP expressing cells appeared to have reduced mitophagy when compared to WT APP. Whereas D23A showed significantly higher mitophagy than 3M and WT. We further found that APP interacts with PINK1 and p62 under conditions of enhanced mitophagy.
Conclusions: APP localization to mitochondria alters ETC function, mitochondrial mass, and mitophagy. Further studies are in progress to elucidate the mechanisms of APP localization at mitochondria, bioenergetic changes, and mitophagy.

Disclosures: T. Strope: None. **H. Wilkins:** None. **C. Lysaker:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.08/J4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P20GM113123
NIH U54GM128729
NIH R01AG048993
NIH RF1AG069378
UND School of Medicine & Health Sciences

Title: Gene expression profiling of changes associated with Alzheimer's Disease-related APP mutations and colitis-associated colon cancer

Authors: *T. TAKAKU, M. SOHRABI, S. CHANDRASEKARAN, C. K. COMBS;
Dept. of Biomed. Sci., Univ. of North Dakota Sch. of Med. and Hlth. Sci., Grand Forks, ND

Abstract: Age-associated diseases such as cancer and Alzheimer's disease (AD) pose significant challenges and demand an urgent need for enhanced treatments and coping strategies. By 2050, these diseases are expected to be the cause of death for approximately one-third of the US population. Epidemiological studies have consistently shown an intriguing inverse relationship between cancer and AD. However, the molecular mechanisms of this negative correlation are largely unknown. Potential causes of disease heterogeneity in both cancer and AD include the influence of sex differences. Notably, women represent two-thirds of AD patients, whereas males face higher incidence and mortality rates of certain cancers, including colorectal cancer.In this study, we attempted to recapitulate the inverse correlation between AD and cancer by employing a mutant APP amyloidosis line combined with an azoxymethane (AOM)/dextran sodium sulfate (DSS) colitis-associated colon cancer model. We conducted a comparative analysis using male and female C57BL/6 wild type and $App^{NL\text{-}G\text{-}F}$ mice with or without an intraperitoneal (IP) injection of AOM and one week of oral DSS treatment, followed by a

recovery period spanning a total of 17 weeks. The tumor numbers and area from male *App*$^{NL-G-F}$ mice were significantly increased in comparison to wild type mice. In contrast, female *App*$^{NL-G-F}$ mice showed a remarkable level of defense against colon cancer, exhibiting significantly reduced tumor number and size when compared to wild type controls. To better understand these findings, RNA-seq was performed using normal and tumor colon tissues from AOM/DSS treated male and female wild type and *App*$^{NL-G-F}$ mice. Differential gene expression analysis revealed striking differences that were specific to mouse genotype or dependent on sex. Pathways involved in steroid hormone formation were up-regulated in tumors from male *App*$^{NL-G-F}$ mice compared to male wild-type tumors. Interestingly, despite the lack of detectable tumor formation, various pathways involved in tumorigenesis were still upregulated in female *App*$^{NL-G-F}$ mice. Steroid hormone-related pathways were down-regulated in female *App*$^{NL-G-F}$ colons compared to male *App*$^{NL-G-F}$ derived tumors. Collectively, these data suggest sex-selective roles for APP and its metabolites in regulating not only AD brain pathology but also tumorigenesis.

Disclosures: **T. Takaku:** None. **M. Sohrabi:** None. **S. Chandrasekaran:** None. **C.K. Combs:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.09/J5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AHA Fellowship PRE34381066
NIH Grant HL66299
NIH Grant HL148069
NIH Grant HL140182
NIH Grant HL160988
OD026560
NIH Grant HL116264
NIH Grant AG058780
W81XWH2110161
W81XWH2110669
OD010944
NIH Grant S10OD025089
NIH Grant HL118334
NIH Grant HL147512
NIH Grant HL007778

Title: Gamma secretase activating protein promotes brain dysfunction after bacterial pneumonia

Authors: M. GWIN[1], M. ALEXEYEV[1], A. GEURTS[2], J. LEE[1], C. ZHOU[1], X.-M. YANG[1], M. COHEN[1], J. DOWNEY[1], R. BARRINGTON[1], D. SPADAFORA[1], J. AUDIA[1], D. FRANK[2], S.

VOTH[3], V. PASTUKH[1], J. BELL[1], L. AYERS[1], D. TAMBE[1], A. NELSON[1], R. BALCZON[1], ***M. LIN**[1], T. STEVENS[1];
[1]Univ. of South Alabama, Mobile, AL; [2]Med. Col. of Wisconsin, Milwaukee, WI; [3]Edward Via Col. of Osteo. Med., Monroe, LA

Abstract: Pneumonia elicits the production of cytotoxic beta-amyloid that contributes to end-organ dysfunction, yet the mechanism(s) linking infection to activation of the amyloidogenic pathway that produces cytotoxic beta-amyloid is unknown. Here, we tested the hypothesis that gamma-secretase activating protein (GSAP), which contributes to the amyloidogenic pathway in the brain, promotes brain dysfunction following bacterial pneumonia. First-in-kind GSAP knockout rats were generated. Wild type and knockout rats possessed similar body weights, organ weights, circulating blood cell counts, arterial blood gases, and cardiac indices at baseline. Intratracheal *Pseudomonas aeruginosa* infection caused acute lung injury. In the hippocampus, GSAP contributed to both pre- and postsynaptic neurotransmission, increasing the presynaptic action potential recruitment, decreasing neurotransmitter release probability, decreasing the postsynaptic response, and preventing postsynaptic hyperexcitability, resulting in greater early long-term potentiation but reduced late long-term potentiation. Infection abolished early and late long-term potentiation in control rats, whereas the late long-term potentiation was partially preserved in GSAP knockout rats. Furthermore, hippocampi from knockout rats, and both the wild type and knockout rats following infection, exhibited a GSAP-dependent increase in neurotransmitter release probability and postsynaptic hyperexcitability. These results elucidate an unappreciated role for GSAP in baseline neurotransmission, excitability, long term potentiation, and brain dysfunction during infection.

Disclosures: M. Gwin: None. **M. Alexeyev:** None. **A. Geurts:** None. **J. Lee:** None. **C. Zhou:** None. **X. Yang:** None. **M. Cohen:** None. **J. Downey:** None. **R. Barrington:** None. **D. Spadafora:** None. **J. Audia:** None. **D. Frank:** None. **S. Voth:** None. **V. Pastukh:** None. **J. Bell:** None. **L. Ayers:** None. **D. Tambe:** None. **A. Nelson:** None. **R. Balczon:** None. **M. Lin:** None. **T. Stevens:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.10/J7

Topic: C.01. Brain Wellness and Aging

Support: NIH 1R01HL137103-01A1
NIH 3R01HL137103-02S1

Title: Evaluating the brain renin-angiotensin system (RAS) and sex differences in pre-clinical models of Alzheimer's Disease

Authors: E. PARONETT[1], H. SMITH[1], L. IYER[1], A. R. DUNN[2], C. C. KACZOROWSKI[2], ***P. J. MARVAR**[1];
[1]George Washington Univ., Washington, DC; [2]The Jackson Lab., Bar Harbor, ME

Abstract: Clinical and pre-clinical evidence supports the involvement of the renin-angiotensin system (RAS), a vital blood pressure regulator, in the pathogenesis of AD and as a potential preventative treatment option. To further evaluate the neurobiological mechanisms of RAS in AD, the current study leveraged two transgenic AD mouse models to compare the behavioral and cognitive deficits and the brain RAS in male and female mice. A triple-transgenic mouse model of AD (3xTg-AD), which exhibits both amyloid beta and tau pathology, was used for in-house assays, while an RNA-sequencing database using a novel mouse model on a genetically diverse background (AD-BXD) (GEO accession number GSE119215) was used to compare expression levels of hippocampal RAS genes at 6 and 14 mos. Morris water maze (MWM) and Y-maze alternation tests were performed at early (3-4 mos) and middle (5-6 mos) time points on 3xTg-AD mice (n=8/sex/age/genotype), followed by collection of plasma and whole brain. Compared to controls, beginning at 3.5 months, 3xTg-AD male and female mice exhibited deficits in learning assessed by MWM escape latency ($p<0.01$). However, beginning at 6 months, only 3xTg-AD females had deficits in memory assessed by Y-maze alternation below 50% (i.e. chance) ($p<0.05$). Sex-dependent deficits are consistent with variability in phenotype beginning at early time points. Next we assessed hippocampal RNA-sequencing data from the AD-BXD database. These data demonstrated a sex-dependent increase in expression of counter-regulatory RAS genes ACE ($p<0.0001$) and ACE2 ($p<0.05$) and decrease in MasR ($p<0.01$) when comparing 6- and 14-month females, as compared to males and wild-type controls. There was no change in classical RAS gene expression, indicating that variation in expression of neuroprotective but not classical RAS genes may be more pronounced in aging female mice. Our results demonstrate sex and stage-specific behavioral deficits in the 3xTg-AD model, which may be linked to changes in hippocampal regulation of the counter-regulatory arm of brain RAS. Additional studies are needed to further evaluate underlying mechanisms for the role of the neuroprotective arm of the brain RAS in AD and its biomarker and therapeutic targeting potential.

Disclosures: E. Paronett: None. **H. Smith:** None. **L. Iyer:** None. **A.R. Dunn:** None. **C.C. Kaczorowski:** None. **P.J. Marvar:** None.

Poster

PSTR462. APP and Metabolites: Function and Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR462.11/J8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Discovery of novel factors on amyloid-beta aggregates in differentiated SH-SY5Y cells using Microscoop optoproteomics

Authors: *C.-C. HUANG, W.-M. CHONG, C.-K. HUANG, Y.-D. CHEN, H.-J. CHANG, J.-C. LIAO;
Syncell, Inc., Taipei, Taiwan

Abstract: Aggregation of amyloid-β peptides (Aβ) observed under a microscope is a hallmark of Alzheimer's disease (AD). Although many proteins are known to be associated with Aβ, it remains unclear whether we have a comprehensive enough proteome of Aβ aggregates to understand the interactions and corresponding signaling pathways. Existing spatial proteomics technologies mostly achieve mapping of known proteins using antibody panels/arrays, lacking the capability of de novo proteomic discovery in high sensitivity and subcellular precision.
Here we used a novel microscopy-based proteomics platform Microscoop to perform ultra-content microscope-guided photo-biotinylation and subsequent subcellular Aβ-associated protein isolation. This platform is effectively a scoopable microscope, isolating spatially-specified proteins from thousands of fields of view automatically so that enough proteins are collected for the following LC-MS/MS-based proteome identification. This unique technology termed optoproteomics allows subcellular proteomic discovery in high specificity, high sensitivity, and high resolution.
In this study, the Aβ1-42 aggregation in human neuroblastoma SH-SY5Y differentiated cells was used as an AD model. Convolutional neural networks (CNNs) based deep learning were implemented to segment specific regions of interest (ROIs) from multiple images of Aβ1-42 aggregates. These Aβ1-42 aggregated regions were illuminated using Microscoop to induce photochemical reactions of proprietary photosensitive probes and trigger spatial covalent labeling of proteins at the Aβ1-42 aggregates. With the complete optoproteomics process, we identified several proteins that have not been reported in existing literature, to the best of our knowledge. At least two of the newly identified proteins were positively validated using antibody staining, confirming that they were indeed colocalized with Aβ1-42. Further studies will be needed to examine the functional relevance of these proteins. Thus, we have discovered novel factors associated with Aβ aggregates in SH-SY5Y differentiated cells using the Microscoop platform-based optoproteomics technology, generating multiple testable hypotheses for Aβ studies and potentially leading to novel biomarker discovery.

Disclosures: C. Huang: None. **W. Chong:** None. **C. Huang:** None. **Y. Chen:** None. **H. Chang:** None. **J. Liao:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.01/J9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA 1R21AG079145-01
Yale Alzheimer's Disease Research Center
American Federation for Aging Research

Title: Nanoscale imaging of pT217-tau in aged rhesus macaque: Trans-synaptic propagation and seeding of tau pathology in entorhinal and dorsolateral prefrontal cortex

Authors: *D. DATTA[1], D. WIJEGUNAWARDANA[4], I. PERONE[2], F. LIANG[5], Y. MOROZOV[6], J. ARELLANO[3], A. DUQUE[6], Z. XIE[8], C. VAN DYCK[6], A. F. ARNSTEN[7]; [1]Psychiatry, [2]Neurosci., [3]Yale Univ., New Haven, CT; [4]Neurosci., Yale Sch. of Med., New Haven, CT; [5]Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA; [7]Sect Neurobiol, [6]Yale Univ. Sch. Med., New Haven, CT; [8]Harvard Univ., Boston, MA

Abstract: Advances in Alzheimer's disease (AD) have revealed a novel fluid biomarker, tau phosphorylated at T217 (pT217-tau), in CSF and plasma, that heralds AD prior to cognitive deficits. Understanding the role of pT217-tau is important in assessing efficacy of novel treatments aimed at early-stage disease. However, it is unknown why pT217-tau is effective in predicting brain pathology, as little is known about early, soluble pT217-tau brain expression. These questions are difficult to address in humans, as soluble p-tau is rapidly dephosphorylated postmortem, and PET scans detect late stage, fibrillated tau. However, the etiology of pT217-tau in aging brains can be probed in rhesus macaques, where perfusion fixation allows capture of phosphorylated proteins in their native state. Aging macaques naturally develop tau pathology with the same qualitative pattern and sequence as humans, including initial cortical pathology in layer II of the entorhinal cortex (ERC) evident early in the aging, and later on in layer III of the dorsolateral prefrontal cortex (dlPFC). We examined the ultrastructural location of pT217-tau in layer II ERC and layer III dlPFC of aged macaques, focusing on potential evidence of propagation between neurons, and exposure to the extracellular space. We used immunohistochemistry with high spatial-resolution immunoelectron microscopy in aged rhesus macaques (18-33y) to localize pT217-tau in ERC layer II and dlPFC layer III. Our results show that pT217-tau labeling is primarily observed in postsynaptic compartments, it accumulated in: 1) dendritic spines on the calcium-storing smooth endoplasmic reticulum spine apparatus near asymmetric glutamatergic-like synapses, and 2) in dendritic shafts, where it aggregated on microtubules, often "trapping" endosomes. The dendrites expressing pT217-tau were associated with autophagic vacuoles and dysmorphic mitochondria, indicative of early neurite degeneration. We observed trans-synaptic pT217-tau trafficking between neurons within omega-shaped bodies and endosomes, specifically near excitatory, but not inhibitory synapses. We also examined pT217-tau in blood plasma in macaques across age-span and observed a statistically significant age-related increase in pT217-tau. These data provide the first evidence of pT217-tau trafficking between neurons to "seed" tau pathology in higher brain circuits, potentially interfacing with the extracellular space to become accessible to CSF and blood as a robust AD biomarker. Illuminating patterns of degeneration with pT217-tau could potentially guide earlier intervention of therapeutics that might mitigate tau hyperphosphorylation in AD.

Disclosures: D. Datta: None. **D. Wijegunawardana:** None. **I. Perone:** None. **F. liang:** None. **Y. Morozov:** None. **J. Arellano:** None. **A. Duque:** None. **Z. Xie:** None. **C. van Dyck:** None. **A.F. Arnsten:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.02/J10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA-K99AG068602 (T.J.Z.)
Harrison Gardner, Jr. Award (T.J.Z./B.T.H.)

Title: Tau accumulates along microvessels in the Alzheimer disease cortex

Authors: *Z. T. HOGLUND[1], T. J. ZWANG[1,2], B. WOOST[1], J. BAILEY[1], B. T. HYMAN, MD,PhD[1,2], R. E. BENNETT[1,2];
[1]Dept. of Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Harvard Med. Sch., Cambridge, MA

Abstract: Alzheimer disease (AD) is a progressive cognitive disorder involving the accumulation of pathological tau protein and its aggregates: neurofibrillary tangles. However, the mechanisms behind pathological tau accumulation and distribution are not well understood. Because vasculature is known to have a role in protein clearance in the brain, we hypothesize that vasculature mediates, in part, tau pathology distribution in AD. To assess the relationship of microvessels with classical Alzheimer pathology, post-mortem human tissue samples (~1 cm^3) from the inferior temporal gyrus of 6 AD and 6 control donors were sliced at thicknesses of 500 μm to 1 mm. Tissue slices were cleared using a modified form of CLARITY, and cleared samples were stained with antibodies for tau (AT8), vasculature (GLUT1), nuclei (DAPI), and neurons (HUD). Images were collected using confocal fluorescence microscopy. A virtual reality tracing method was developed to segment individual blood vessels across large tissue volumes and 17-21 individual vessels were isolated in each sample. We used the Ilastik machine learning object classifier to segment individual tau tangles and neurons. Novel MATLAB scripts calculated the intensity of tau, tau tangle density, and neuron density as a function of distance from the blood vessels. Using this 3D cleared tissue technique in human Alzheimer tissue, we found that, surprisingly, there are thin streaks of tau immunostaining associated with microvessels which would be difficult to discern in a 2D slide. Quantitative analysis of these 3D images revealed vascular tau accumulation correlates with tau intensity in the surrounding tissue and may correlate with neuronal loss. These results suggest that extracellular tau, in a way perhaps analogous to extracellular amyloid, can accumulate along microvessels. In addition, the results suggest an unexpected perivascular clearance mechanism for tau in the Alzheimer brain.

Disclosures: Z.T. Hoglund: None. **T.J. Zwang:** None. **B. Woost:** None. **J. Bailey:** None. **B.T. Hyman:** 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.; His laboratory is supported by sponsored research agreements with AbbVie and F Prime., His laboratory is supported by research grants from the National Institutes of Health, Cure Alzheimer’s Fund, Tau Consortium, and the JPB Foundation.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); He has a family member who works at Novartis and owns stock in Novartis; he serves on the scientific advisory board of Dewpoint and owns stock.. F. Consulting Fees (e.g., advisory boards); He serves on a

scientific advisory board or is a consultant for AbbVie, Avrobio, Axon, Biogen, BMS Cell Signaling, Genentech, Ionis, Novartis, Seer, Takeda, the US Dept of Justice, Vigil, Voyager.. **R.E. Bennett:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.03/K1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K01AG066847
NIH Grant P30-AG066530
Epstein Family Foundation Alzheimer's Research Collaboration Gene Therapy Program

Title: Characterizing Alzheimer's Disease Neurofibrillary Tangles in Subregions of the Human Hippocampus Using Machine Learning

Authors: *B. BRENINGSTALL[1,3], T.-L. STEPHEN[1,3], L. KOROBKOVA[2,3], K. NGUYEN[1,3], M. BIENKOWSKI[1,3,4];
[2]Neurol., [1]USC, Los Angeles, CA; [3]Keck Sch. of Med., Stevens Neuroimaging and Informatics Inst., Los Angeles, CA; [4]Keck Sch. of Med., Zilkha Neurogenetic Inst., Los Angeles, CA

Abstract: Neurofibrillary tangles (NFTs) are one of the neuropathological hallmarks of Alzheimer's Disease (AD), first described by Alois Alzheimer using silver-based histological staining techniques. By the 1980's, the discovery of hyperphosphorylated tau protein as a major constituent protein of NFTs and the development of immunohistochemistry allowed for the visualization of NFTs using antibody staining. Currently, there are many tau antibodies that have been developed which recognize different forms of tau protein expression (eg. AT8) and have been associated with different NFT maturation states (ex. pretangles, mature tangles, ghost tangles). Despite the frequent use of tau markers to stage AD in post-mortem human brain tissues, it is unclear how tau immunohistochemistry compares to silver staining methods and how this could affect the study of the progressive spread of NFT pathology and its relationship to AD. Here, we developed a digital pathology machine learning approach (Stephen et al., 2023) to analyze whole slide images of Gallyas silver stained and AT8 immunostained post-mortem human hippocampus sections from the same 40 USC ADRC patients to compare and assess the detection of NFT distribution within the hippocampus. We found a significant relationship between Gallyas staining and ABC score, indicating that NFTs contributes to overall pathological score. Interestingly, however, there was not a significant relationship between memory score on the Clinical Dementia Rating (CDR) and NFT levels, despite the fact that the memory scores and ABC scores were significantly related. NFT density was highest in the subiculum subregion in patients with intermediate and high ABC scores. In paired comparison between AT8 and Gallyas staining, we found differences across hippocampal subregions, likely

relating to the differences between pretangles and ghost tangles between regions. Overall, these findings contribute to characterization of tau spread across hippocampal subregions in AD. Additionally, we demonstrate that NFT pathology in AD patients are influenced by the type of staining used to characterize this neuropathology. Consideration for staining type is critical to neuropathological assessment and will allow for better characterization of tau spread across stages of AD, memory scores, and risk factors like sex and APOE.

Disclosures: B. Breningstall: None. **T. Stephen:** None. **L. Korobkova:** None. **K. Nguyen:** None. **M. Bienkowski:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.04/K2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: New Jersey Health Foundation Grants (Z.L.M., V.M.)
Connecticut Science Fund Grant (V.M.)

Title: Tunneling nanotube-like structures connecting daughter cells that do not complete cytokinesis may facilitate neuron-to-neuron transfer of pathogenic species in neurodegenerative diseases

Authors: P. VAID, Z. LADESCU MURESAN, *V. MURESAN;
Pharmacology, Physiol. and Neurosci., New Jersey Med. School, Rutgers Univ., Newark, NJ

Abstract: Alzheimer’s Disease (AD), a severe form of dementia that causes brain damage and cognition loss at old age, is characterized by initial accumulation of toxic protein species - amyloid-β peptide (Aβ) and hyperphosphorylated Tau protein (pTau) – in a small population of neurons in the entorhinal cortex and/or the locus coeruleus. As the disease progresses, the toxic protein species gradually spread throughout the brain, likely through a process that involves neuron-to-neuron transmission. It was proposed that the transfer of the toxic species from the neuron where they have been generated to the recipient neuron could occur through thin, channel-like connections, known as tunneling nanotubes (TNTs). TNTs are thought to be generated via two different mechanisms: (1) extension of a filopodium from one cell to another, followed by membrane fusion; and (2) membrane fusion between two cells in close contact, followed by distancing of the connected cells. Using metabolically stressed, locus coeruleus-derived neuronal cells (CAD), we identified a novel mechanism of generation of cell-to-cell connections that structurally resemble TNTs. We found that such connections form between daughter cells that fail to complete cytokinesis after mitosis and remain permanently connected. The TNT-like connections increase in length as the cells migrate in different directions and withstand extreme mechanical stress. We showed that these TNT-like connections contain actin filaments and neurofilaments, and are enriched in acetylated microtubules. They also contain a

large variety of proteins and protein aggregates typically present in AD and other proteinopathies (e.g., phosphorylated Tau, TDP-43, SOD1, FUS, α-synuclein), as well as organelles that participate in the generation of such aggregates (e.g., endocytic/autophagic compartments, elements of the endoplasmic reticulum). These findings are consistent with the possibility that these connections are bona fide, functional TNTs that support neuron-to-neuron propagation of potentially pathogenic species. The CAD cells used in this study reproduce the AD-specific formation of Aβ and pTau aggregates in the absence of disease-causing mutations, and are considered a valid cell culture model for sporadic AD. We propose that the metabolically stressed CAD cells could be used to study the neuron-to-neuron transfer of pathogenic species in AD and other neurodegenerative diseases via these newly identified TNT-like structures, and identify strategies to prevent such transfer.

Disclosures: P. Vaid: None. **Z. Ladescu Muresan:** None. **V. Muresan:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.05/K3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Predicting the evolution of tau distribution and cortical thinning in Alzheimer's: comparing the impact of incorporating anatomical and functional data on models' accuracy with a Bayesian approach

Authors: *J.-P. SOUCY[1], Z. CAI[2], G. Y. BEZGIN[3], J. STEVENSON[2], N. RAHMOUNI[2], F. LUSSIER[2], C. TISSOT[2], P. ROSA-NETO[2], H. RIVAZ[4], C. BELASSO[4], H. BENALI[4]; [1]Montreal Neurolog. Inst., Montreal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada; [3]McConnell Brain Imaging Ctr., McConnell Brain Imaging Ctr., Montreal, QC, Canada; [4]Concordia Univ., Montreal, QC, Canada

Abstract: Accumulation and spread of tau protein aggregates across the brain in Alzheimer's disease (AD) is a proximate driver of synaptic and cellular loss leading to cognitive impairment. Such anomalies can be assessed with tau PET and MRI imaging, but in practice those cannot be repeated at multiple close time points because of cost and radiation protection considerations, reducing their research and clinical usefulness. Even if tau distribution generally progresses over time following the canonical Braak pattern, exceptions are not rare, and modelling progression in groups of patients from initial imaging studies remains difficult. Here, we investigate whether using models that include progressively more informed describers of progression pathways actually improves their accuracy.
We analyzed data from 113 subjects (68 females; 18 AD dementia, 23 MCI and 72 cognitively normal) from the Translational Biomarkers in Aging and Dementia (TRIAD) longitudinal cohort from McGill University who have been tested with tau PET ([^{18}F]MK-6240) and structural MRI twice at a year interval. Four probability models were studied: 1- a basic model, wherein we

assume that all data points stem from a single overarching data distribution; 2- one where subjects' observations are clustered within regions of interest (ROIs). Each ROI's distributional parameters are assumed to come from an overarching probability distribution; 3- one where subjects' observations are clustered within networks. Each network's distributional parameters are assumed to come from an overarching probability distribution; 4- finally, one where subjects' observations are clustered within ROIs that are also clustered within functional networks. We used Bayesian data analysis to compare the predictive accuracy of those models for progression at 1 year from baseline tau PET and MRI data.
Model 4 was the best model for both tau and cortical thickness predictions. We therefore used it to perform posterior predictions across hemispheres, showing that the prediction curves of the left and right hemispheres for the pericalcarine cortex behave differently. We also noticed a decreasing trend in the CN curve for the left hemisphere as the rate of cortical thinning increases. In contrast, there is an increasing trend in the AD curve as the rate of cortical thinning increases.
In conclusion, the model that incorporated both ROI-level and network-level information was the best predictor of progression, and such an approach can reveal underappreciated properties of the disease (laterality and changes across diagnosis over time).

Disclosures: **J. Soucy:** None. **Z. Cai:** None. **G.Y. Bezgin:** None. **J. Stevenson:** None. **N. Rahmouni:** None. **F. Lussier:** None. **C. Tissot:** None. **P. Rosa-Neto:** None. **H. Rivaz:** None. **C. Belasso:** None. **H. Benali:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.06/K4

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

Support: Shanahan Foundation Fellowship to AA
NIA Grant U19AG060909 to SEA-AD consortium

Title: Mapping the progression of Alzheimer's Disease combining detailed neuropathology and statistical models

Authors: *A. AGRAWAL[1,2], V. M. RACHLEFF[1,2], K. J. TRAVAGLINI[1], S. MUKHERJEE[2], P. K. CRANE[2], D. KEENE[2], E. LEIN[1], G. MENA[3], M. GABITTO[1,2];
[1]Allen Inst., Seattle, WA; [2]Univ. of Washington, Seattle, WA; [3]Univ. of Oxford, Oxford, United Kingdom

Abstract: Alzheimer's Disease (AD) is a severe neurodegenerative disease affecting a large population of older adults. Pathological proteins, like amyloid-beta (Aβ) and tau aggregates, are hallmark indicators of disease progression. Current pathological metrics of progression categorization, such as Braak staging [1] and Thal phase [3], focus on a selected few disjoint

features. Neuropathological staging metrics disregard the aggregation dynamics of protein pathologies and create a coarse-grained staging characterization. In this work, we propose a latent hierarchical statistical model to study AD progression that is able to incorporate biophysical dynamics of pathology markers as priors into a joint model, which is capable of predicting a continuously graded time course of pathology progression. We use the extensive quantitative neuropathology dataset [2] from the SEA-AD consortium to align the 84 donors in the cohort along a progression scale, quantifying prediction uncertainty. Our model is able to leverage multiple pathology markers, ranging from layer quantification of tau and Aβ accumulation to layer NeuN+ve quantification in the MTG (middle temporal gyrus) area. We show that this joint model incorporating multiple features and their covariances is robust in predicting the temporal course of disease progression compared to a model which treats pathology features as independent. Importantly, we are able to quantify the correlations between dynamic parameters of progression dynamics across features and use them to interpret the interdependence of pathology markers. We show that for MTG, utilizing the covariances between layers through our joint estimation model provides better estimates of parameters across layers even in the presence of outliers.**References**[1] H. Braak and E. Braak. Neuropathological stageing of alzheimer-related changes. *Acta neuropathologica*, 82(4):239-259, 1991.[2] M. Gabitto, K. Travaglini, J. Ariza, E. Kaplan, B. Long, V. Rachleff, Y. Ding, J. Mahoney, N. Dee, J. Goldy, et al. Integrated multimodal cell atlas of alzheimer's disease. 2023.[3] D. R. Thal, U. Rüb, M. Orantes, and H. Braak. Phases of aβ-deposition in the human brain and its relevance for the development of ad. *Neurology*, 58(12):1791-1800, 2002.

Disclosures: A. Agrawal: None. **V.M. Rachleff:** None. **K.J. Travaglini:** None. **S. Mukherjee:** None. **P.K. Crane:** None. **D. Keene:** None. **E. Lein:** None. **G. Mena:** None. **M. Gabitto:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.07/K5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1 F31 AG074649-01
NIA-RF1

Title: Sleep disruption precedes forebrain synaptic Tau burden and contributes to cognitive decline in a sex-dependent manner in the P301S Tau transgenic mouse model

Authors: *S. MARTIN[1], K. JOYCE[2], K. HARPER[3], V. NIKOLOVA[3], T. COHEN[4], S. MOY[3], G. DIERING[2];
[1]Cell Biol. & Physiol., Univ. of North Carolina Chapel Hill, CHAPEL HILL, NC; [2]Cell Biol. & Physiol., [3]Psychiatry, [4]Neurol., Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Sleep is an essential physiological behavior that supports brain health and cognitive function. Alzheimer's disease (AD) patients experience accelerated sleep loss which correlates with disease onset and progression. Tau is an axonal microtubule stabilizing protein that forms aggregates in AD and contributes to cognitive decline, synapse loss and neuronal death. In AD, tau mislocalization and aggregation at synapses may further impair sleep and restorative sleep-dependent homeostatic plasticity. However, the synaptic biochemical consequences of sleep loss have not been explored in the context of tau pathology. I hypothesize that sleep disruption occurs early in AD progression and subsequently drives disease pathology and cognitive decline. Sleep recordings from transgenic mice expressing human Tau P301S (PS19) shows that deficits in sleep behavior arise as early as 3 months in females and 6 months in males in the form of hyperarousal during the dark phase. Synaptic accumulation of tau in the cortex becomes apparent between 6-9months and is not correlated with sleep disruption. Moreover, experimentally induced acute 4hr sleep deprivation (SD) or 30day chronic sleep disruption (CSD) had no measured effect on synaptic Tau levels, showing that sleep disruption is not a direct driver of synaptic Tau accumulation. In response to CSD treatment, female PS19 mice, and not males, show an increase in expression of synaptic proteins in the hippocampus, suggesting that the sexes may have differential vulnerability to sleep loss. Indeed, we find that CSD treatment is able to accelerate cognitive decline in PS19 males, whereas females and WT littermates were found to be resilient. These results highlight sex differences in the onset of, and sensitivity to sleep disruption and support sleep disruption as an early-stage symptom that accelerates cognitive decline in a sex dependent manner. Endocannabinoids provide an intriguing avenue for therapeutic intervention because of their role in promoting sleep and anti-inflammatory signaling. Preliminary work shows that hyperarousal in PS19 mice can be acutely reversed by increasing the endocannabinoid anandamide. These studies will provide a deeper understanding of the behavioral and molecular changes that occur during abnormal sleep in AD and highlight endocannabinoids as a suitable signaling pathway for enhancing the restorative benefits of sleep.

Disclosures: S. Martin: None. **K. Joyce:** None. **K. Harper:** None. **V. Nikolova:** None. **T. Cohen:** None. **S. Moy:** None. **G. Diering:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.08/K6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA R01AG067762
NIH/NIA R01AG044372
NIH/NIA R01NS082730

Title: Insights into the distribution and potential interaction between tau and Protein Phosphatase 1 in primary hippocampal neurons

Authors: *M. A. PEREZ, N. M. KANAAN;
Translational Neurosci., Michigan State Univ., Grand Rapids, MI

Abstract: Tau is a microtubule-associated protein that has largely been upheld as playing a role in maintaining microtubule stability. However, more recent studies show that tau is involved in regulating microtubule dynamics at the labile domain rather than stabilizing microtubules. Additional studies also support the idea that tau acts as a scaffolding protein thereby regulating various biological pathways under physiological conditions. Traditionally, tau is described as an axon-specific protein, however, there is ample evidence supporting the distribution of tau throughout other neuronal compartments (dendrites, spines, nucleus, soma, axon, and synapses) and in glial cells. Protein phosphatase 1 (PP1) is a member of the serine/threonine phosphatase family with three isoforms expressed in the mammalian brain: PP1α, β, and γ1. Like tau, PP1 is found throughout multiple compartments of neurons where it mediates protein dephosphorylation thereby regulating several biological pathways including protein synthesis, axonal transport, synaptic activity, and nuclear functions. There are over 200 proteins confirmed to be PP1 interacting partners with tau categorized as a PP1 substrate. Interestingly, data suggests that tau can act as a PP1 scaffolding protein that directly affects the function of PP1. Using primary hippocampal neuron cultures from E16 human tau knock-in (hTau-KI) mice, we first sought to establish the distribution of tau and PP1 in this model. Confocal fluorescence microscopy using total tau and PP1 isoform-specific antibodies in (DIV 14) hTau-KI neurons confirms the two are present within many of the same subcellular compartments. Preliminary data obtained using a proximity ligation assay further supports that tau and PP1 interact in neurons, potentially in an isoform-dependent manner. To understand how factors such as neuronal activity may affect the distribution and interaction of tau and PP1, we will utilize hTau-KI cultures that have undergone pharmacological stimulation or inhibition. To determine the motifs responsible for the interaction with and activation of PP1 by tau, we will generate tau deletion and point mutants and employ the use of in-cell interaction assays to measure protein interaction and PP1 activity. Together, these works will provide additional insight as to the complexity of the tau-PP1 interaction and the physiological role of tau in modulating PP1-dependent functions in multiple cellular compartments.

Disclosures: M.A. Perez: None. **N.M. Kanaan:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.09/K7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BrightFocus Foundation PT-AABO0071

Title: Expression of pathological Tau in different compartments of the tripartite synapse results in distinct electrophysiological impairments

Authors: *E. K. ARGYROUSI[1,2], L. KIM[2], R. E. NICHOLLS[3], O. ARANCIO[4];
[1]Columbia Med. Ctr., New York, NY; [2]Taub Inst. for Res. on Alzheimer's Dis. and the Aging Brain, New York, NY; [3]Dept. of Pathology and Cell Biol., [4]Dept of Pathol, Columbia Univ., New York, NY

Abstract: Toxic forms of tau are known to interfere with synaptic function, producing cognitive impairments in Alzheimer's disease (AD). The glutamatergic tripartite synapse consists of a presynaptic terminal, a postsynaptic site, and an astrocyte that interact with one another to regulate synaptic plasticity. Therefore, it is important to understand the pathological actions of toxic forms of tau in a cell-type-specific manner. However, the physiological effects of pathological tau expression in distinct compartments of the tripartite synapse are unknown. In this study, we demonstrate that hyperphosphorylated tau expression in the tripartite synapse results in distinct electrophysiological impairments, in a cell-type-specific manner. To restrict mutant tau expression at specific components of the tripartite synapse, we utilized three transgenic mouse models that express *Cre* recombinase at the presynaptic, postsynaptic, or astrocytic cells of the hippocampus. Adult mice were stereotaxically injected at the hippocampus bilaterally with an adeno-associated virus that expresses the human 4R0N tau isoform with the P301L mutation flanked by loxP sites. Thereafter, the electrophysiological phenotype was assessed in the different mouse models 2 weeks post-injection. Specifically, we examined long-term synaptic plasticity by means of long-term potentiation (LTP) and long-term depression (LTD), as well as short-term synaptic plasticity by evaluating paired-pulse facilitation (PPF), post-tetanic potentiation (PTP) and synaptic fatigue (SF). A differential pattern of defects was observed when P301L tau was expressed at the different cells of the tripartite synapse. Specifically, expression of mutant tau at the presynaptic cells of the hippocampus causes impairments in LTP, LTD, PPF and SF, while expression of mutant tau at the postsynaptic cells induces defects in LTP, PPF and PTP. Finally, astrocytic expression of mutant tau resulted in deficits in all measurements of short-term synaptic plasticity and LTP, but not LTD. Considering that tau could leave the cell that is expressed and be present at the extracellular space, we bath-applied a tau-specific antibody in hippocampal slices prior to LTP recordings, confirming that the observed defects were indeed due to cell-specific expression of mutant tau. Collectively, these results contribute to the existing knowledge regarding the effects of pathological tau in the different cells comprising the tripartite synapse and most importantly, highlight the importance of combinatorial therapeutic approaches for effectively combating the multifaceted impairments of tau in synaptic plasticity.

Disclosures: E.K. Argyrousi: None. **L. Kim:** None. **R.E. Nicholls:** None. **O. Arancio:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.10/K8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R01AG072719-01

Title: Wolframin-1$^+$ pyramidal neurons propagate tau from entorhinal cortex to CA1 neurons resulting in sex dependent cognitive impairments and transcriptomic changes.

Authors: J. DELPECH[1], S. VENKATESAN KALAVAI[3], ***A. RAVULA**[4], C. MADORE[1], E. HAYS[6], A. RAU[2], S. IKEZU[8], M. MEDULLA[6], J. I. LUEBKE[7], T. IKEZU[5];
[1]INRAE, Bordeaux, France; [2]INRAE, Jouy-en-josas, France; [3]New York Univ. Neurosci. & Physiol., New York, NY; [4]Neurosci., [5]Mayo Clin. Florida, Mayo Clin., Jacksonville, FL; [7]Boston Univ., [6]Boston Univ., Boston, MA; [8]Mayo Clin., Dept. of Neuroscience, Mayo Clin. in Florida, Jacksonville, FL

Abstract: Abnormally phosphorylated tau (pTau) plays a key role in Alzheimer's disease (AD) pathology where pTau levels correlates with cognitive impairments. Tau pathology first appears in the transentorhinal cortex and entorhinal cortex layer II (EC II), then spreads to the hippocampal Cornu Ammonis 1 (CA1) region at the prodromal stage of AD (Braak stage I-II). Tau propagation animal models have demonstrated tau transfer from ECII stellate neurons to dentate gyrus, often found in advanced stages of AD. Our recent study showed that Wolfram syndrome-1 (Wfs1) positive cells in EC II project to CA1 via the stratum lacunosum moleculare along the temporammonic pathway and mediate tau propagation to the CA1, mimicking the early stages of tau pathology in AD. This study further investigated sex dependent differences in behavioral, histological, transcriptomic and electrophysiological changes in ECII-CA1 tau mouse model.
Four-month-old Wfs1-Cre mice were injected with Cre-inducible AAV2/6-SYN1-Flex-P301L tau expressing human P301L mutant tau (AAV-tau) or AAV2/6-Flex-TdTomato (AAV-tdTomato) in ECII. One month post injection, behavioral test on memory impairments were assessed by Y maze, novel object recognition (NOR) and trace fear conditioning (TFC) tests. Mice were euthanized for electrophysiology (CA1 pyramidal neuron excitability), bulk-RNAseq, transcriptomics, RNAscope (T cell infiltrations) and immunochemistry of HT7, AT8 and Mac2 were performed to assess tau propagation, pTau, and microglial activation respectively.
AAV-tau injected Wfs1-Cre mice exhibited significant sex differences. Y maze, NOR and TFC tests revealed deficits in hippocampal and amygdala memory acquisition and retention in the AAV-tau injected female group compared AAV-tdTomato female group. Male mice in AAV-tau group showed behavioral deficits only in FTC. AAV-tau injected Wfs1-Cre mice exhibited numerous human tau$^+$ CA1 pyramidal neurons, which had altered excitatory and inhibitory post synaptic currents, action potential amplitudes and durations. Bulk RNAseq of the hippocampal brain region revealed total of 411 significantly differentially expressed genes (DEG) while the females showed fewer DEGs with 129 in AAV-tau group compared to the AAV-tdTomato group. RNAscope and IHC analysis revealed significant CD8$^+$ T cell infiltration and microglial activation in the CA1 region with the AAV-tau compared to tdTomato group.
These data indicate that there are sex-dependent cognitive impairments and hippocampal immune-related gene expression changes in the ECII-CA1 tau mouse model, suggesting the sex-dependent immune-related amelioration of cognitive function in tauopathy.

Disclosures: J. Delpech: None. **S. Venkatesan Kalavai:** None. **A. Ravula:** None. **C. Madore:** None. **E. Hays:** None. **A. Rau:** None. **S. Ikezu:** None. **M. Medulla:** None. **J.I. Luebke:** None. **T. Ikezu:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.11/K9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P01 AG014449

Title: Early pre-tangle tau pathology in postmortem tissue from default mode network hubs

Authors: *B. KARA[1], T. GRABINSKI[1], N. M. KANAAN[2], S. COUNTS[1];
[2]Translational Sci. & Mol. Med., [1]Michigan State Univ., Grand Rapids, MI

Abstract: There is an urgent need for disease-modifying treatments for Alzheimer's disease (AD), especially since AD can remain silent for decades while soluble aggregates of a-beta and tau fibrillize into plaques and tangles, respectively, which are ultimately detectable by PET tracers. Previous studies show that early, soluble forms of pathological tau are more toxic than later-stage tangles. To begin understanding the extent to which these prefibrillar tau moieties accrue during the incipient stages of AD, we used custom ELISA assays to quantify pathological tau S422 phosphorylation, tau oligomers, N-terminal tau misfolding, or C-terminal tau truncation in the posterior cingulate cortex (PCC) and precuneus (PreC) obtained postmortem from cognitively intact control, early-stage AD, or moderate AD subjects (n = 12/group) who displayed a range of Braak stages 0/I to V/VI. Notably, PCC and PreC comprise the posterior hubs of a large-scale brain network called the default mode network (DMN), and communication among these DMN hubs falters very early in AD patients. Hence, our study also sought to help understand the relationship between the early pathological tau in the DMN and changes in cognitive function. Our initial findings indicated that early pathological tau starts to accumulate in PCC and PreC as early as Braak stage III/IV ($p=0.002$), which is significantly earlier than predicted by NFT staging. Moreover, tau pathology load inversely correlated with antemortem Mini-Mental State Exam (MMSE) global cognitive scores ($p=0.004$). We also detected trends for earlier tau pathology accrual in PreC compared to PCC and for females bearing more pathological tau than males in these regions across Braak stages. While validating these findings in a larger cohort, we are also investigating the proteomic profiles of these two regions. Taken together, these results may help shed light on the timeline of early pathological tau accumulation within the DMN, mechanisms of early tau pathology accrual, and how these outcomes might impact cognitive decline in AD and inform novel therapeutics.

Disclosures: B. Kara: None. **T. Grabinski:** None. **N.M. Kanaan:** None. **S. Counts:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.12/K10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant R01AG054073
NIA Grant R01AG058533
NIA Grant U19AG078109

Title: Spatial patterns of cortical tau pathology differ by age, sex, and type II diabetes status in a multiethnic study of cognitively impaired older adults

Authors: *B. HALL[1], M. TUBI[1], N. LEE[1], V. TENNANT[1], A. MAHAJAN[1], M. BRASKIE[1], A. W. TOGA[2], L. JOHNSON[3], R. NANDY[3], R. VINTIMILLA[3], R. VIG[4], K. YAFFE[5], S. O'BRYANT[3];
[2]USC Stevens Neuroimaging and Informatics Inst., [1]USC, Los Angeles, CA; [3]Univ. of Northern Texas, Fort Worth, TX; [4]Imaging Midtown Med. Imaging, Fort Worth, TX; [5]Univ. of California San Francisco, San Francisco, CA

Abstract: Neurofibrillary tau accumulation is a hallmark of Alzheimer's disease (AD). The spatial pattern of tau varies and has been categorized into 4 patterns: typical, limbic predominant (LP), hippocampal sparing (HS), and sub-median tau (SMT). These patterns differ by mean age, disease duration, and symptom profiles. Some metabolic risk factors for AD, such as type 2 diabetes (T2DM), also correlated with increased tau neuropathology. To study how these factors may relate to tau patterns in AD, we created classification models to identify differences in BMI, hypertension, and T2DM between tau patterns in older adults with MCI and AD.We used PI2620 tau PET images for 222 participants with MCI or AD from the Health and Aging Brain - Health Disparities cohort (**Table 1**). We used Freesurfer v5.3 to segment T1 MRI and calculated PET regional standardized uptake value ratios with the inferior cerebellar gray matter as a reference region. We algorithmically categorized participants' tau pathology into patterns. In separate multinomial logistic regression models adjusting for age and sex (R v4.2), we measured whether hypertension, T2DM, and BMI differentiated the participants' tau patterns.See **Table 1** for the distribution of tau patterns. HS participants were more likely to have T2DM ($z = 1.9$, $p = 0.06$) and had higher BMI ($z = 1.8$, $p = 0.07$) compared to typical participants (**Table 1**). LP and HS patterns differed in age ($z = -3.3$, $p = 0.004$) and sex ($z = -3.0$, $p = 0.003$).This study suggests that in an ethnically diverse cohort of older adults with MCI or AD, cortical tau deposition patterns may be differentiated by sex, age, BMI, and T2DM status. The HS pattern is of particular interest, with the highest proportion of females, the lowest mean age, the highest mean BMI, and the highest proportion of T2DM participants. To better understand why female sex, T2DM diagnosis, and BMI are risk factors for AD, it may be beneficial to focus on this pattern of tau pathology.

Table 1

Category	Total	Typical	Limbic Predominant	Hippocampal Sparing	Sub-median Tau
Demographics					
N	221 (100%)	95 (43%)	30 (13%)	26 (12%)	70 (32%)
Age	64.8 (±8.6)	65.9 (±8.9)	67.7 (±9.4)	62.6 (±9.1)	62.9 (±6.5)
Sex (Female)	116 (52%)	59 (62%)	10 (33%)	17 (65%)	30 (43%)
Race/Ethnicity					
Hispanic	62 (28%)	34 (36%)	10 (33%)	3 (12%)	15 (22%)
Non-Hispanic White	61 (27%)	25 (26%)	6 (20%)	13 (50%)	17 (24%)
Hispanic Black	3 (1%)	2 (2%)	0 (0)%	0 (0%)	1 (1%)
Non-Hispanic Black	95 (43%)	34 (36%)	14 (47%)	10 (38%)	37 (53%)
Cognitive diagnoses					
MCI	169 (76%)	73 (77%)	23 (77%)	17 (65%)	56 (80%)
AD	52 (24%)	22 (23%)	7 (23%)	9 (35%)	14 (20%)
Metabolic factors					
BMI	31.6 (±8.0)	31.3 (±8.4)	31.5 (±6.0)	36.1 (±11.1)	30.2 (±6.2)
Hypertension +	161 (73%)	65 (68%)	25 (83%)	22 (85%)	49 (70%)
Diabetes +	66 (30%)	24 (25%)	11 (37%)	11 (42%)	20 (29%)

Table 1: a demographics table that summarizes the characteristics of the sample from the HABS-HD cohort. Acronyms: MCI -- Mild Cognitive Impairment, AD -- Alzheimer's Disease, Typical -- the classic progression of AD pathology from medial temporal to cortical regions, Sub-median tau -- where tau accumulation is below the median of a larger HABS cohort sample in both the hippocampal and neocortical regions (see Figure 1).

Disclosures: B. Hall: None. **M. Tubi:** None. **N. Lee:** None. **V. Tennant:** None. **A. Mahajan:** None. **M. Braskie:** None. **A.W. Toga:** None. **L. Johnson:** None. **R. Nandy:** None. **R. Vintimilla:** None. **R. Vig:** None. **K. Yaffe:** None. **S. O'Bryant:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.13/L1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG079141
AG077610
AARF-61441339

Title: Pathological Tau propagation regulated by excitatory neuronal BIN1 expression

Authors: *M. PONNUSAMY[1,2], M. DELOZIER[2,1], O. PATEL[2,1], V. SKOROBOVENKO[2,1], S. SMIRNOU[2,1], L. COLLIER[2,1], G. THINAKARAN[2,1];
[1]Dept. of Mol. Med., Univ. of South Florida, Tampa, FL; [2]Byrd Alzheimer's Ctr. and Res. Inst., Dept. of Mol. Medicine, USF Morsani Col. of Med., Univ. of South Florida, FL

Abstract: Pathological Tau propagation regulated by excitatory neuronal BIN1 expression
Abstract Aggregation of intracellular hyperphosphorylated tau is a hallmark pathogenic feature of Alzheimer's disease (AD). The Bridging Integrator-1 *(BIN1)* gene is the second strongest genetic risk factor for Late-Onset AD (LOAD, after *APOE*). Recently we reported that microglial BIN1 regulates proinflammatory activation, whereas neuronal BIN1 positively promotes hippocampal tau pathology. BIN1 was also found to limit trans-cellular tau propagation in cultured neurons. However, the molecular events underpinning neuronal BIN1 function in disease progression i*n vivo* are yet to be defined. Based on our lab's previous observation that BIN1 localizes to presynaptic terminals and modulates excitatory synaptic transmission, we hypothesized that neuronal BIN1 regulates neuron-to-neuron propagation of tau seeds. In order to establish a direct connection between neuronal BIN1 and the degree of neurofibrillary tangle (NFT) pathology, we injected tau seeds into the brains of Tau P301S mice lacking BIN1 expression in the forebrain excitatory neurons (PS19:*Bin1*-cKO). Brain extracts containing tau aggregates (from pooled PS19 mice) were characterized using immunofluorescence and immunoblotting analysis, and the presence of tau seeds was verified using an *in vitro* FRET assay for tau seeding. A stage before overt tau pathology develops in the PS19 model, tau seeds were unilaterally injected into the hippocampus of 2-month-old male PS19:Emx-Cre (control) and PS19:*Bin1*-cKO mice, and the spread of tau pathology was examined 60 days post-injection. Quantitative analysis revealed in PS19:Emx-Cre mice injected with brain-derived tau seeds had intense Ser202/Thr205 (p-tau) immunoreactivity in CA1 pyramidal neurons in the ipsilateral hippocampus, which readily spread to connected brain regions, including the contralateral hippocampus. However, PS19:*Bin1*-cKO mice showed the induction of tau templating in the ipsilateral hippocampus but diminished tau pathology in the contralateral side and limited tau spreading in neuroanatomically connected areas. Our observation *in vivo* that loss of excitatory neuronal BIN1 limits tau release and spread from the site of injection in the hippocampus contrasts with the data from cultured neurons. Our study demonstrating neuronal BIN1's role in promoting tau pathogenesis *in vivo* by spreading pathogenic tau through neuroanatomically connected brain areas has important therapeutic implications for AD and other tauopathies.
Key words: Alzheimer's disease; tau pathology; BIN1; GWAS risk factor; Tau spreading.

Disclosures: M. Ponnusamy: None. **M. Delozier:** None. **O. Patel:** None. **V. Skorobovenko:** None. **S. Smirnou:** None. **L. Collier:** None. **G. Thinakaran:** None.

Poster

PSTR463. Tau: Distribution

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR463.14/L2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Context-specific differences in cellular internalization and responses to tau in iPSC-derived neurons and glia

Authors: *J. MCINNIS[1], J. M. BONNER[1], A. LOTUN[1], Y. LI[1], R. KRISHNAN[2], S. DUJARDIN[3];
[2]Sanofi-Aventis, [3]Neurol., [1]Sanofi, Cambridge, MA

Abstract: Context-specific differences in cellular internalization and responses to tau in iPSC-derived neurons and glia John McInnis, Julia Maeve Bonner, Anoushka Lotun, Yihang Li, Rajaraman Krishnan, Simon Dujardin Tauopathies are neurodegenerative disorders where tau is hyperphosphorylated, takes on pathological conformations, and aggregates. In these tauopathies, pathological tau propagates in a prion-like fashion between neurons and the progression of symptoms correlates strongly with tau pathology. Though tau is neuronally expressed, and the *in vivo* spread of phosphorylated/aggregated tau is seen primarily in neurons, recent studies implicate both astrocytes and microglia in tau-mediated dysfunction. Despite these findings, the interplay between these different cell types and their relative contribution to pathology has not been fully characterized. Using tau labelling, we showed differential kinetics of tau uptake and corresponding cell-signaling responses across different human iPSC-derived cells, both neuronal and glial. Tau uptake was also altered in cells edited to contain MAPT mutations. Interestingly, the addition of anti-tau antibodies had differential effects on tau fibrils uptake depending on cell type. To better elucidate tau trafficking and corresponding inflammatory responses in a complex system, we then extended our characterization to co- and tri-culture systems. We show phosphorylated tau induction and corresponding inflammatory profiles in response to treatment with tau fibrils. Neuronal cell-type and genotype altered the response to tau fibrils in tri-cultures in the presence of the same astrocytes and microglia. This has important implications for how antibody treatments could alter tau dynamics and the role that different cell-types in the brain could play in the progression of tauopathies.

Disclosures: **J. McInnis:** A. Employment/Salary (full or part-time):; Sanofi. **J.M. Bonner:** A. Employment/Salary (full or part-time):; Sanofi. **A. Lotun:** A. Employment/Salary (full or part-time):; Sanofi. **Y. Li:** A. Employment/Salary (full or part-time):; Sanofi. **R. Krishnan:** A. Employment/Salary (full or part-time):; Sanofi. **S. Dujardin:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.01/L3

Topic:

Support: NIH Grant NS117469

Title: Resveratrol and celastrol modify synaptic and inflammatory genetic expression in a model of Parkinsonian rat midbrain primary neurons

Authors: *D. G. S. BARNETT, S. A. LECHNER, C. KELM-NELSON;
Dept. of Surgery | Div. of Otolaryngology, Univ. of Wisconsin - Madison, Madison, WI

Abstract: Parkinson disease (PD) is highly prevalent and lacks disease-modifying therapies. Prompt treatment of the earliest manifestations of brainstem pathology are ideal to halt progression of disease. We have previously identified early-stage altered gene expression in caudal CNS nuclei, independent of nigrostriatal pathology, in the *Pink1-/-* rat model of prodromal PD. We used these transcriptomic data to power a drug repurposing technique designed to identify therapeutic candidates capable of reversing altered genetic expression due to loss of *Pink1*. The top results included naturally occurring resveratrol and celastrol. To screen the efficacy of these drugs in primary midbrain neurons germane to our previous work, we developed a relatively brief and inexpensive in vitro siRNA knockdown model. After validation, we tested the hypotheses that resveratrol and celastrol will reverse gene expression changes in midbrain neurons due to reductions of *Pink1* in vitro. Midbrains were dissected from wild-type E18 Long-Evans rats. Each midbrain yielded approximately $0.5 – 1 \times 10^6$ cells with > 90% viability and cells were plated on either poly-D-lysine coated culture plates for qPCR, or chamber slides for immunocytochemistry (ICC). After treatment with siRNA directed against *Pink1* or control sequences, cells were treated with either resveratrol (10 µM in EtOH), celastrol (0.5 µM in EtOH), or vehicle followed by RNA harvest or ICC. We achieved significant neuronal predominance (microtubule associated protein [Map] expression, beta-III tubulin ICC) with few astrocytes (aldehyde dehydrogenase 1 member L1 [Aldh1l1] expression, glial fibrillary acidic protein [Gfap] ICC). Levels of *Pink1* siRNA knockdown were significant compared to control. Resveratrol treatment led to significant increases in genes necessary for normal synaptic function (*Snap25, S100b*) and activation of the transcription factor *Sirt1*, all decreased in the *Pink1-/-* rat. Celastrol similarly increased *S100b* and *Sirt1*, although not significantly. This study remains underway with addition of E18 *Pink1-/-* rat midbrain cultures for further validation of the in vitro model in a complete knockout. Here, we highlight the utility of validating drug candidates prior to relatively lengthy and costly in vivo testing. These data support investigation of in vivo resveratrol and celastrol in the *Pink1-/-* rat and corroborate our previous in silico drug repurposing efforts. The nimble primary midbrain neuron culture model described can be quickly optimized or altered to address novel hypotheses while reducing animal subjects and reliance on immortalized cell lines with adulterated phenotypes.

Disclosures: D.G.S. Barnett: None. **S.A. Lechner:** None. **C. Kelm-Nelson:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.02/L4

Topic: C.03. Parkinson's Disease

Support: NIH NINDS R01 NS117469

Title: Resveratrol alters NF-κB signaling target mRNA profiles in Parkinsonian Pink1-/- rats

Authors: S. A. LECHNER[1], D. G. S. BARNETT[2], S. C. GAMMIE[3], ***C. A. KELM-NELSON**[4];
[1]Surgery, Univ. of Wisconsin, Madison, Madison, WI; [2]Surgery, [3]Integrative Biol., Univ. of Wisconsin-Madison, Madison, WI; [4]Surgery, Univ. of Wisconsin Madison Dept. of Surgery, Madison, WI

Abstract: Parkinson disease (PD) is the fastest growing neurodegenerative disease. Identifying early-stage pathology and gene signatures is necessary for the development of novel and effective disease-modifying treatments. The *Pink1-/-* rat, a mitochondrial dysfunction model of prodromal PD, demonstrates the early-stage deficits in cranial motor (vocal) function with significant upregulation of inflammatory signaling pathways in the vocal fold as well as caudal brainstem vocal motor nuclei. Using CNS transcriptomic data and a drug repurposing approach, we first identified therapeutic candidates that could reverse pathologic gene expression patterns in the *Pink1-/-* model. The top match, resveratrol, has anti-inflammatory, antioxidant, and anti-apoptotic properties and in previous work has demonstrated a therapeutic effect in humans with PD. Next, we tested the hypothesis that systemic resveratrol administration will ameliorate vocalization deficits and decrease inflammation-related transcription profiles in *Pink1-/-* rats, via whole blood measurement of readily-accessible blood biomarkers. This study was performed at 4- and 10-months of age on separate groups of rats (total $N = 40$). Male *Pink1-/-* rats were randomly assigned into drug condition groups, vehicle ($n = 10$) or resveratrol (20 mg/kg dose; $n = 10$) and were given a 4-gram sugar cookie + drug daily for 8 weeks. Vocalization testing was done at three timepoints: baseline, 4-, and 8-weeks. The RT^2 Profiler PCR Array for NF-κB Signaling Targets was used to analyze whole blood mRNA expression changes after the 8 weeks of treatment. RM-ANOVAs were used to analyze differences in vocalization variables between timepoints and drug condition. Fisher's LSD was used for post-hoc comparisons; level of significance was set *a priori* at 0.05. There were no interactions between timepoint and treatment for any vocalization variables at 4-months; however, 10-month data collection is ongoing. Rats receiving resveratrol had significant beneficial transcription changes including downregulation of *Myd88* (microglial activation), *Bcl2a1* and *Irf1* (apoptosis), and upregulation of *Ifnb1* (anti-inflammatory). This is the first study to suggest that resveratrol acts to reduce systemic inflammation in the *Pink1-/-* rat but did not change vocalizations. We hypothesize the lack of vocal deficit amelioration may be due to nonideal blood brain penetration or insufficient/subtherapeutic concentrations within the CNS. Our lab is presently testing this hypothesis with direct *in vivo* administration of resveratrol to brain parenchyma via stereotaxic microcannulation.

Disclosures: **S.A. Lechner:** None. **D.G.S. Barnett:** None. **S.C. Gammie:** None. **C.A. Kelm-Nelson:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.03/L5

Topic: C.03. Parkinson's Disease

Support: DOD PD200005

Title: Development of a chronic paraquat and lectin-induced rat model recapitulating the human pathogenesis of Parkinson's Disease

Authors: *V. PESHATTIWAR[1], C. SWAIN[1], K. LE[1], K. VENKITESWARAN[2], T. SUBRAMANIAN[3];
[1]Dept. of Neurol., [2]Dept. of Neurol. & Neurosci., [3]Dept. of Neurology, Neurosci. and Bioengineering, The Univ. of Toledo, Toledo, OH

Abstract: Environmental neurotoxicants have been implicated in the development of idiopathic Parkinson's Disease (PD). The aim of our present study was to mimic the effects of possible multiple low dose exposure of environmental neurotoxin Paraquat (P) and Lectin (L) on the induction of idiopathic PD-like pathophysiological manifestations in rats. To achieve this, subthreshold doses of Paraquat and lectin (P+L; p.o.) supplemented with Cholecystokinin (i.p.) was administered daily to Sprague Dawley rats over a period of 90 days. The animals underwent a vibrissae-evoked forelimb placement test (VEFPT) to detect the initiation and progression in motor deficits at baseline and every 2 weeks during the 90 days treatment period and until 2 months after the last P+L+CCK dose. The animals were further evaluated for Levodopa (LD) responsiveness and sleep abnormalities (REM sleep behavior disorder (RBD)) with around the clock levodopa (ATC-LD) administration (LD 4 mg/kg + Benserazide 15mg/kg), i.p every 4 h for 48 h). The animals were euthanized, perfused with 4% PFA, and their brains were processed for histological examination of TH+ immunohistochemistry and quantification of pSyn in the SN. Onset of parkinsonian motor deficits was observed from week 4 (i.e., day 28, $p< 0.05$) from the start of P+L+CCK administration. A gradual progression in severity of motor deficits starting from hemiparkinsonism to bilateral symptoms was observed over the entire duration of 90 days of P+L+CCK treatment. The motor symptoms continued to progress for 2 months beyond cessation of P+L+CCK treatment. Parkinsonian motor deficits were levodopa responsive ($p<0.05$). RBD was observed in all animals regardless of the variation in their motor deficit severity. RBD was attenuated by ATC-LD treatment. These preliminary results suggest that the subthreshold doses of paraquat and lectin administered daily for 90 days in rats results in an animal model that simulates the natural disease progression as observed in human PD inclusive of the RBD, a key non-motor feature of PD and associated ascending synucleinopathy along the gut brain axis.

Disclosures: **V. Peshattiwar:** None. **C. Swain:** None. **K. Le:** None. **K. Venkiteswaran:** None. **T. Subramanian:** F. Consulting Fees (e.g., advisory boards); Thyagarajan Subramanian has received honoraria for serving on scientific advisory board for Teva, Neurocrine and Supernus; honoraria for study section service from the National Institutes of Health., He has received research funding from National Institutes of Health and from the Department of Defense, UCB pharma, Bukwang and BlueRock.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.04/L6

Topic: C.03. Parkinson's Disease

Title: Eaat2 activation promotes glutamate and calcium homeostasis and improves cognition in a rodent model of parkinson's disease

Authors: *S. DAS[1], S. KORTAGERE[2];
[1]Microbiology & Immunol., Drexel Univ., Philadelphia, PA; [2]Microbiology and Immunol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: EAAT2 activation promotes glutamate and calcium homeostasis and improves cognition in a rodent model of Parkinson's diseaseSanjay Das, Kyle Mccloskey, Sandhya KortagereDrexel University College of Medicine, Philadelphia, PAGlutamate is a major excitatory neurotransmitter involved in cognition, mood, anxiety and depression to name a few. Glutamate homeostasis is tightly regulated by a network of receptors and transporters in the brain. Excitatory amino acid transporter (EAAT2 or GLT-1 in rodents) is predominantly localized to astrocytes and is responsible for clearing ~90% of glutamate from the synapse. Under conditions of Parkinson's Disease (PD) and other neurodegenerative diseases, studies have shown that EAAT2 is downregulated, and aberrant activation of glutamatergic neurons lead to excitotoxicity. Dysregulation of dopamine and glutamate neurotransmission are not only implicated in motor and cognitive impairment in PD but also in promoting compulsive and impulsive behaviors. Therefore, we hypothesized that small molecule activators of EAAT2 that can effectively reduce excitotoxicity will be beneficial to treat motor and cognitive impairment in PD without promoting impulsive behaviors. We tested GTS467 - a novel small molecule activator of EAAT2 that was recently developed in our laboratory in a unilateral lesioned rodent model of PD. Results from the study confirms that GTS467 treatment significantly improved performance in a 5-choice serial reaction time cognitive task with reduced premature impulsive responses and omissions in comparison to vehicle treated PD animals. Ex vivo biochemical analysis of the tissue from prefrontal cortex and striatum from these treated animals showed a reduction in glutamate levels with an increase in EAAT2 protein expression and normalization of intracellular calcium levels with an increase in expression of Calbindin, Calcineurin, an increase in synaptic NMDA expression which altogether promote the survival of neurons. Under the normalized glutamate and calcium level the enhanced postsynaptic NMDAR further helps in

maintaining the homeostasis and improvement in cognitive ability. These results suggest that GTS467 can rescue cognitive deficits in PD by normalizing aberrant glutamate and calcium levels through EAAT2 activation.

Disclosures: S. Das: None. **S. Kortagere:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.05/L7

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA
Center of Behavioral Brain Sciences

Title: Examining accumulation rate of neuromelanin in the locus coeruleus as a critical factor for neurodegeneration

Authors: *C. NOVÁK[1], A. M. JARAMILLO[1], C. GONZÁLEZ-CABRERA[1], E. DURÁN[1], M. BETTS[3], C. SEIDENBECHER[2], M. PRIGGE[1];
[1]Neuromodulatory Networks Res. Group, [2]Dept. of Neurochemistry and Mol. Biol. and Dept. of Behavioral Neurol., Leibniz Inst. for Neurobio., Magdeburg, Germany; [3]German Ctr. For Neurodegenerative Dis. (DZNE), Magdeburg, Germany

Abstract: One-Sentence-Summary: The rate of neuromelanin accumulation in the locus coeruleus is a critical parameter that determines vulnerability of noradrenergic neurons to neurodegeneration. Throughout an individual's lifespan, all dopaminergic and noradrenergic neurons in the human brain accumulate neuromelanin (NM), a black pigment. While NM generally serves a protective function, excessive levels can contribute to neuronal degeneration and increase susceptibility to Parkinson's disease. Since rodents do not naturally produce NM, we investigated whether inducing NM accumulation at varying rates would have a protective effect or trigger neurodegeneration. To explore this, we developed a conditional expression system involving an oxidoreductase (tyrosinase) that converts catecholaminergic metabolites into reactive quinones and semiquinones, ultimately forming NM. By applying this approach, we examined the behavioral consequences of NM accumulation in the highly vulnerable locus coeruleus of rodents. We administered a Cre-dependent virus expressing tyrosinase into noradrenergic neurons of male DBH-Cre mice at two different dilutions. With a low titer virus injection, NM accumulation was gradual, reaching detectable levels after 1.5 months and consistently high levels at 7 months post-injection. Despite the accumulation of NM, neurodegeneration remained minimal. Assessing anxiety-like and olfactory behaviors at various time points (2 weeks, 3 months, 7 months, and 11 months post-injection), we found mostly intact functions, except for a pronounced preference for the light compartment in the light/dark box test

at 11 months. Even after a year, no changes were observed in basic autonomic functions or taste perception. In contrast, injecting the same virus at a high titer resulted in rapid NM accumulation, leading to significant degeneration of the locus coeruleus as early as 6 weeks post-injection. This degeneration was accompanied by increased anxiety-like behaviors. In conclusion, the expression of tyrosinase through viral vectors induces progressive NM accumulation and neurodegeneration in the locus coeruleus of mice, accompanied by functional alterations. The extent of NM accumulation, neurodegeneration, and symptom severity are heavily influenced by the concentration of the injected virus.

Disclosures: **C. Novák:** 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.; Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA. **A.M. Jaramillo:** None. **C. González-Cabrera:** 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.; Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA. **E. Durán:** 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.; Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA. **M. Betts:** None. **C. Seidenbecher:** None. **M. Prigge:** 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.; Center of Behavioral Brain Sciences, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.06/L8

Topic: C.03. Parkinson’s Disease

Title: Characterization of the physiological effect induced by Tio2 nano-matrices in models with induced hemiparkinsonism.

Authors: *E. A. RODRIGUEZ PEREZ, G. REYNOSO GALVEZ, A. VEGA GARCIA, P. VERGARA-ARAGON, R. BUSTAMANTE-GARCIA, M. JAIME FONSECA;
Univ. Nacional Autonoma De Mexico, México City, Mexico

Abstract: We are interested in developing new and improved biotechnological strategies for the treatment of central nervous system diseases. Titanium dioxide (TiO2) has been used to construct

nanomatrices (NM) capable of releasing and transporting dopamine (DA) to the caudate nucleus of animal models with induced hemiparkinsonism (HP), which affects motor behavior, the uptake index of the 11[C] DTBZ marker, and immunoreactivity for tyrosine hydroxylase. Significant statistical differences were observed between the experimental groups, obtaining a beneficial effect on both fine and gross motor skills. This effect is likely caused by the release of dopamine from the TiO2 matrix directly into the caudate nucleus. The beneficial effect was also evident in the analysis of THir+ immunoreactivity and in the conducted tomographies.

Disclosures: E.A. Rodriguez Perez: None. **G. Reynoso Galvez:** None. **A. Vega Garcia:** None. **P. Vergara-Aragon:** None. **R. Bustamante-Garcia:** None. **M. Jaime Fonseca:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.07/M1

Topic: C.03. Parkinson’s Disease

Support: CIHR Grant 507489
NSERC Grant 506730
CIHR Canada Graduate Scholarship-Master's

Title: Characterization of olfactory function following alpha-synuclein fibril inoculation in the anterior olfactory nucleus in a prodromal Parkinson's Disease mouse model

Authors: *R. TRAN, J. ARSENAULT, J. KIM;
Univ. of Toronto, Toronto, ON, Canada

Abstract: Parkinson’s disease (PD) presents a significant challenge in terms of timely diagnosis, as motor impairments typically emerge at stage 3 or 4 when extensive neuropathology is already evident. Approximately 90% of PD patients experience olfactory impairment in the first stage of the disease, preceding the onset of cardinal motor symptoms by at least 4 years. Alpha-synuclein (a-syn) aggregates form Lewy bodies and neurites, the pathological hallmark of PD, and these inclusions first appear in the olfactory system, specifically, within the anterior olfactory nucleus (AON). The AON is a hub that receives top-down input from the hippocampus and bottom-up input from the olfactory bulb, thereby integrating contextual and odour sensory information. Thus, the AON plays a role in simple odour detection and discrimination as well as complex odour functions such as olfactory episodic memory. Albeit a-syn preferentially accumulates in the AON at the earliest stage of PD, its role in pathology and manifestations of prodromal symptoms remains unknown. To address this knowledge gap, the present study aims to investigate the progressive spread of a-syn aggregates and neuroimmune responses following intracerebral injections of a-syn fibrils in the AON of transgenic (Tg) heterozygous A53T mice, overexpressing mutant a-syn as well as its impact on olfactory function over time. In the initial phase of the experiment, we developed a robust go/no-go operant conditioning paradigm to

evaluate the effect of the transgene A53T on olfactory function. As anticipated, the mixed two-way ANOVA revealed no significant differences between wild-type (WT) and Tg animals in their performance in odour detection and discrimination. In the next phase, Tg animals are receiving bilateral inoculation of either a-syn fibrils or control injections in the AON. Olfactory sensitivity, discrimination and episodic-like memory are evaluated longitudinally using our olfactory operant conditioning apparatus. Immunofluorescence staining is being performed to detect misfolded a-syn, microglia and astrocytes using phosphorylated-serine129 (Pser129), ionized calcium-binding adaptor protein-1 (IBA-1), and glial fibrillary acidic protein (GFAP) antibody markers, respectively. The outcomes of the current study will enhance our understanding of the underlying mechanisms contributing to olfactory dysfunction in PD. Moreover, the findings have the potential to inform the development of a reliable and cost-effective diagnostic tool, thereby aiding in identifying individuals at high risk for PD at the earliest possible stage and facilitating intervention strategies.

Disclosures: **R. Tran:** None. **J. Arsenault:** None. **J. Kim:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.08/M2

Topic: C.03. Parkinson’s Disease

Support: ERC starting grant 805426

Title: Endoplasmic reticulum stress and inflammation in a low-dose 6-hydroxydopamine rat model

Authors: *T. VILJAKAINEN, M. H. VOUTILAINEN;
Fac. of Pharm., Univ. of Helsinki, Helsinki, Finland

Abstract: Parkinson’s disease is a progressive neurodegenerative disease characterized by the degeneration of dopaminergic neurons of the nigrostriatal pathway. 6-hydroxydopamine (6-OHDA) is a neurotoxin that is widely used to model Parkinson's disease *in vitro* and *in vivo*. When injected into the striatum, substantia nigra or medial forebrain bundle, it causes a loss of dopaminergic neurons, which recapitulates the pathophysiology of Parkinson's disease. Here we studied the effects of a low-dose 6-OHDA model in rats.
6-OHDA was unilaterally injected to three sites in the striatum (3x2 µg) of male Wistar rats. Two weeks later, rotational behaviour was assessed after a subcutaneous amphetamine injection by using automatic rotometry. The rats were sacrificed 3 or 12 weeks post-lesioning by transcardial perfusion. Free-floating coronal substantia nigra and striatum sections were cut to perform immunohistochemical stainings.
Intrastriatal 6-OHDA injections caused a significant loss of tyrosine hydroxylase (TH) positive neurons in the substantia nigra. We also assessed the number of microglia and astrocyte;

however, we did not see any changes. Next, we wanted to study whether the endoplasmic reticulum stress and unfolded protein response (UPR) were increased. There was an increase in certain UPR markers, such as XBP1s and p-eIF2α, in non-TH positive cells in the substantia nigra three weeks after 6-OHDA injections.
The low-dose 6-OHDA lesion model produced a robust loss of TH-positive neurons without causing long-term activation of microglia or astrocytes. Increased UPR activation was observed; however, a more elaborate time course of the activation needs to be studied.

Disclosures: T. Viljakainen: None. **M.H. Voutilainen:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.09/M3

Topic: C.03. Parkinson’s Disease

Support: F31NS129277-02
R01AG081433-01
Parkinson's Association of Alabama
T32N095775

Title: Mutant GBA1 mice show impaired contextual memory and increased hippocampal alpha-synuclein pathology in a Parkinson’s Disease model

Authors: *S. BOBBA, C. MAHONEY-CRANE, L. VOLPICELLI-DALEY;
Neurol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson’s disease (PD) impacts over 2 million people in the United States alone and over 10 million people worldwide. PD is pathologically characterized by the accumulation of neuronal α-synuclein (α-syn) aggregates, termed Lewy pathology. Lewy pathology is significantly correlated with decreased cognitive function. The most common genetic risk factor for PD is mutations in the gene glucocerebrosidase-1 (GBA1), which encodes for the lysosomal enzyme Glucocerebrosidase (GCase). Approximately, 7-11% of all PD cases show a GBA1 mutation. Patients heterozygous for the GBA1 L444P ($GBA1^{+/L444P}$) mutation demonstrate a 5.6-fold increased risk for developing dementia. The effects of GBA1 L444P expression on the hippocampus, a brain region that plays an integral role in cognitive function, are not fully understood. Thus, we sought to determine the effects of the $GBA1^{+/L444P}$ mutation on spatial learning, memory, and hippocampal α-syn pathology. To analyze behaviors associated with hippocampal function, behavior paradigms such as fear conditioning and Barnes maze were used. Our data show that by 3-mo of age, $GBA1^{+/L444P}$ showed reduced freezing when compared to their wildtype ($GBA1^{+/+}$) controls, suggesting impairments in associative memory. Concurrently, data derived from immunoblot experiments suggest that $GBA1^{+/L444P}$ mice exhibit a lower expression of the presynaptic protein vGLUT1 in the hippocampus. We also injected

mice with α-syn preformed fibrils to induce formation of α-syn inclusions. Immunofluorescence and confocal microscopy revealed that hippocampal α-syn aggregates were significantly increased in $GBA1^{+/L444P}$ mice compared to $GBA1^{+/+}$ 9-months post-fibril injection. Overall, these data reveal that heterozygosity for GBA1 L444P contributes to impairments in behaviors associated with hippocampal function, and selectively increased α-syn pathology in the hippocampus. Recent advancements in research and healthcare have developed treatments for PD symptoms; however, studying how to prevent the formation of α-syn aggregates is crucial in developing novel treatments that will slow the progression of PD.

Disclosures: S. Bobba: None. **C. Mahoney-Crane:** None. **L. Volpicelli-Daley:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.10/M4

Topic: C.03. Parkinson’s Disease

Support: MOST 109-2314-B-182-029-MY3
MOST 111-2622-B-182-002
CMRPD1M0252
CMRPD1M0701
CMRPD1K0671

Title: Longitudinal Analysis of the Electroencephalogram in the 6-OHDA-Induced Rat Model of Parkinson's Disease

Authors: L.-H. PAN[1], C.-W. KUO[1], *T.-H. HSIEH[1,2];
[1]Sch. of Physical Therapy and Grad. Inst. of Rehabil. Sci., Chang Gung Univ., Taoyuan, Taiwan; [2]Neurosci. Res. Ctr., Chang Gung Mem. Hosp., Taoyuan, Taiwan

Abstract: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disorder. Currently, the diagnosis of PD progression relies mainly on functional behavioral symptoms. Previous studies on PD have suggested that alterations in quantitative electroencephalography (EEG) profiles might reflect underlying neuronal dysfunction. However, the existence of a rapid, recurrent, and individualized EEG indicator for PD remains uncertain. This study developed a longitudinal EEG recording platform to identify whether abnormal quantitative EEG parameters can be used to monitor disease progression in a 6-hydroxydopamine (6-OHDA)-induced rat model of PD. The results demonstrated that, compared to rats with sham PD lesions, PD rats exhibited significantly lower gamma (30-95 Hz) power as early as 1 week post-PD lesion, maintaining relatively low levels throughout the four-week observation period. The alteration of gamma power correlated well with apomorphine-induced rotational changes over the four-week observation period ($R2= 0.65$, $p<0.001$). These findings could serve as reliable and widely

accessible indicators for monitoring the progression of PD, potentially facilitating early diagnosis and aiding in the assessment of the effectiveness of novel treatment protocols for PD.

Disclosures: **L. Pan:** None. **C. Kuo:** None. **T. Hsieh:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.11/M5

Topic: C.03. Parkinson's Disease

Support: Swedish Research Council VR-MH Grant 2022-04198
Swedish Research Council VR-TVAR Grant 2021-03293
Swedish Brain Foundation Grant FO2021-0318
Science for Life Laboratory

Title: Mass spectrometry imaging of brain signalling systems reveals abnormal alterations induced by parkinsonism and L-DOPA-induced dyskinesia

Authors: T. VALLIANATOU[1], I. KAYA[1], A. NILSSON[1], R. SHARIATGORJI[1], P. SVENNINGSSON[2], E. BEZARD[3], ***P. E. ANDREN**[1];
[1]Dept. of Pharmaceut. Biosci., Uppsala Univ., Uppsala, Sweden; [2]Dept. of Clin. Neurosci., Karolinska Institutet, Stockholm, Sweden; [3]Inst. of Neurodegenerative Dis., Bordeaux Univ., Bordeaux, France

Abstract: We employed ultrahigh-mass resolution Fourier-transform ion cyclotron resonance matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) to comprehensively map different molecular species (spatial omics), such as neurotransmitters, neuropeptides, metabolites, and lipids in specific brain regions. Using brain samples from an experimental Parkinson's disease (PD) model (MPTP, *Macaca mulatta*) with L-DOPA-induced dyskinesia (LID), we previously observed abnormal elevations of L-DOPA and its metabolite, 3-O-methyldopa, in the whole brain of LID animals. This resulted in increased dopamine and downstream metabolites in all brain regions, except putamen and caudate. Dopamine formation correlated with serotonin in specific layers of the hippocampus and cortex in LID. Furthermore, we found that the abundance of selected neuropeptides was associated with L-DOPA concentrations in the putamen, emphasizing their sensitivity to L-DOPA. Levels of truncated neuropeptides, i.e., dynorphins and tachykinins, correlated with dyskinesia severity and may constitute a functional compensatory mechanism for balancing the increased L-DOPA levels across the whole basal ganglia. In the present study, utilizing tissue from the same individual animals, we observed reduced putaminal acetylcholine levels in PD and LID that persisted after L-DOPA treatment. LID showed decreases in metabolites crucial for brain homeostasis, including S-adenosylmethionine, glutathione, adenosine monophosphate, and acylcarnitines. The vasculature marker heme B was upregulated in LID, suggesting blood-brain barrier

modifications and increased blood flow in the dyskinetic putamen. Furthermore, we extensively imaged various lipid types, detecting specific distributions of sulfatide lipids in MPTP-lesioned brains. Hydroxylated sulfatides with polyunsaturated chains were depleted in motor-related regions, while non-hydroxylated sulfatides were elevated. Comparing LID with non-dyskinetic animals, plasmalogen phosphatidylcholines decreased, while polyunsaturated fatty acid-containing phospholipids increased in the internal segment of globus pallidus. This MALDI-MSI study provides insights into signaling system dynamics during PD and its treatment.

Disclosures: **T. Vallianatou:** None. **I. Kaya:** None. **A. Nilsson:** None. **R. Shariatgorji:** None. **P. Svenningsson:** None. **E. Bezard:** None. **P.E. Andren:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.12/Web Only

Topic: C.03. Parkinson’s Disease

Support: DST-SERB Sanction No. EMR/2017/003490

Title: Effect of bacillus calmette gaurinevaccine against rotenone induced parkinson's disease focusing on neuroinflammation and neurodegeneration

Authors: *N. G. YEDKE[1], P. KUMAR[2], D. SONI[2];
[1]Dept. of Pharmaceut. Sci., Maharaja Ranjit Singh Punjab Tech. Univ., Bathinda, India;
[2]Pharmacol., Central university of punjab, Bathinda, India

Abstract: Parkinson’s disease (PD) is an extrapyramidal neuronal movement disorder (MD). PD is the foremost hypokinetic condition upraised by features of impairment of dopaminergic neurons in the striatal region of the brain. The biological impairment is profound scarcity in dopamine (DA), serotonin (5-HT), glutathione (GSH), elevated lipid peroxidation (LPO), catalase, superoxide dismutase (SOD), apoptosis, and oxidative stress. In the present study, we found that when Rotenone (ROT) was given in a toxic dose by subcutaneously (*s.c*) for 28 days, it significantly induced motor symptoms like open field test (OFT), narrow beam walking (NBW), Rotarod, biochemical imbalance (GSH, LPO, SOD, Nitrite, Catalase), neuronal (DA, 5-HT, nor-epinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), 5-hydroxy indoleacetic acid (5-HIAA), cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) impairments in the striatum. A previous study found that the Bacille Calmette Gaurine (BCG) vaccine attenuated the level of different neurons. As a result, we looked into the neuroprotective effects of the BCG vaccine in ROT-induced PD. Moreover, the BCG vaccine-treated group revealed the effect of the ROT by reversing to the normal impaired biological concentrations and motor functions as well. Our findings show that the BCG vaccine has an encouraging protective role against progressive degeneration in exploratory PD, with a beneficial role facilitated by neurochemistry restoration and anti-

inflammatory properties. Finally, the histological study was done with hematoxylin and eosin (H and E) staining. In the ROT group, neuronal damage was observed in the striatum compared to the control and BCG vaccine-treated groups.

Disclosures: **N.G. Yedke:** None. **P. Kumar:** None. **D. Soni:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.13/M7

Topic: C.03. Parkinson’s Disease

Title: Gender-dependent differences in the performance of action and stimulus-based reversal learning, and prepulse inhibition response in female and male Wistar rats undergoing unilateral dopaminergic depletion within the Substantia Nigra compacta with 6-OHDA.

Authors: *D. LIEVANO, J. RIVERA, F. CÁRDENAS PARRA;
Psychology, Los Andes Univ., Bogota, Colombia

Abstract: Motor disruption in Parkinson's disease (PD) is regularly accompanied by deficits in Reversal Learning from the onset of the illness, and more recently there have been reports of changes in Prepulse Inhibition response occurring years prior to the onset of the motor disorder. Due to the difficulty of predicting the beginning of motor impairment in PD, animal models are a beneficial tool to explore the dynamics of non-motor symptoms before and after dopamine manipulation. Fifty-one adult female and fifty-four adult male Wistar Rats underwent unilateral infusion of either 6-OHDA or saline in the Substantia Nigra Compacta. One week prior to the surgical procedure, motor performance was measured in a horizontal ladder, and a thirty-minute protocol was applied to assess Prepulse Inhibition one day before surgery. Following surgery, the rats were given apomorphine, and the number of right and left turns were registered by Any Maze. Afterwards, the rats were divided into three groups to assess Reversal Learning at three different moments post-surgery: two, four, and six weeks. Action-based and stimulus-based deterministic reversal learning were evaluated in a two-option nose-poke chamber. Finally, Prepulse Inhibition assessment was conducted after the protocol of reversal learning had been completed.

Disclosures: **D. Lievano:** None. **J. Rivera:** None. **F. Cárdenas Parra:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.14/M8

Topic: C.03. Parkinson's Disease

Title: Assessing changes in whole-brain structural connectivity in the unilateral 6-hydroxydopamine rat model of Parkinson's Disease using diffusion imaging and tractography

Authors: M. MOSHCHIN[1], R. J. SCHULTZ[1], K. P. CHENG[1], S. OSTING[2], J. KOEPER[1], M. LALUZERNE[1], J. K. TREVATHAN[1], A. BRZECZKOWSKI[1], J.-P. YU[3], W. B. LAKE[1], S. A. HURLEY[4], K. A. LUDWIG[1], *A. J. SUMINSKI[1];
[1]Neurolog. Surgery, [2]Neurol., [4]Radiology, [3]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Parkinson's disease (PD) is caused by degeneration of neurons in the substantia nigra pars compacta (SNc) that leads to striatal dopamine deficiency. In humans, degeneration of SNc neurons triggers motor symptoms, including bradykinesia, resting tremor, rigidity and freezing of gait. Unfortunately, there is no naturally occurring animal model of PD. However, unilateral injection of 6-hydroxydopamine (6-OHDA) in the median forebrain bundle (MFB) of the rat lesions nigro-striatal dopaminergic (DA) neurons producing similar motor symptoms to those seen in PD patients. It remains unclear whether the physiological changes from unilateral 6-OHDA lesions are limited to the cells local to the lesion or if they extend throughout the brain. This work aims to fill this gap by using whole brain diffusion magnetic resonance imaging (dMRI) and correlational tractography to detect microstructural changes in 6-OHDA lesioned rats. A total of 10 male, Long Evans rats were used in this study. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin-Madison. While under isoflurane anesthesia, rats received an injection of 6-OHDA (n=5; 2µg/µl) or saline (n=5) into the left MFB (-4.3mm AP, -1.6mm ML, -8.6mm DV). Limb use asymmetry and apomorphine induced rotation tests were performed 19 and 20 days following creation of the lesion to verify the PD phenotype. On day 21, rats were transcardially perfused with 4% paraformaldehyde. Following fixation, their brains were prepared for diffusion weighted imaging and tyrosine hydroxylase (TH) staining. Consistent with a PD phenotype, 6-OHDA lesioned animals showed a significant increase in ipsilateral forelimb use ($p < 0.005$ - two-sample t-test) and rotational rate ($p < 0.005$, two-sample t-test) compared to vehicle. Differences in behavior were corroborated by qualitative histology with sections through the striatum having significantly less stained area in the 6-OHDA group compared to vehicle ($p < 0.005$, two-sample t-test). Correlational tractography found a significant negative correlation (FDR < 0.05) in quantitative anisotropy between 6-OHDA and vehicle cohorts in the fibers bilaterally throughout the cortico-thalamo-basal ganglia network including the left MFB. Thus, our work shows significant structural changes in nigro-striatal pathways related to toxin injection that extend beyond the injected fibers. Furthermore, it suggests that connectomic measures may be appropriate tools to evaluate the effects of neurodegenerative disease and subsequent therapeutic interventions on structural connectivity across the whole brain network.

Disclosures: M. Moshchin: None. **R.J. Schultz:** None. **K.P. Cheng:** None. **S. Osting:** None. **J. Koeper:** None. **M. LaLuzerne:** None. **J.K. Trevathan:** None. **A. Brzeczkowski:** None. **J. Yu:** None. **W.B. Lake:** None. **S.A. Hurley:** None. **K.A. Ludwig:** None. **A.J. Suminski:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.15/M9

Topic: C.03. Parkinson's Disease

Title: Development and characterization of intracranial α-synuclein seeding mouse models

Authors: *C. COMMINS, H. SANDERS, L. NORWOOD, P. BOSCH, J. STOEHR, T. DELLOVADE;
AbbVie, Cambridge, MA

Abstract: Parkinson's disease (PD), characterized by progressive motor and cognitive decline, is the second most prevalent age-related neurodegenerative disease, affecting more than 10 million people worldwide with no currently approved disease modifying treatments. Developing therapeutics relies on the availability of robust and translational in vivo animal models to enable testing of drug candidates aiming to impact disease progression. Histopathologic, genetic and biomarker data in PD patient cohorts highlight the importance of aggregation of the protein alpha synuclein (aSyn) in the brain of PD patients as a central biological pathway in initiation and progression of PD. To model this pathological cascade of aSyn aggregation in vivo, we are developing disease relevant mouse models in which mice are intracerebrally injected (or 'seeded') with commercially available recombinant human or mouse misfolded aSyn (so-called pre-formed fibrils, PFFs). Direct injection of human PFFs into the hippocampal CA1 or striatum of heterozygous M83 transgenic mice that overexpress human A53T variant of aSyn resulted in pathology at multiple brain sites, as measured by deposits of phosphorylated (serine 129 (pS129)) aSyn immunoreactivity after one month with sufficient assay window, without causing neuronal loss. Similarly, direct injection of mouse PFFs into the hippocampal dentate gyrus of mice that constitutively express CRISPR associated protein 9 (Cas9), Rosa26-Cas9 knock in (Cas9 KI), resulted in increased pS129-immunoreactivity. While the M83 seeding models are aimed at testing efficacy of potential small molecules or biologics, the goal of the Cas9 KI model is validation of novel targets using specific guide RNAs (gRNAs) to introduce single or multiple genetic modifications. We validated this approach using an AAV9-SNCA gRNA to knockdown aSyn, which was able to almost completely prevent PFF-induced pS129-immunoreactivity in the Cas9 KI mice when injected into the dentate gyrus 2 weeks prior to seeding. Together, these highly reproducible in vivo model systems will enable future testing of novel small molecules, biologics, and genetic targets in a pharmaceutical industry setting.

Disclosures: **C. Commins:** A. Employment/Salary (full or part-time):; AbbVie. **H. Sanders:** A. Employment/Salary (full or part-time):; AbbVie. **L. Norwood:** A. Employment/Salary (full or part-time):; AbbVie. **P. Bosch:** A. Employment/Salary (full or part-time):; AbbVie. **J. Stoehr:** A. Employment/Salary (full or part-time):; AbbVie. **T. Dellovade:** A. Employment/Salary (full or part-time):; AbbVie.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.16/M10

Topic: C.03. Parkinson’s Disease

Support: Aligning Science Across Parkinson's (ASAP)
NIH NIGMS Grant 5K12GM000680-23

Title: Changes in glutamatergic and catecholaminergic innervation within cortical motor areas using the progressive mitoPark mouse model of parkinsonism

Authors: *A. J. LOPEZ[1], H. CHEHADE[2], O. GOTTIPALLI[3], H.-Y. CHU[2], Y. SMITH[3]; [1]Neurol., Emory Univ., Atlanta, GA; [2]Van Andel Inst., Grand Rapids, MI; [3]Neurol., Emory University, Emory Natl. Primate Res. Ctr., Atlanta, GA

Abstract: Striatal dopamine (DA) loss in Parkinson’s disease (PD) induces functional and anatomical changes throughout the motor basal ganglia-thalamo-cortical loop. The resulting dysfunction of this loop induced by striatal DA loss impacts processing in primary and secondary motor cortices (M1 and M2 in rodents). Existing functional and anatomical data mainly originates from parkinsonian animals with severe DA loss. Thus, it remains unclear when cortical abnormalities develop during the course of the development of the parkinsonian state. The goal of this study is to assess longitudinal changes in glutamatergic and catecholaminergic innervation of M1 and M2 at varying stages of striatal DA loss using a progressive mouse model of parkinsonism (i.e., mitoPark mice). To date, 9 control (4 female) and 9 mitoPark (5 female) mice greater than (>)24 weeks old, and 6 control (3 female) and 6 mitoPark (3 female) mice between 12-14 weeks old, have been perfused and brain tissue collected for immunohistochemistry (IHC). To determine the extent of glutamatergic and catecholaminergic cortical innervation, serial sections at the level of M1 and M2 from control and mitoPark mice were immunostained with specific antibodies against vesicular glutamate transporter 2 (vGluT2), norepinephrine transporter (NET), and tyrosine hydroxylase (TH). Digital images of stained tissue slides were imported into ImageJ, converted into 8-bit grayscale format, and grayscale units were converted to optical density (O.D.) units using the Rodbard function. O.D. measurements, which are proportional to stain intensity, were obtained from Layer I, Layers II-III, and Layer V in M1 and M2 for both groups. To date, preliminary analysis has been completed on 2 control mice and 2 mitoPark mice. As data analysis on the processed stained tissue continues, we predict that for vGluT2, NET, and TH labeling, mitoPark mice will show reductions for staining intensity in both M1 and M2 across all cortical layers, which will be associated with motor and non-motor symptoms in PD. Ongoing work will continue to assess and quantify changes in glutamatergic and catecholaminergic cortical innervation at varying stages of striatal DA loss: 6-8 weeks old, 12-14 weeks old, and >24 weeks old.

Disclosures: A.J. Lopez: None. **H. Chehade:** None. **O. Gottipalli:** None. **H. Chu:** None. **Y. Smith:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.17/N1

Topic: C.03. Parkinson’s Disease

Title: Sleep-wake disorders in a primate model of parkinson’s disease

Authors: C. E. GROSS[1], A. SADOUN[1], ***B. BIOULAC**[2], E. Y. PIOLI[4], E. BEZARD[3]; [1]Motac France, Bordeaux, France; [3]Inst. of Neurodegenerative Dis., [2]Inst. of Neurodegenerative Dis., Bordeaux, France; [4]MOTAC, Bordeaux, France

Abstract: Non-motor symptoms of Parkinson’s disease have been studied for decades in several animal models. The MPTP *Macaca mulatta* model offers an opportunity to study most of the non-motor symptoms observed in humans, particularly regarding sleep-wake disorders, sleep fragmentation, and daytime sleepiness. In the present study, we analysed the sleep/wake structure and alterations for 24 hours in 12 macaques (*Macaca mulatta).* Six of them have been MPTP-rendered parkinsonian by daily injection of MPTP hydrochloride. We used a radio-telemetric system allowing continuous recordings of EEG, EOG (electro-oculographic), and EMG (electromyographic) signals in freely behaving and unrestrained animals. Sleep/Wake states and the EEG Power Spectral Density in each frequency band (Delta, Theta, Alpha, Sigma, Beta, and low and high Gamma) were quantified during the night [20h-08h] and the daytime [08h-20h] periods for both MPTP-treated and Controls. A non-parametric test was used to compare the two groups.Results showed, in the MPTP monkeys, a significant increase in wake durations during the night period, a decrease in sleep duration, particularly regarding NREM (SWS, Slow Wave Sleep), an increase in sleep fragmentation and poor distribution.Results showed, in the MPTP monkeys, significant alterations in the power spectrum in several frequency bands, in particular the Delta band during the sleep stages, and the Sigma and Beta bands during the Wake stage.Our results confirm the disorganisation of the slow wave sleep in MPTP- treated monkeys and report that the EEG Power Spectral Density of slow wave sleep is reduced in MPTP animals, suggesting that the slow wave sleep is less efficient.The present study demonstrates that the MPTP-treated macaque is a good model that replicates non-motor symptoms of Parkinson’s Disease and paves the way for further studies.

Disclosures: C.E. Gross: None. **A. Sadoun:** None. **B. Bioulac:** None. **E.Y. Pioli:** None. **E. Bezard:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.18/N2

Topic: C.03. Parkinson's Disease

Title: Induction of alpha-synuclein pathology in the adult rodent brain with AAV-mediated gene transfer and preformed fibrils

Authors: *S. BÄCK[1], R. PUSSINEN[1], T.-K. STENIUS[2], Y. SINGH[2], F. KHAN[2], T. BRAGGE[2], J. RYTKÖNEN[2];
[2]Charles River Discovery Services, [1]Charles River Discovery Services, Kuopio, Finland

Abstract: Induction of α-synuclein overexpression, phosphorylation, and/or aggregation in the adult rodent brain can be used in pre-clinical drug discovery to study novel treatments targeting α-synuclein- and Parkinson's disease-related pathologies. The aim of the present study was to compare the behavioral and biomarker phenotype of three rodent models of α-synuclein pathology induced with viral vector-mediated overexpression or with pre-formed fibrils (PFF). Alpha-synuclein pathology was induced by either AAV1/2-mediated overexpression of A53T α-synuclein in the rat and mouse substantia nigra, or by delivery of α-synuclein PFF into the mouse striatum. During in-life, the fine motor and kinematic gait performance of the animals was evaluated using MotoRater (TSE Systems) coupled to an in-house developed analysis pipeline. Endpoint readouts included evaluation of α-synuclein pathology and neurodegeneration in the nigrostriatal dopaminergic tract using immunohistochemistry and neurochemical assessment of striatal dopamine and dopamine metabolites.
In both rats and mice, AAV1/2-mediated overexpression of A53T α-synuclein in the substantia nigra resulted in a mild but significant change in the animals' fine motor and kinematic gait performance, accompanied by a decrease in total tissue dopamine in the striatum. Although injection of PFFs lead to an increase in phosphorylated S129-α-synuclein throughout the mouse brain, including aggregation in the substantia nigra, no significant model phenotype was observed in the fine motor and kinematic gait analysis or in striatal dopamine levels.
In summary, α-synuclein pathologies could be detected in all models, whereas only AAV-mediated overexpression of α-synuclein resulted in clear deficits in the dopaminergic system. When choosing the α-synuclein model for pre-clinical research projects, the mode-of-action of the therapy as well as the treatment window in the targeted readout(s) need to be taken into consideration.

Disclosures: S. Bäck: A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **R. Pussinen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **T. Stenius:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **Y. Singh:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **F. Khan:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **T. Bragge:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland. **J. Rytkönen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services Finland.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.19/N3

Topic: C.03. Parkinson's Disease

Support: NIH grant NS045962

Title: Dopamine depletion alters GluN2-subunits contribution to NMDA receptor currents in striatal projection neurons

Authors: *S. COLETTA[1], C. G. SINON[1], V. GHIGLIERI[3], F. CAMPANELLI[5], G. MARINO[5], T. YABUMOTO[1], B. KOCHOIAN[1], C. BURE[1], B. PICCONI[4], P. CALABRESI[5], S. F. TRAYNELIS[6], S. M. PAPA[2];
[1]Neurol., Emory Univ., ATLANTA, GA; [2]Neurol., Emory Univ., Atlanta, GA; [3]Neurophysiol. Lab., [4]Fondazione Santa Lucia, Rome, Italy; [5]Univ. Cattolica del Sacro Cuore, Rome, Italy; [6]Dept Pharmacol, Sch. of Med., Atlanta, GA

Abstract: Dopamine depletion results in a dysregulated pattern of activity in spiny projection neurons (SPNs) in animal models and patients with Parkinson's Disease (PD). Specifically, both direct and indirect pathways SPNs (d-SPNs and i-SPNs, respectively) are characterized by hyperactivity when compared to normal condition. The elevated firing is thought to arise from adaptive changes in excitatory glutamate NMDAR signaling. In fact, NMDAR inhibitors counteract the hyperactivity and showed antiparkinsonian properties. The expression of NMDAR subunits is thought to be cell type-specific and likely undergo reorganization in PD. Thus, a clear understanding of functional changes in NMDAR signaling in d- and i-SPNs represents the first step for developing a more specific pharmacological strategy to restore the physiological pattern of SPN activity in PD. Here we aimed at characterizing GluN2A, GluN2B, and GluN2D subunits' contribution to synaptic currents in i-SPNs following dopamine depletion.Whole-cell patch clamp recordings of i-SPNs in the dorsolateral striatum compared intact versus dopamine-denervated striatum using adult rats. Rats received a sham or unilateral nigrostriatal 6-OHDA lesion. In addition, both groups received an injection of rAAV-D2SP-eYFP that labels i-SPNs with a ~90% specificity. SPNs were held at a membrane potential of +40 mV and EPSCs were evoked by stimulating cortico-striatal fibers every 30s. NMDAR currents were isolated by adding Picrotoxin and NBQX to the recording aCSF solution, and further tested by bath application of DL-APV at the end of the recording. The contribution of each NMDAR subunit was tested pharmacologically by bath application of a selective antagonist. In this study, we used MPX-004, CP101606, and NAB-14. The peak amplitude of NMDA-mediated currents in i-SPNs was reduced by 1/3 following bath application of MPX-004. The peak reduction was significant compared to the baseline, but not between intact and lesioned groups. A significant reduction of the peak response from baseline was observed also following bath application of CP101606 or NAB-14. Moreover, a trend emerged suggesting a significant contribution of GluN2B and GluN2D subunits to the overall NMDA-mediated currents following dopamine depletion.These results suggest that dopamine loss leads to a reorganization of NMDAR subunits in i-SPNs characterized by an increased expression of GluN2B- and GluN2D-subunits, but not GluN2A-subunits and that these changes may underlie the altered activity of these neurons. Comparison of these results to findings in d-SPNs will provide further insights into the pathophysiology of striatal dysfunction in PD.

Disclosures: **S. Coletta:** None. **C.G. Sinon:** None. **V. Ghiglieri:** None. **F. Campanelli:** None. **G. Marino:** None. **T. Yabumoto:** None. **B. Kochoian:** None. **C. Bure:** None. **B. Picconi:** None. **P. Calabresi:** None. **S.F. Traynelis:** None. **S.M. Papa:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.20/N4

Topic: C.03. Parkinson's Disease

Support: the John and Maurine Cox Endowed Chair.

Title: Characterization of gut changes in a parkinsonian rat model of 6-hydroxydopamine

Authors: *C. KAUFHOLD[1], A. SHAIK[2], E. LEPE[2], R. SRINIVASAN[2], F. SOHRABJI[3]; [1]Texas A&M Univ., Bryan, TX; [2]Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [3]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX

Abstract: Parkinson's disease (PD) is the most common movement disorder, characterized by motor dysfunction due to a loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). Multiple studies have demonstrated that gastrointestinal dysfunction persists throughout the evolution of PD and is an important feature during the prodromal phase of PD, prior to the onset of motor symptoms. In this study, we investigated changes in the gut histoarchitecture using an established preclinical 6-hydroxydopamine (6-OHDA) model of parkinsonism in rats. Adult (5-7m) and middle-aged (9-12m), male Sprague Dawley rats were unilaterally injected with 6-OHDA into the dorsolateral striatum (DLS), while control rats for both age groups received a unilateral injection of 0.2% ascorbic acid. To assess progressive SNc DA loss in 6-OHDA injected rats, we administered the dopamine receptor agonist, apomorphine, and compared apomorphine-induced contralateral rotations between control and 6-OHDA rats at 7 days pre-6-OHDA injection, and at 7, 14, 21, and 28 days post. Additionally, we collected serum from all rats at each of the timepoints to measure gut-related metabolites. Finally, rats were terminated at 30 days post and brain, ileum and colon were collected. Our preliminary results showed 6-OHDA rats display a progressive time-dependent increase in apormophine-induced rotations, validating our preclinical rat parkinsonian model ($p < 0.0001$). The blood-gut barrier was assessed by measuring the serum levels of LPS and iFABP. 6-OHDA rats showed no change in LPS and iFABP at pre, 7 and 28 days post. Next, we assessed gut tissue for the localization and expression villin, a marker for epithelial brush border cells, and for ZO-1, a tight junction protein important for maintenance of intestinal mucosal barrier integrity. Preliminary results (n=3-4 per group) show a trend ($p < 0.1$) towards increased dysregulated expression of ZO-1 in the colon, and downregulation of ZO-1 expression in the ileum, in 6-OHDA rats compared to controls. These preliminary experiments suggest that although 6-OHDA does not cause an increase in gut leakiness using the surrogate markers LPS and iFABP, specific changes

in gut architecture at a molecular level could potentially alter other gut metabolites and the gut microbiome.
This project is supported by the John and Maurine Cox Endowed Chair.

Disclosures: C. Kaufhold: None. **A. Shaik:** None. **E. Lepe:** None. **R. Srinivasan:** None. **F. Sohrabji:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.21/N5

Topic: C.03. Parkinson’s Disease

Support: UNAM DGAPA PAPIIT IA201421
UNAM DGAPA PAPIIT IN213923
CONACyT CF6653
CONACyT CF154039
CONACyT CVU:828305
The Kavli Foundation
Graduate Program in Biological Sciences (PDCB, UNAM)
Instituto de Neurobiología (INB)
Microscopy Unit (INB)
Proteogenomics Unit (INB)

Title: Impairment of visually guided behavior in a mouse model of Parkinson's disease

Authors: *G. I. MENDOZA, L. CARRILLO-REID;
Developmental Neurobio. and Neurophysiol., Inst. de Neurobiología, UNAM, Queretaro, Mexico

Abstract: Impairment of visually guided behavior in a mouse model of Parkinson’s disease. Parkinson’s disease (PD) is an increasing neurodegenerative disorder caused by the degeneration of dopaminergic neurons in the brain. PD is characterized by motor signs such as tremor, bradykinesia, instability, and locomotion unbalance. However, PD patients frequently suffer from non-motor symptoms (NMS) such as sensory and cognitive impairments. It has been demonstrated that PD patients show visual abnormalities like degraded orientation discrimination and visual hallucinations that limit their ability to perform visually guided behaviors. Despite that there is no cure for PD, the most effective therapy that has been used to treat the motor symptoms is L-DOPA at high concentration. L-DOPA is a dopaminergic precursor that crosses the blood-brain barrier increasing dopamine levels and improving motor symptoms. It is known that a high concentration of L-DOPA induces dyskinesias in more than 60% of PD patients after years of treatment. However, the effect of a low concentration of L-DOPA in visually dependent NMS remains unknown. We used a visually guided Go/No-Go task in a mouse model of PD

(unilateral substantia nigra pars compacta lesion with 6-OHDA) to characterize the effect of a low concentration of L-DOPA in visually dependent NMS. Our experiments demonstrate that control mice learned the visually guided Go/No-task developing high rates of licking responses that are related to a high task performance (above 80%) after 20 days, whereas parkinsonian mice were unable to learn the visually guided Go/No-Go task even after 30 days of training (task performance below 20%).On the other hand, we found that parkinsonian mice administered with a low concentration of L-DOPA developed high rates of licking responses and high task performance (above 80%) similarly to control animals. Importantly, L-DOPA at low concentration didn't induce dyskinesias after 30 days of treatment. Finally, using the pupillary diameter we show that a low concentration of L-DOPA normalized pupillary responses in parkinsonian mice.Our results indicate that: 1) unilateral dopamine depleted mice are unable to learn a visually guided Go/No-Go task. 2) a low concentration of L-DOPA reverses the impairment of visually guided behavior. 3) a low concentration of L-DOPA doesn't induce dyskinesias.

Disclosures: G.I. Mendoza: None. **L. Carrillo-Reid:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.22/N6

Topic: C.03. Parkinson's Disease

Support: Center for Development and Behavioral Neuroscience at Binghamton University

Title: Motivational properties of L-DOPA in a bilateral 6-OHDA lesion rat model of Parkinson's disease

Authors: *I. DIROSA, J. MASLINSKI, E. KLAYMAN, N. LIPARI, C. BISHOP; Binghamton Univ., Vestal, NY

Abstract: Parkinson's Disease (PD) is the second most prevalent neurodegenerative disorder causing motor and non-motor dysfunction consequent in part, to the loss of dopamine (DA) neurons in the nigro-striatal pathway. The gold-standard treatment, levodopa (L-DOPA) is highly efficacious for early PD. However, when taken chronically, L-DOPA can lead to unwanted complications, including L-DOPA-induced dyskinesia (LID) and dopamine dysregulation syndrome (DDS). While DDS results in patterns of impulsive and compulsive behaviors, particularly related to L-DOPA self-administration, its underlying causes remain opaque. To gain insight into the potential relationship between LID and DDS, 19 Sprague-Dawley rats received bilateral 6-hydroxydopamine (6-OHDA) or sham lesion of the left medial forebrain bundle (MFB). To confirm a parkinsonian phenotype, we determined whether 6-OHDA or sham lesioned rats displayed significant motor impairments as assessed by the total

steps taken on the forepaw adjusting steps (FAS) test and by use of the rotarod test where rats were recorded for ability to stay on a rotating rod. Thereafter, the conditioned place preference (CPP) paradigm was used to investigate the reinforcing aspects of L-DOPA by examining if pairing L-DOPA treatment (12 mg/kg) with a specific context would lead sham or parkinsonian rats to prefer that context, interpreted as a preference for the drug (CPP). To observe LID expression, the animals were rated during an abnormal involuntary movement (AIMs) test after CPP to assess axial, limb, and orolingual (ALO) behaviors. Our central hypothesis was that 6-OHDA bilateral lesions will produce motor deficits and L-DOPA treatment will produce CPP in subjects with the greatest motor impairment and LID. Preliminary results reveal that there may be reinforcing effects of L-DOPA in both sham and parkinsonian rats and additional analyses should indicate whether LID is associated with L-DOPA preference in DA-lesioned subjects. Overall, findings from this study will provide further evidence of L-DOPA effects in PD, interrogate relationships between DDS and LID, and illuminate potential overlapping and orthogonal mechanisms that could be targeted for treatment.

Disclosures: I. DiRosa: None. **J. Maslinski:** None. **E. Klayman:** None. **N. Lipari:** None. **C. Bishop:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.23/N7

Topic: C.03. Parkinson’s Disease

Title: Intestinal barrier impairment in Parkinson’s Disease pathologies

Authors: *H. TEMPLETON[1], A. CHERWIN[2], C. HENRY[2], S. A. TOBET[3];
[1]Biomed. Sci., [2]Colorado State Univ., Fort Collins, CO; [3]Colorado State Univ., FORT COLLINS, CO

Abstract: Accumulating evidence suggests that Parkinson’s disease (PD) pathology may arise in the gut. This likely occurs through the enteric nervous system (ENS), which facilitates bidirectional communication between the brain and intestines. A hallmark of PD is neuronal accumulation of misfolded α-synuclein (aSyn) proteins that may be able to travel from the ENS to the brain via several options. However, it is still unclear how aSyn aggregation in the gut is initiated. Deterioration of gut barrier integrity may provide one key for aSyn aggregation as it is hallmark of PD patients. Specialized epithelial cells, known as goblet cells (GCs), produce and secrete mucin to create a mucosal barrier that prevents unregulated passage of luminal contents into underlying tissue. Intestinal mucosal barrier defects are associated with the development of PD. The goal of the study is to investigate intestinal barrier disruption in neuronal and aSyn phenotypes of PD in ex vivo mouse models. One class of candidates for barrier disruption is matrix metalloproteases (MMPs) among other proteases. Several can degrade collagen and MMP1 has been shown to partially cleave aSyn leading to aggregate formation. Gut bacteria

such as enterococcus faecalis can secrete MMPs into the intestinal lumen. For this study we start with a broad-spectrum bacterial collagenase to disrupt barrier integrity in ex vivo mouse intestinal tissue. Ex vivo colon tissue was exposed to collagenase for 48hr using both an organotypic slice model and a separate microfluidic barrier device model. Following collagenase exposure, lectin- and immuno- histochemistry were performed. GC mucopolysaccharides were fluorescently labeled using the lectin Ulex Europaeus Agglutinin I (UEA) conjugated to rhodamine. Collagenase treatment decreased GC number by 25% (15.5 +/- 0.5) compared to control (20.5 +/- 0.5). Mucin 2 (MUC2) is the main mucin protein of CGs in the gut. Apical MUC2-immunoreactivity (ir) decreased by 14% with collagenase treatment (51.5 +/- 17.5) compared to control (59.5 +/- 7.5). These results suggest substantial alterations in the mucosal barrier. Enteric neuronal fibers were identified via ir-peripherin. The average area of peripherin-ir fibers was decreased by 36% (5.95 +/- 0.2) after collagenase exposure compared to control (8 +/- 0.1). The area of aSyn-ir in enteric fibers was increased by 27% (1.07 +/-0.02) after collagenase exposure compared to control (1.8 +/- 0.8). These results are consistent with the hypothesis that disruptions to barrier integrity may be involved in neuronal alterations and aSyn aggregation in the gut.

Disclosures: H. Templeton: None. **A. Cherwin:** None. **C. Henry:** None. **S.A. Tobet:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.24/N8

Topic: C.03. Parkinson’s Disease

Support: DST/CSRI/2017/33

Title: The combination of probiotics and rTMS ameliorates cognitive and motor deficits in 6 hydroxydopamine induced Parkinson’s disease rats

Authors: *S. SHARMA[1], S. JANGRA[1], S. JAIN[1], R. CHAUDHRY[2], S. DAS[1], T. NAG[3], S. SINGH[3], D. M RADHAKRISHNAN[4], S. PANDEY[5], K. KOCHHAR[1];
[1]Physiol., [2]Microbiology, [3]Anat., [4]Neurol., [5]Biostatistics, All India Inst. of Med. Sci., New Delhi, India

Abstract: Non-invasive repetitive Transcranial Magnetic Stimulation(rTMS) and probiotics intervention have been demonstrated to improve motor functions and alleviate cognitive deficits in 6-OHDA rat model of PD. Despite this, it is still unknown whether both interventions together will have a synergistic effect on the gut-brain axis. Hence, in this study we have investigated the combined effect of rTMS and probiotics intervention on motor and cognitive functions, neuronal survival, expression of neurotrophic factors along with morphology of enteric nervous system in bilateral 6-OHDA rat model of PD. Adult male Wistar rats(280-320gm) underwent either sham surgery or received 2µL 6-OHDA(7.5µg/µL dissolved in 0.2% ascorbate saline) bilaterally into

striatum at coordinates (AP:-0.2;ML:±2.5;DV:-5.5). One-week post surgery, rats were divided into five experimental groups: a sham group(sham), a 6-OHDA group that received probiotics(PD+Probiotics), a 6-OHDA group that received rTMS(PD+rTMS), a 6-OHDA group that received both probiotics & rTMS(PD+Probiotics+rTMS) and a 6-OHDA without any intervention (PD). High frequency rTMS(10 Hz, 20 min/day) and probiotics mix(0.270 µL daily) were administered for 4 weeks. Pre and post-intervention motor and cognitive performances were examined by using rotarod and passive avoidance behavior, elevated plus maze, respectively. Morphology of the neurons in brain and gut was assessed by cresyl violet and hematoxylin and eosin staining, respectively. Immunohistochemistry was done to assess the expressions of tyrosine hydroxylase (TH), brain derived neurotrophic factor (BDNF), glial cell derived neurotrophic factor (GDNF). Except PD group, all groups showed improved memory ($p \leq 0.05$; PD vs all groups), motor behavior (($p \leq 0.05$; PD vs all groups) along with low anxiety (un-even number of entries in all arms in maze except PD group; $p \leq 0.05$; PD vs all groups) and improved gait. Cresyl violet staining revealed improved morphology of neurons in brain and gut after intervention. Expression of all the beneficial factors (BDNF and GDNF) enhanced along with increased TH expression. We have found that the administration of probiotics and rTMS improves the motor and cognitive functions of rats with Parkinson's disease. Plausibly, probiotics combined with rTMS may facilitate the remodelling of the gut-brain axis that improves the functions of dopaminergic neurons in PD. Thus, we suggest that the combination of probiotics and rTMS may be a promising regenerative therapeutic strategy for the treatment of PD by neuromodulating the brain-gut axis.

Disclosures: **S. Sharma:** None. **S. Jangra:** None. **S. Jain:** None. **R. Chaudhry:** None. **S. Das:** None. **T. Nag:** None. **S. Singh:** None. **D. M Radhakrishnan:** None. **S. Pandey:** None. **K. Kochhar:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.25/O1

Topic: C.03. Parkinson's Disease

Support: Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 821522
DZNE
Alexander von Humboldt foundation

Title: Alpha synuclein driven mitochondrial alterations in dopaminergic neurons of the substantia nigra

Authors: *S. A. O'SULLIVAN[1], A. ULUSOY[2], R. PINTO-COSTA[3], S. S. LEE[4], D. A. DI MONTE[2];
[1]German Ctr. for Neurodegenerative diseases, Bonn, Germany; [2]German Ctr. For

Neurodegenerative Dis. (DZNE), Bonn, Germany; [3]German Ctr. for Neurodegenerative diseases (DZNE), Bonn, Germany; [4]German center for neurodegenerative diseases, Bonn, Germany

Abstract: Parkinson's disease (PD) is characterised by progressive degeneration of discrete neuronal populations, in particular, dopaminergic (DA) cells in the substantia nigra pars compacta (SN). PD pathogenesis is also linked to accumulation and aggregation of alpha-synuclein (αSyn) within neurons of the SN as well as other brain regions. Clinical and experimental evidence supports a key role for mitochondrial dysfunction and, in particular, mitochondrial complex I impairment in the pathogenesis of sporadic and familial PD. Our aim was to investigate the relationship between αSyn accumulation and mitochondrial injury by assessing levels of key mitochondrial proteins (including complex I subunits) within nigral DA neurons in a mouse model of αSyn overexpression.To overexpress αSyn, adeno-associated viral vectors encoding for human αSyn were injected into the SN. Animals were sacrificed 4 and 12 weeks after treatment. Stereological cell counting was used to determine the effects of αSyn overexpression on nigral cell integrity/survival. For semi-quantitative assessments of mitochondrial proteins within DA neurons, quadruple immunofluorescent staining was carried out on coronal sections of the SN using antibodies against (a) tyrosine hydroxylase (TH, a marker of DA cells), (b) human αSyn, (c) Grp75 (a mitochondrial marker), and (d) GRIM19 (a complex I marker) or MTCO1 (a complex IV marker). Proximity ligation assays (PLAs) were developed and used to assess αSyn-mitochondria interactions *in-situ*.Our results show that αSyn overexpression caused the degeneration of approx. 30% of DA neurons in a time dependent manner. Semi-quantitative fluorescence measurements of mitochondrial proteins were done within surviving nigral cells, immunoreactive for both TH and αSyn. Levels of general mitochondrial markers, such as Grp75, remained unchanged throughout the 12-week period of our experiments. Quite in contrast, data revealed a significant decrease in GRIM19:Grp75 as well as MTCO1:Grp75 ratios at 12 weeks post-injection. These decreases, indicating complex I and IV losses, were not seen at the 4-week time point. A negative correlation was found between levels of neuronal αSyn expression and GRIM19:Grp75 ratio. Additionally, PLA results demonstrated interactions of αSyn with mitochondrial proteins at 12, but not 4 weeks post injection. Altogether, these findings support a relationship between αSyn accumulation and specific mitochondrial alterations in the form of loss of complex I and IV expression. αSyn-induced abnormalities require time to develop and could be due, at least in part, to a progressive burden of αSyn-mitochondria interactions.

Disclosures: S.A. O'Sullivan: None. **A. Ulusoy:** None. **R. Pinto-Costa:** None. **S.S. Lee:** None. **D.A. Di Monte:** None.

Poster

PSTR464. Parkinson's Disease: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR464.26/Web Only

Topic: C.03. Parkinson's Disease

Support: DGAPA IN216821

Title: Mitochondrial hypoactivity and ultrastructural changes in a Parkinson disease model by manganese inhalation

Authors: *C. GARCIA-CABALLERO[1], J. ORDOÑEZ-LIBRADO[2], I. HERNÁNDEZ-URBINA[2], E. MONTIEL-FLORES, Sr.[5], J. PEDRAZA-CHAVERRI[3], O. APARICIO-TREJO[6], L. GARCÍA-GONZÁLEZ[7], L. REYNOSO-ERAZO[4], M. AVILA-COSTA[2];
[1]Neuromorphology Lab., Univ. Nacional Autonoma de Mexico, Tlalnepantla de Baz, Mexico; [2]Neuromorphology Lab., UNAM, Tlalnepantla, Mexico; [3]Biol., UNAM, CDMX, Mexico; [4]Hlth. Educ. Project, UNAM, Tlalnepantla, Mexico; [5]UNIVERSIDAD ESTATAL DEL VALLE DE ECATEPEC, UNIVERSIDAD ESTATAL DEL VALLE DE ECATEPEC, México, Mexico; [6]Fisiopatología Cardio-Renal, Inst. Nacional de Cardiología, CDMX, Mexico; [7]Clin. Lab., Hosp. Infantil de México "Federico Gómez", CDMX, Mexico

Abstract: The increasing life span of the global population has brought consequently the rising incidence of Parkinson disease (PD) around the world. This represents a health problem that is growing worldwide. Despite this, the exact mechanisms responsible for neuronal injury remain not understood, whereby it is necessary to develop animal models that reproduce most features of this disorder. In our laboratory has been developed a rodent model by manganese chloride ($MnCl_2$) and manganese acetate ($Mn(OAC)_3$) which has shown a depletion of substantia nigra compacta dopaminergic neurons, the exposed animals show tremor, rigidity, and bradykinesia, these changes were gradual and bilateral consistent with what was reported in PD patients. This work aimed to determine mitochondrial IV complex activity in substantia nigra, striatum and globus pallidus. Additionally, we evaluate the ultrastructural features of mitochondria and their cristae. To achieve this, we used male CD1 mouses divided into two groups; a) mouses which inhaled deionized water (n=25) and b) mouses that inhaled ($MnCl_2$)/ ($Mn(OAC)_3$) (n=25), one hour, two times a week during five months. Our results show that Mn mixture inhalation induces hypoactivity in complex IV within the substantia nigra pars compacta. Meanwhile, manganese exposition increases the number of mitochondria and their area, decreases mitochondrial circularity, and intensifies the occurrence of damaged cristae in the three analyzed nuclei. In conclusion, the PD model by manganese mixture inhalation proves to be a valuable tool for analyzing this pathology, as it successfully reproduces certain mitochondrial injuries observed in PD patients.

Disclosures: C. Garcia-Caballero: None. **J. Ordoñez-Librado:** None. **I. Hernández-Urbina:** None. **E. Montiel-Flores:** None. **J. Pedraza-Chaverri:** None. **O. Aparicio-Trejo:** None. **L. García-González:** None. **L. Reynoso-Erazo:** None. **M. Avila-Costa:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.01/O2

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, 5 R01 ES024745-07 (MCH)
NIH/NIEHS, 5 R01 ES033462-02 (MCH)
MJFF-020697 (MCH)

Title: Characterizing the impact of microglia restricted Nlrp3 activity in a rotenone-based model of Parkinson's disease

Authors: *E. CARLONI[1], M. C. HAVRDA[2];
[1]Dartmouth Col., Hanover, NH; [2]Dartmouth Med. Sch., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Parkinson's disease (PD) is a neurodegenerative motor disorder characterized by bradykinesia, rigidity, and tremors. It affects 1% of the world population over 60 years old. Environmental factors and normal aging increase the risk of PD. Immune cells of both the central and peripheral immune systems are posited to drive the well-documented neuroinflammatory pathology observed in PD. The NLRP3 inflammasome is a protein complex that is activated in response to cellular damage and stress such as that caused by many environmental toxicants. We model PD-related exposure using the pesticide rotenone, a mitochondrial complex I inhibitor that activates the NLRP3 inflammasome and is associated with an increased risk of PD in agricultural workers. Mice systemically exposed to rotenone harbor detectable changes in peripheral immune cell phenotype and develop *Nlrp3*-dependent neuroinflammation and nigral cell loss. We do not yet know the specific contributions of peripheral and central nervous system immune cells in the development of PD symptoms in rotenone treated mice. We developed cell type specific *Nlrp3* gain-of-function models to determine whether peripheral *NLRP3* is required for the development of neuroinflammation and neurodegeneration in rotenone treated mice. We leveraged the inducible CAPS-associated hyperactive $Nlrp3^{L351PneoR}$ allele that is functionally null prior to CRE recombinase-mediated deletion of a reverse oriented neomycin cassette. We crossed these animals with $Tmem119^{em1(cre/ERT2)Gfng}$ mice on an $Nlrp3^{-/-}$ background to generate microglia-only *Nlrp3*animals. We confirmed CRE-mediated induction of *NLRP3* expression and cytokine induction in brain tissues in mice challenged with LPS and inflammation was assayed in plasma and brain tissues. Preliminary data in mice exposed to rotenone for 14 days identified behavior deficits in wild-type mice in the open field and pole test paradigms. These deficits were not observed in $Nlrp3^{-/-}$ animals consistent with our previous findings. Interestingly, no behavioral changes were observed in similarly treated $Tmem119/NLRP3^{L351P}$ animals. These initial data suggest that microglial Nlrp3 may not be sufficient to drive PD-related phenotypes associated with systemic rotenone exposure. Ongoing studies will evaluate animals at advanced age to identify behavioral, histological, and mechanistic outcomes of rotenone exposure in this model.

Disclosures: E. Carloni: None. **M.C. Havrda:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.02/O3

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, 5 R01 ES024745-07 (MCH)
NIH/NIEHS, 5 R01 ES033462-02 (MCH)
MJFF-020697 (MCH)
NIH/NCI 5P30CA023108-40 (Dartmouth Cancer Center)

Title: Function of BRI3 in CD16 monocytes in Parkinson's disease

Authors: *K. PAUL[1], O. M. WILKINS[2], K. E. BIGGS[1], T. S. TURNER[1], F. ANDERSON[1], S. L. LEE[3], F. W. KOLLING[4], D. M. KASPER[1], M. C. HAVRDA[1];
[1]Dept. of Mol. and Systems Biol., [2]Dartmouth Col. Geisel Sch. of Med., Lebanon, NH; [3]Dept. of Neurol., Dartmouth Hitchcock Med. Ctr., Lebanon, NH; [4]Genomics Shared Resource, Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease and is characterized by varying motor and non-motor symptoms. PD is associated with a chronic inflammatory state. Inflammation is easily observed in degenerating tissues of the PD central nervous system, but peripheral immune involvement and dysregulation of peripheral innate immune cells in PD remains poorly understood. Dyshomeostasis of classical and non-classical monocyte proportions and aberrant classical monocyte functioning has been described previously, but the non-classical CD16+ monocyte phenotype in PD has not been investigated. We evaluated peripheral blood mononuclear cells from three male PD patients and two healthy controls by single cell RNA-sequencing to identify a PD-specific transcriptional profile of circulating immune cells. We identified hundreds of differentially expressed genes in PD peripheral immune cells, indicative of the existence of a PD-specific transcriptomic state across multiple cell lineages. Further analysis of monocyte sub-clusters revealed non-classical CD16+ monocytes were proportionally reduced in PD. Analysis of differentially expressed genes in the CD16+ monocyte subtype identified significant elevation of *Brain Protein I3* (*BRI3*) in PD patients compared with healthy controls. We found that *BRI3* was inducible in a cultured human monocyte-like cell line stimulated towards the CD16 phenotype. *BRI3* inactivation impaired the secretion of inflammatory cytokines and altered pathways related to arginine metabolism, a metabolic program characteristic of invasive monocyte derived macrophages, a cell type found in the PD brain. More recently, we utilized CRISPR/Cas9 technologies to inactivate Bri3 in zebrafish expressing macrophage and vascular fluorescent reporters and established an *in vivo* inflammatory injury paradigm to assess macrophage function. Forward looking studies will determine the role of BRI3 in monocyte phenotype *in vitro* and *in vivo* to determine its function and whether it is an immune modulator of translational importance in PD.

Disclosures: **K. Paul:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/318,612. **O.M. Wilkins:** None. **K.E. Biggs:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/318,612. **T.S. Turner:** None. **F. Anderson:** None. **S.L. Lee:** None. **F.W. Kolling:** None. **D.M. Kasper:** None. **M.C. Havrda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 63/086,765, 63/318,612.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.03/O4

Topic: C.03. Parkinson's Disease

Title: Differences in glycosphingolipid metabolism are revealed after transient inhibition of beta-glucocerebrosidase with conduritol beta-epoxide

Authors: *M. COGSWELL, J. CLARKE, C. VIEL, R. CHIANG, H. PARK, K. RANDALL, Y. WANG, B. KIRAGASI, S. AIVAZIDIS, B. WANG, P. SARDI, C. KAYATEKIN; Sanofi, Cambridge, MA

Abstract: *GBA* mutations are the most common genetic risk factor for developing synucleinopathies including Parkinson's disease (PD). Mutations in *GBA* reduce the activity of the lysosomal enzyme beta-glucocerebrosidase (GCase) which cleaves glucosylceramide into glucose and ceramide. However, lipid substrate accumulation has not been consistently observed in either brain tissue samples collected post-mortem or in accessible biofluids from patients. These challenges make it difficult to use conventional plasma biomarkers to assess which patients might benefit from therapeutic intervention. Here, we report a novel method, the CBE challenge, to overcome these challenges and uncover defects in glycosphingolipid metabolism by transiently inhibiting GCase activity with the irreversible inhibitor conduritol beta-epoxide (CBE) and measuring the time course of sphingolipid accumulation and restoration. Since CBE is an irreversible inhibitor, this method evaluates the combined efficiency of new enzyme synthesis, trafficking to the lysosome, and catalytic activity. Our results show that the CBE challenge can discriminate between mice with and without *GBA* mutations, whereas baseline measurements cannot. Peak lipid accumulation was higher in homozygous mice ($Gba^{D409V/D409V}$) compared to heterozygous ($Gba^{D409V/+}$) and wild-type mice in the brain, plasma, and liver. The rate of substrate recovery was also dependent on genotype, with WT mice returning to normal levels the fastest. Differences in lipid flux were also detected across different WT mouse strains. C57BL/6 mice showed an increased sensitivity to GCase inhibition compared to BALB/cJ and FVB mice, demonstrating that the CBE challenge can differentiate between different genetic backgrounds without a *GBA* mutation. We also show the CBE challenge can be used to evaluate viral delivery of exogenous GCase to the brain. Lastly, our results show that the CBE challenge can be performed in healthy human PBMCs isolated from whole blood, opening the door to a clinically-transferrable assay in which patients with defects in glycosphingolipid processing can be targeted for therapeutic intervention.

Disclosures: **M. Cogswell:** A. Employment/Salary (full or part-time):; Sanofi. **J. Clarke:** A. Employment/Salary (full or part-time):; Sanofi. **C. Viel:** A. Employment/Salary (full or part-time):; Sanofi. **R. Chiang:** A. Employment/Salary (full or part-time):; Sanofi. **H. Park:** A. Employment/Salary (full or part-time):; Sanofi. **K. Randall:** A. Employment/Salary (full or part-time):; Sanofi. **Y. Wang:** A. Employment/Salary (full or part-time):; Sanofi. **B. Kiragasi:** A.

Employment/Salary (full or part-time):; Sanofi. **S. Aivazidis:** A. Employment/Salary (full or part-time):; Sanofi. **B. Wang:** A. Employment/Salary (full or part-time):; Sanofi. **P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi. **C. Kayatekin:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.04/O5

Topic: C.03. Parkinson's Disease

Support: National Medical Research Council, Singapore under the Open Fund Individual Research Grant (MOH-000559)

Title: Functional characterisation of SV2C and its variants as a Parkinson's Disease-associated gene

Authors: *C. CHANG[1,2], E. G. CHEW[2], M. TANDIONO[2], T. ZHANG[2], X. GONG[2], Y. XIA[2], J. FOO[2,3];
[1]Neuroscience, Interdisciplinary Grad. Programme, Nanyang Technological Univ., Singapore, Singapore; [2]Lee Kong Chian Sch. of Medicine, Nanyang Technological Univ. Singapore, Singapore, Singapore; [3]Genome Inst. of Singapore, Agency for Science, Technol. and Research, A*STAR, Singapore, Singapore

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta in the midbrain. Extensive effort has been devoted to identifying genetic risk factors for PD to allow for early intervention and management of PD patients. In a genome-wide association study conducted in the East Asian population (Foo et al., 2020), *SV2C* was identified as a novel risk locus for PD where its lead SNP (rs246814) tagged a missense variant (rs31244) p.Asp543Asn (D543N) in the *SV2C* gene that could potentially introduce a new N-glycosylation site (Asn-X-Ser/Thr) in the luminal domain of the encoded synaptic vesicle glycoprotein. SV2C was reported to be highly expressed in the midbrain and the loss of SV2C in mice showed reduced dopamine release in the striatum. However, the exact function of SV2C in dopaminergic neurons of the midbrain is still unclear. This study aims to characterise the functional effect of *SV2C* and its variants in human stem cell-derived midbrain dopaminergic (mDA) neurons. *SV2C* gene was first knocked out in H9 human embryonic stem cells and the differentiated SV2C-KO mDA neurons formed neuronal-like projections and were positive for mature midbrain markers TH and NURR1, suggesting that the loss of SV2C did not affect the direct differentiation of mDA neurons. Due to the heterogeneity of cells upon differentiation, a midbrain reporter H9-PITX3-mCherry knock-in cell line was generated to enrich the population of mDA neurons for functional characterisation. CRISPR/Cas9-mediated site-specific mutagenesis will also be performed to generate *SV2C* D543N variant in H9 cells and the N-glycosylation of SV2C will be

investigated. Ongoing functional assessment of SV2C-KO and SV2C-D543N mDA neurons will include measuring the dopamine release and synaptic vesicle (SV) trafficking using a fluorescent SV marker synapto-pHlourin. These data would give insights into the role of *SV2C* as a PD risk gene and its contribution to PD pathogenesis.

Disclosures: C. Chang: None. **E.G. Chew:** None. **M. Tandiono:** None. **T. Zhang:** None. **X. Gong:** None. **Y. Xia:** None. **J. Foo:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.05/O6

Topic: C.03. Parkinson's Disease

Support: Deutsche Forschungsgemeinschaft (German Research Foundation)

Title: Role of FBXO7(PARK15) in the synaptic integrity of olfactory bulb and characterization of the FBXO7 interactome

Authors: *S. GAGNEJA[1,2], V. SARIK[2], J. STEGMÜLLER[2];
[1]RWTH Aachen Univ., Aachen, Germany; [2]Neurol., RWTH Univ. Hosp., Aachen, Germany

Abstract: Parkinson's disease(PD) is characterized by multiple symptoms including motor and non-motor dysfunctions. F-box only protein 7(FBXO7) is encoded by *PARK15* and is a subunit of the SCF(SKP1/Cullin-1/F-box protein) E3 ubiquitin ligase that plays an important role in the ubiquitin proteasome system. Mutations in FBXO7 protein cause Parkinsonian Pyramidal syndrome(PPS) which is an autosomal recessive juvenile form of parkinsonism. Among the non-motor symptoms, hyposmia shows the highest prevalence in PD. Our research focuses on the synaptic alterations in the olfactory bulb by using an established parkinsonism mouse model in which PARK 15 is deleted from specific neurons. In addition,we investigate the effects of FBXO7 in the functioning of vesicle fusion protein MUNC 18-1(mammalian uncoordinated 18-1) also known as STXBP1(Syntaxin-binding protein 1),which is a crucial protein for exocytosis at synaptic level. We hypothesize to find changes in the synaptic proteome of the olfactory bulb and that FBXO7 affects the function of MUNC 18-1.

Disclosures: S. Gagneja: None. **V. Sarik:** None. **J. Stegmüller:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.06/O7

Topic: C.03. Parkinson's Disease

Title: Molecular identification of candidate modulators changing in Parkinson's Disease mouse models

Authors: *M. GRAZIANO[1], I. MANTAS[2], Y. MASARAPU[3], S. FRAPARD[3], S. GIACOMELLO[3], K. MELETIS[1];
[1]Karolinska Inst., Stockholm, Sweden; [2]Karolinska Institutet, Stockholm, Sweden; [3]KTH Royal Inst. of Technol., Stockholm, Sweden

Abstract: Molecular identification of candidate modulators changing in Parkinson's Disease mouse models Marta Graziano[1], Ioannis Mantas[1], Yuvarani Masarapu[2], Solène Frapard[2], Stefania Giacomello[2], Konstantinos Meletis[1]
[1] Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden[2]Science for Life Laboratory, Department of Gene Technology, KTH Royal Institute of Technology, Stockholm, Sweden
Parkinson's disease (PD) is induced by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Nevertheless, little is known about how molecular signals, including neuromodulators, are altered in the prodromal phase and their role at the circuit or behaviour level. To address this, we aimed to characterize the molecular events in mouse models of prodromal PD. We have used spatial transcriptomics and single-nucleus RNA sequencing in two models of the disease: a) a transgenic model with human alpha-synuclein overexpression (SNCA-OVX mice), and b) a model with mild unilateral striatal dopamine depletion after low dose 6-hydroxydopamine (6-OHDA) injection in the medial forebrain bundle (MFB). We have found primarily a dysregulation of activity-dependent genes and immediate early genes in the striatum such as Nr4a1, Rgs2 and Egr1. We have validated the downregulation of candidate genes in striatum (e.g. Nr4a1) and we confirm these findings with single-nucleus RNA sequencing in another mouse model of PD.

Disclosures: M. Graziano: None. **I. Mantas:** None. **Y. Masarapu:** None. **S. Frapard:** None. **S. Giacomello:** None. **K. Meletis:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.07/O8

Topic: C.03. Parkinson's Disease

Support: NIH Grant F31NS124269

Title: Phosphodiesterase 9 regulation of dopamine signals in striatal neurons.

Authors: *B. KOCHOIAN[1], T. YABUMOTO[2], S. COLETTA[2], C. BURE[2], S. M. PAPA[2]; [1]Emory Univ., Decatur, GA; [2]Emory Univ., Atlanta, GA

Abstract: BACKGROUND: Striatal phosphodiesterases (PDEs) regulate selectively the cyclic nucleotides, cAMP and/or cGMP, second messengers involved in dopaminergic signaling cascades of striatal projection neurons (SPNs). Previous work has revealed decreased striatal expression levels of the cyclic nucleotides in models of Parkinson's Disease (PD). PDE isotypes have different substrate specificity and tissue distributions and may be selectively targeted in order to regulate DA signaling in SPNs. PDE9 has substrate affinity for cGMP and is highly expressed in the striatum. To understand the impact of PDE9 function on striatal SPN responses to DA, we assessed the effect of PDE9 inhibition directly on the SPN activity in the non-human primate (NHP) model of PD, focusing on advanced parkinsonism. METHODS: An MPTP-treated macaque with severe parkinsonism was surgically implanted with a recording chamber, and the activity of single SPNs was recorded before and after local microinjection of a selective PDE9 inhibitor (PDE9-I). Our approach was to analyze firing changes of SPNs with continuous recordings from the OFF state to 1 min and 5 min after local PDE-I injection, to 20 min following a systemic L-DOPA administration ('ON' state onset) and to the peak of L-DOPA action ('ON' state peak). RESULTS: In this severely parkinsonian animal, the activity of SPNs consistently displayed aberrant activity, firing at higher-than-normal frequencies. We found that inhibition of PDE9 in the striatum induced SPN activity increases and decreases resembling L-DOPA actions. DISCUSSION: Previous work from our lab has demonstrated that systemic co-administration of PDE9-I (2.5 to 7.5 mg/kg) and L-DOPA (threshold and suboptimal doses) prolongs the therapeutic action of L-DOPA ("ON" state). In the analysis of neuronal effects, SPN firing frequencies changed after the local injection of PDE9i with a similar response as that induced by L-DOPA. Our findings from our analysis of SPN activity following microinjection of a PDE9-I may help demonstrate the mechanism for the antiparkinsonian effects of PDE9-Is that we have previously observed.

Disclosures: B. Kochoian: None. **T. Yabumoto:** None. **S. Coletta:** None. **C. Bure:** None. **S.M. Papa:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.08

Topic: C.03. Parkinson's Disease

Support: 2022 Nu Rho Psi Undergraduate Research Grant
2022 Parkinson's Foundation Grant

Title: Insight into Synucleinopathies: Comparative Evaluation of Wild-Type a-, b-,and g-Synucleins in a Yeast Model

Authors: *I. MOONLIGHT, R. OSSELBORN, F. BERTOLOTTI, T. NASSUNA, M. CAMBA ALAMBAN, S. GACEK, S. DEBBURMAN;
Lake Forest Col. Neurosci., Lake Forest, IL

Abstract: Synucleinopathies are a group of neurodegenerative disorders linked with the misfolding and aggregation of α-synuclein, the most well-known among them being Parkinson's disease (PD). α-Synuclein belongs to a larger family of proteins that include β- and *γ*;-synuclein. Mutant forms (P123H and V70M) of β-synuclein have been shown to cause Dementia with Lewy Bodies (DLB). However, the extent to which β- and *γ*;-synuclein are neurotoxic is still highly understudied compared to α-synuclein. While specific alterations in cellular environments (including nitrative stress, lysosomal degradation, mitochondrial dysfunction) and post-translational modifications alter the toxicity and aggregation of α-synuclein, less is known of their impacts on β- and *γ*;-synuclein. Here, we used our lab's budding yeast (*Saccharomyces cerevisiae*) model to comparatively evaluate these three wild-type synucleins and explore their pathological potential through the assessment of toxicity, localization, and expression. We report that: 1) Both wildtype α- and β-synuclein are differentially toxic in control yeast strains, whereas *γ*;-synuclein is non-toxic. 2) Expression levels of these proteins are tied to extent of aggregation and cytotoxicity observed. 3) Toxicity potential of all synucleins are impacted to varying degrees by specific synucleinopathy-related altered cellular environments in genetically altered yeast strains. 4) β-synuclein, when expressed in budding yeast, displays a higher molecular weight than expected. This study adds to a growing body of research exploring disease association of the synuclein family of proteins and illustrates the importance of further evaluation of β-synuclein's role in neurodegeneration.

Disclosures: I. Moonlight: None. **R. Osselborn:** None. **F. Bertolotti:** None. **T. Nassuna:** None. **M. Camba Alamban:** None. **S. Gacek:** None. **S. DebBurman:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.09/P1

Topic: C.03. Parkinson's Disease

Support: 2023 ASBMB Undergraduate Research Award
2023-2024 Nu Rho Psi Undergraduate Research Grant

Title: Insight into synucleinopathies: Molecular dissection of β- and γ-Synuclein for potential toxicity in a yeast model

Authors: *F. BERTOLOTTI, T. NASSUNA, R. OSSELBORN, I. MOONLIGHT, H. A. KIERNAN, L. CASARES, S. F. GACEK, S. DEBBURMAN;
Neurosci., Lake Forest Col., Lake Forest, IL

Abstract: The family of synucleinopathies is linked to the misfolding and aggregation of proteins within the family of synucleins (α-, β-, and γ-), including Parkinson's Disease (PD), the second most prevalent neurodegenerative disease. While α-synuclein is well-studied for its direct contribution to PD, less is known about the role in neurodegeneration and toxicity potential of β- and γ-synuclein. Two β-synuclein mutants (P123H and V70M) were recently linked with Dementia with Lewy Bodies (DLB), and recently, γ-synuclein inclusions were reported observed with ALS pathology. Lessons from α-synuclein pathogenicity demonstrate that it is not only enhanced by point mutations within it, but modified by post-translational modifications and altered cellular environments linked with mitochondrial dysfunction, altered lysosomal pathways, nitrative stress, and lipid metabolism. Here, we further evaluated the toxicity potential of β- and γ-synuclein in these various neurodegeneration-related cellular environment strains, using our *Saccharomyces cerevisiae* (budding yeast) PD model system. Additionally, we evaluated substitution mutants for V70M and P123H β-synuclein, where the original amino acid was mutated to representatives of all four amino acid classes (A, R, N, E), for whether loss of the original amino acid (V70, P123) or gain of the new mutant (70M, 123H) is key to toxicity. Finally, we swapped several known familial mutations in α-synuclein and β-synuclein onto each other and onto γ-synuclein as assessed their toxicities. We report that: 1) V70M and P123H β-synuclein mutants aggregate and are more toxic than WT β-synuclein, in a strain- and expression-dependent manner. 2) Evaluation of β-synuclein substitution mutants demonstrates that the gain of histidine is key to P123H-β-synuclein toxicity. 3) The V70M β-synuclein mutation when swapped into α-synuclein makes the latter differentially more toxic than swapping in the P123H mutation. 4) WT and mutant β-synuclein toxicities are differentially aggravated by altered nitrative stress. Overall, this study expands the evaluation of β- and γ-synuclein, as well as genetically modified mutants in a yeast model, to understand mechanisms of toxicity for these two nervous system proteins and illuminates mutant toxicity in β-synuclein.

Disclosures: F. Bertolotti: None. **T. Nassuna:** None. **R. Osselborn:** None. **I. Moonlight:** None. **H.A. Kiernan:** None. **L. Casares:** None. **S.F. Gacek:** None. **S. DebBurman:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.10/P2

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS038065
NIH Grant NS086074
NIH Grant NS092093
NIH Grant NS108686

Title: Integrated Stress Response in alpha synucleinopathy

Authors: *C. FANG[1], D. JANGIR[1], P. GRIFFIN[1], M. KARIM[1], J. MEINTS[1], E. TIEGS[1], S. MORE[2], M. LEE[1,3];
[2]Ctr. for Drug Design, [1]Univ. of Minnesota, Minneapolis, MN; [3]Inst. for Translational Neurosci., Minneapolis, MN

Abstract: Abnormalities in α-synuclein (αS) is directly linked to the pathogenesis of Parkinson's disease (PD) and related disorders called α-synucleinopathies. We showed that α-synucleinopathy in a αS transgenic mouse model (TgA53T) and humans causes chronic endoplasmic reticulum stress (ERS)/unfolded protein response (UPR)/Integrated Stress Response (ISR). Treatment of TgA53T with salubrinal, an inhibitor eIF2α dephosphorylation, can significantly delay onset of αS pathology and motor deficits. Thus, we further studied the mechanistic relationships between of eIF2α phosphortulation and α-synucleinopathy. First, we examined if the loss of eIF2α phosphorylation by the Protein kinase R-like ER Kinase (PERK) in neurons exacerbates α-synucleinopathy. We show that conditional deletion of PERK in neurons of TgA53T accelerates the onset of α-synucleinopathy. Further, loss of PERK was associated with increased severity of the disease, including the levels of phosphor-Ser129 αS (pS129αS). The result show the pathologic importance of PERK-eIF2α pathway in α-synucleinopathy. Because Salubrinal inhibits both the protein phosphatase 1 regulatory subunit 15A (PPP1R15A, Gadd34) and PPP1R15B (CReP), we tested the pathological importance of each of these phosphatases independently. While inhibition of Gadd34 is neuroprotective multiple models of neurodegeneration, inclusing motor neuron disease and multiple sclerosis, we show that neither the pharmacological inhibition of Gadd34, using Guanabenz and Sephin-1, or genetic loss Gadd34 function attenuated α-synucleinopathy in TgA53T model. To test the role of CReP, we treated the TgA53T mouse model with Raphin1, a selective inhibitor of CReP. Treatment of TgA53T model with an Raphin1 significantly delays disease onset. Further, while pathology at end stage was not affected by Raphin1, analysis of intermediate stages show that Raphin1 treatment leads to decrease in pS129αS and neurodegeneration. The neuroprotective effects Raphin1 as Raphin1 treatment reduces pS129αS in neuronal cells via activation of autophagy and attenuates αS preform fibril induced neurotoxicity. Our data show that PERK-eIF2α pathway is an important pathological axis for α-synucleinopathy and different phosphatases are differentially involved in various neurodegenerative conditions. Moreover, we show that ISR components, particularly inhibition of CReP, is a therapeutic target for α-synucleinopathy.

Disclosures: C. Fang: None. **D. Jangir:** None. **P. Griffin:** None. **M. Karim:** None. **J. Meints:** None. **E. Tiegs:** None. **S. More:** None. **M. Lee:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.11/P3

Topic: C.03. Parkinson's Disease

Title: Age-related vulnerability of nigral dopaminergic neurons to Zn^{2+} influx through GluR2-lacking AMPA receptors

Authors: *A. TAKEDA, S. NAKAJIMA, R. NISHIO, H. TAMANO;
Univ. of Shizuoka, Shizuoka, Japan

Abstract: We have reported that H_2O_2 is produced by 6-hydroxydopamine (6-OHDA) taken up through dopamine transporters in the SNpc, is retrogradely transported to presynaptic glutamatergic terminals, activates the transient receptor potential melastatin 2 (TRPM2) channels, accumulates glutamate in the extracellular compartment, and induces intracellular Zn^{2+} dysregulation via AMPA receptor activation, resulting in nigral dopaminergic degeneration prior to movement disorder. Here we compared vulnerability to neurodegeneration after exposure to NMDA and AMPA. Apomorphine-induced movement disorder and dopaminergic degeneration in the SNpc, which are associated with Parkinson's syndrome, were induced after injection of AMPA into the SNpc of rats, but not after injection of NMDA. Co-injection of 1-naphthyl acetyl spermine (NASPM), a selective blocker of Zn^{2+}-permeable GluR2-lacking AMPA receptors rescued dopaminergic degeneration and increase in intracellular Zn^{2+} by AMPA. Next, we tested the effect of capturing reactive oxygen species (ROS) produced by Zn^{2+} on neuroprotection in vivo. The levels of ROS, which were determined by HYDROP, a membrane-permeable H_2O_2 fluorescence probe and Aminophenyl Fluorescein (APF), a fluorescence probefor hydroxyl radical and peroxynitrite, were increased after injection of AMPA, but not after co-injection of CaEDTA, an extracellular Zn^{2+} chelator, suggesting that increase in Zn^{2+} influx by AMPA elevates the levels of intracellular ROS. AMPA-mediated dopaminergic degeneration was completely rescued by co-injection of either HYDROP or APF. The present study indicates that neurotoxic signaling of the influx of extracellular Zn^{2+} through GluR2-lacking AMPA receptors is converted to ROS production and that capturing the ROS completely protects dopaminergic degeneration after exposure to AMPA, but not NMDA. It is likely that regulation of the conversion from Zn^{2+} influx into ROS production plays a key role to preventing Parkinson's syndrome. Furthermore, we used a low dose of AMPA, which does not increase extracellular Zn^{2+} influx in the SNpc of young adult rats. When AMPA (1 mM) was injected at the rate of 0.05 μl/min for 20 min into the SNpc, intracellular Zn^{2+} level was increased in the SNpc of aged rats followed by increase in turning behavior in response to apomorphine and nigral dopaminergic degeneration. In contrast, young adult rats do not show movement disorder and nigral dopaminergic degeneration. Intracellular Zn^{2+} dysregulation, which is induced by GluR2-lacking AMPA receptor activation, may be accelerated in the SNpc of aged rats, resulting in age-related vulnerability to Parkinson's syndrome.

Disclosures: **A. Takeda:** None. **S. Nakajima:** None. **R. Nishio:** None. **H. Tamano:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.12/P4

Topic: C.03. Parkinson’s Disease

Support: ASAP#P38300054

Title: The Puzzle of Parkinson's Disease: Spatial proteomics identifies common pathobiology preceding degeneration

Authors: *D. SHONAI[1], J. KENT[4], E. J. SODERBLOM[2], S. SODERLING[3];
[1]Mol. Genet. and Microbiology, [2]Proteomics and Metabolomics Core Facility, [3]Cell Biology, Neurobio., Duke Univ., Durham, NC; [4]Cell Biol., Duke university, Durham, NC

Abstract: Parkinson's disease (PD) is characterized by the selective loss of dopamine neurons (DANs) in the Substantia nigra pars compacta (SNc) region of the midbrain. Missense mutations found in familial PD cases suggest that DAN-specific degeneration is associated with intracellular protein trafficking abnormalities. However, our understanding of how these missense PD-risk mutations alter protein distributions within DANs, leading to selective cell death, remains limited.In this study, we developed a novel approach to investigate the subcellular spatial proteomes of DANs in living brains. Our strategy combined cell-type specific expression of TurboID, an engineered biotin ligase, with the in vivo Localization of Organelle Proteins by Isotope Tagging after Differential ultracentrifugation (in vivo LOPIT-DC) method. This unique combination allowed us to capture temporal transitions in protein distribution across organelles in DANs, providing insights into the unique pathobiology underlying progressive degeneration in PD.By applying this method to three different PD model mice (Tg-α-Synuclein A30P/A53T, Tg-LRRK2 G2019S, and VPS35 D620N Knock-in models), we, for the first time, revealed significant protein mislocalization in all three PD model mice as early as 1 month old, preceding the reported manifestation of PD-related phenotypes. These mislocalized proteins are enriched in biological pathways involved in PD pathology, further supporting the feasibility of our strategy. Furthermore, comparative analysis of the spatial proteomics data from the three PD models identified alterations in the proteasomal and calcium-dependent signaling pathways as early events in DANs.Our findings suggest that alterations in these biological pathways may potentially precede DAN-specific neurodegeneration in PD and could serve as common upstream factors across different forms of PD. These insights provide valuable evidence for shared underlying biology among different PD subtypes and highlight the potential of these altered pathways to serve as early indicators of pathological changes in sporadic PD. Understanding the early molecular events contributing to DAN-specific degeneration opens new avenues for targeted therapeutic interventions. It paves the way for developing diagnostic tools to detect PD at early stages.

Disclosures: D. Shonai: None. **J. Kent:** None. **E.J. Soderblom:** None. **S. Soderling:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.13/P5

Topic: C.03. Parkinson's Disease

Support: NIA Intramural Research Program

Title: Single-cell spatial transcriptomics reveal cell type-specific phenotypic changes in a mouse model of alpha-synucleinopathy

Authors: L. HORAN-PORTELANCE[1], M. IBA[1], J. DING[2], R. GIBBS[2], M. COOKSON[3], *E. MASLIAH[1];
[1]Lab. of Neurogenetics, Mol. Neuropathology Unit, [2]Lab. of Neurogenetics, Computat. Biol. Group, [3]Lab. of Neurogenetics, Cell Biol. and Gene Expression Section, Natl. Inst. on Aging, Bethesda, MD

Abstract: With the advent of single-cell-resolution *in situ* transcriptomic technologies, identifying, phenotyping, and targeting specific cell types in specific brain areas involved in disease progression is feasible and practical. Here, we used these technologies to gain an understanding of spatially-dependent transcriptional changes occurring in neurons and glia in Parkinson's disease (PD). We used a mouse model of PD which overexpresses human α-synuclein under the neuronal Thy1 promoter ("Line 61"). These mice are well-characterized as developing extensive α-synuclein pathology, microgliosis, astrogliosis, T-cell recruitment, and neurodegeneration. Prior bulk and microglia-specific RNAseq experiments have revealed complex changes to pathways implicated in neuronal homeostasis as well as to immune-related signaling cascades. We utilized two spatial technologies, 10x Genomics' Xenium, and Nanostring's CosMx, with their standard mouse brain panels (Xenium: 248 genes; CosMx: 950 genes), to analyze FFPE brain tissues from α-synuclein transgenic mice and non-transgenic control animals. Both platforms provided robust *in situ* identification of transcripts, providing high data quality, as measured by number of transcripts and number of unique genes detected per cell. Both platforms allowed for cell typing and clustering using both supervised and unsupervised methods, and mapping these cell types and clusters onto the brain morphology enabled visualization of the composition of different brain regions by cell type, including the hippocampus and laminal structure of the neocortex. Finally, differential gene expression analysis revealed cell type-specific expression changes in neuronal and glial pathways relevant to neurodegeneration and neuroinflammation in PD, shedding light on spatially-dependent phenotypic changes occurring in the proximity of α-syn-burdened cells. In conclusion, single-cell *in situ* transcriptomic technologies represent a promising approach for studying neurodegenerative disease in mouse and human models.

Disclosures: L. Horan-Portelance: None. **M. Iba:** None. **J. Ding:** None. **R. Gibbs:** None. **M. Cookson:** None. **E. Masliah:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.14/P7

Topic: C.03. Parkinson's Disease

Support: NIH NINDS R35 NS122257
NIH NIA F30 AG066333
NIH NINDS K99 NS109252
NIH NINDS R00 NS109252
DFG SCHW 866/6-1
DFG SCHW 866/7-1

Title: Vps13c regulates lysosomal dynamics and function in human dopaminergic neurons

Authors: *L. F. SCHROEDER[1,2], W. PENG[1], Y. C. WONG[1], M. SCHWAKE[2], D. KRAINC[1]; [1]Neurol., Northwestern Univ. - Feinberg Sch. of Med., Chicago, IL; [2]Biochem. III/Faculty of Chem., Bielefeld Univ., Bielefeld, Germany

Abstract: Rare homozygous and compound heterozygous mutations in Vacuolar Protein Sorting 13 Homolog C (VPS13C) have been linked to early-onset Parkinson's disease. Patients with VPS13C loss-of-function mutations presented clinically with rapid disease progression, early cognitive decline, severe neuronal loss in the substantia nigra and diffuse Lewy Body disease. While VPS13C has been previously studied in non-neuronal cells, the neuronal role of VPS13C in disease-relevant human dopaminergic neurons has not been elucidated. Here, we use human iPSC-derived dopaminergic neurons to investigate the role of VPS13C in regulating lysosomal dynamics and function using live-cell microscopy. VPS13C-deficient dopaminergic neurons exhibited significantly enlarged lysosomes and increased inter-lysosomal contacts, leading to impaired lysosomal motility and cellular distribution. These enlarged lysosomes further demonstrated lysosomal dysfunction including defective lysosomal hydrolytic activity, acidification, and an impaired lysosomal stress response upon loss of VPS13C. Our work highlights an important role of VPS13C in regulating lysosomal homeostasis.

Disclosures: L.F. Schroeder: None. **W. Peng:** None. **Y.C. Wong:** None. **M. Schwake:** None. **D. Krainc:** F. Consulting Fees (e.g., advisory boards); Vanqua Bio, The Silverstein Foundation, Intellia Therapeutics, AcureX Therapeutics.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.15/P8

Topic: C.03. Parkinson's Disease

Support: JST SPRING, Grant Number JPMJSP2108 (L.X.)
KAKENHI 19H01015 (T.T.)
KAKENHI 23H00394 (T.T.)
AMED 22dm0207072 (T.T.)

Grants-in-Aid for Scientific Research (C), Grant number 17K08265, 21K06558 (G.I.)

Title: Comprehensive identification of proximal proteins and functional analysis of Rab29

Authors: *L. XU[1], G. ITO[2], T. TOMITA[1];
[1]Lab. of Neuropathology and Neuroscience, Grad. Sch. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Biomolecular Chemistry, Fac. of Pharma-Science, Teikyo Univ., Tokyo, Japan

Abstract: Parkinson disease (PD) is a major neurodegenerative disorder characterized by bradykinesia, resting tremor and muscle rigidity. PD is pathologically characterized by selective degeneration of dopaminergic neurons in the substantia nigra and the formation of intracytoplasmic inclusions called Lewy bodies. Genome-wide association studies (GWAS) in sporadic PD have identified single nucleotide polymorphisms (SNPs) in the PARK16 locus that are associated with the risk of developing PD. Some of these SNPs are located in the putative Rab29 promoter region and are predicted to alter transcription factor binding sites. Collectively, Rab29 has been suggested to play an important role in the pathogenesis of PD. The Rab29 protein is a member of the Rab family, which is known to be involved in the regulation of intracellular vesicle trafficking. However, it is not clear how Rab29 is involved in the pathogenesis of PD. In this study, we comprehensively identified proximal proteins of Rab29 and investigated how they affect the cell biological properties of Rab29.For this purpose, we used TurboID, a promiscuous biotin ligase. TurboID fused to Rab29 at its amino terminus (TurboID-Rab29) was expressed in cultured cells to label Rab29 proximal proteins with biotin. Biotinylated proteins were isolated using streptavidin beads and identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Rab29 proximal proteins contained numerous endoplasmic reticulum (ER)- and Golgi-resident proteins, suggesting that Rab29 is involved in vesicular trafficking between ER and Golgi. Consistent with this, Rab29 localized to the ER and Golgi in fluorescent immunocytochemistry. The majority of these proteins are known to be involved in ER-Golgi vesicle trafficking, suggesting that changes in Rab29 expression levels may affect ER-Golgi vesicle trafficking, leading to neurodegeneration in PD.

Disclosures: L. Xu: None. **G. Ito:** None. **T. Tomita:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.16/P9

Topic: C.03. Parkinson’s Disease

Support: NIH grant NINDS R56NS115767
NIH grant RF1NS115767

Title: Role of Rab27b in lysosomal function and α-synuclein clearance

Authors: *R. PATTANAYAK[1], K. SCHOLZ[2], T. A. YACOUBIAN[3];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Alabama, Birmingham, Hoover, AL; [3]Univ. of Alabama At Birmingham, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Alpha synuclein (α-syn) is one of the key proteins implicated in Parkinson's disease (PD)and Dementia with Lewy bodies (DLB), two common neurodegenerative disorders whichlack therapies to slow neurodegeneration. Prion-like spread of α-syn from cell to cell hasbeen implicated in neurodegeneration, yet little is known about the mechanismsunderlying the release and uptake of α-syn between cells. Rab proteins are smallGTPases that play critical roles in vesicular trafficking and have been implicated insynucleinopathies. We previously identified Rab27b as a potential regulator of α-syntransmission. Rab27b protein levels were increased in postmortem human brain lysatesfrom PD and DLB subjects compared to healthy controls. A doxycycline-inducibleneuroblastoma line (called isyn line) was created that, upon doxycycline treatment,induces α-syn overexpression and consequent secretion of α-syn that is toxic toseparately cultured cells. Upon shRNA-mediated knockdown (KD) of Rab27b in thismodel, we observed increased α-syn-mediated paracrine toxicity and the release of highmolecular weight α-syn.In this study, we investigated the role of Rab27b in α-syn clearance and lysosomal activity.We observed a significant increase in α-syn in lysosomal fractions from isyn cells in whichRab27b was knocked down compared to non-target controls, while total levels of α-synwere not altered. Given the increase in α-syn within lysosomal fractions, we thenmeasured lysosomal function in isyn cells with and without Rab27b KD. Isyn cells withRab27b KD showed significant reductions in lysosomal acid lipase and cathepsin Dactivity compared to control isyn cells. Isyn cells with Rab27b KD showed reducedlysosomal degradation as measured by the DQ-BSA assay. Similar deficits in lysosomalfunction were observed in primary hippocampal neurons from Rab27b knockout mice.Rab27b knockout mice also demonstrated defects in anterograde lysosomal trafficking inaxons.We next tested the effect of Rab27b overexpression (OE) in isyn cells. Rab27b OEreduced α-syn paracrine toxicity. Levels of Triton-X100 soluble α-syn was also reduced inisyn cells upon Rab27b OE. Rab27b OE also increased autophagic flux, as those cellsdisplay more robust LC3II buildup than control cells when treated with theautophagosome-lysosome fusion inhibitor chloroquine. Preliminary results also showRab27b OE also resulted in a dramatic increase in DQ-BSA degradation activity. Basedon these results, we conclude Rab27b enhances lysosome trafficking and function to promote α-syn clearance. Our findings highlight Rab27b as potential target for therapeuticintervention in synucleinopathies.

Disclosures: R. Pattanayak: None. **K. Scholz:** None. **T.A. Yacoubian:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.17/P10

Topic: C.03. Parkinson's Disease

Support: NIH NINDS R56NS115767
NIH NINDS RF1NS115767
NIGMS 5T32GM135028-02

Title: Role of Rab27a in Alpha-Synuclein pathology

Authors: *K. J. SCHOLZ[1], T. A. YACOUBIAN[2];
[1]Univ. of Alabama, Birmingham, Birmingham, AL; [2]Univ. of Alabama At Birmingham, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Parkinson's Disease is a neurodegenerative disorder in which the key pathogenic protein alpha-synuclein (asyn) is hypothesized to promote neurodegeneration by aggregating and spreading between cells. There are currently no disease-altering therapies available for this devastating disease, and while the spread of asyn is critical to disease progression, little is known about the mechanism of its propagation. One candidate protein involved in asyn propagation is Rab27a. Rab GTPases are a large family of proteins with several members implicated in PD, and we have both found that Rab27a is expressed in PD-relevant areas of the brain and shown via proximity ligation assay that it interacts with asyn. While we previously demonstrated that knockdown (KD) of its sister isoform, Rab27b, results in impaired autophagy and exacerbated asyn pathology, we surprisingly found that knockout (KO) of Rab27a is protective against asyn pathology in primary neurons treated with pre-formed asyn fibrils (PFFs). In this model, neurons are treated with PFFs and then stained for phosphorylated, toxic asyn (psyn). We have found that Rab27a-KO neurons accumulate less psyn than wild-type (WT) controls upon PFF treatment. Since Rab27a promotes endocytosis in non-neuronal cells, we hypothesized that KO of Rab27a could reduce the uptake of asyn and thus ameliorate pathology. Preliminary data indicate that KO of Rab27a reduces uptake of dextran in primary neurons, pointing to reduced endocytosis in Rab27a-KO neurons. We are currently examining the uptake of asyn PFFs in Rab27a-KO neurons. We are additionally testing the effects of Rab27a-KO *in vivo*: Rab27a-KO and WT mice were injected bilaterally with PFFs into the striatum, and we are currently assessing psyn inclusion formation and dopaminergic cell loss at 6-months-post injections. Like other GTPases, Rab27a acts through effector proteins, and we have identified the nonclassical Rab27 effector Coronin1C (Coro1c) as an additional potential player in asyn uptake. Coro1c works with Rab27a to promote endocytosis in non-neuronal cells. We have determined that Coro1c levels are elevated in the temporal cortical lysates from human PD subjects compared to age-matched controls. Additionally, KD of Coro1c in an asyn-overexpressing neuroblastoma line reduces the amount of asyn in the cell lysate while increasing the amount of asyn in the conditioned culture media, indicating a potential reduction in uptake. Our findings so far suggest that both Rab27a and its effector Coro1c may play a role in cell-to-cell transmission of asyn in PD.

Disclosures: K.J. Scholz: None. **T.A. Yacoubian:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.18/Q1

Topic: C.03. Parkinson's Disease

Support: ASAP Grant 02663614

Title: Exploring the effect of ATP13A2 loss of function mutation: unraveling astrocytic changes and the significance of the methylation pathway in Parkinson's Disease

Authors: *E. COCCIA[1], G. PARFITT[1], T. MEIMOUN[1], S. SOHAIL[1], T. AHFELDT[2], P. VANGHELUWE[3], J. BLANCHARD[1];
[1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Recursion, Salt Lake City, UT; [3]Dept. of Cell. and Mol. Med., KU Leuven, Leuven, Belgium

Abstract: Parkinson's disease (PD) is a multifaceted neurodegenerative disorder characterized by the loss of dopaminergic neurons (DN) in the *substantia nigra*. Even though neurodegeneration is cell type-specific, it is becoming clear that other cell types, such as astrocytes, are also altered and have an essential contribution to the pathology. Around 5% of PD cases are caused by mutations of specific genes, which have been used to help uncover the mechanisms underlying the disease, whereas 95% of Parkinson's disease has unknown origins. A rare genetic form of early-onset familial PD arising from loss-of-function (LOF) of *ATP13A2* implicates lysosomal polyamine transport in PD pathogenesis, but the precise mechanism remains elusive. Here, we investigated the impact of ATP13A2 LOF mutation in human pluripotent stem cell-derived midbrain astrocytes.We observed that ATP13A2 loss of function induced key PD hallmarks, including increased neuroinflammatory markers, lysosomal dysfunction, and a-synuclein accumulation in midbrain astrocytes. With co-cultures and conditioned media experiments, we show that ATP12A2 LOF astrocytes are selectively toxic to DN, and not to other types of neurons. ATP13A2 LOF causes polyamines to be trapped in the lysosomal space. This leads to the upregulation of *de novo* polyamine synthesis which depletes the ubiquitous methyl donor SAM. We, therefore, hypothesized a link between ATP13A2 LOF and alterations in the cell methylation pathway and the epigenetic state of midbrain astrocytes. Strikingly, ATAC-seq analysis revealed an increase in open chromatin regions and potential transcription factors associated with pathological phenotypes, such as BHLHE40. To target the shared pathway between polyamine synthesis and methylation, we employed a specific inhibitor and demonstrated its potential to rescue some of the pathological phenotypes caused by ATP13A2 mutation. Overall, our developed in vitro model recapitulates hallmark signatures of PD, providing a platform for future studies and screens to elucidate and target PD pathogenesis mechanisms. Moreover, our findings provide insights into the underlying mechanisms of ATP13A2 mutation-induced astrocytic dysfunction and its impact on neurons. Altered polyamine levels and DNA and histone methylation have been observed in sporadic PD patients. Our results demonstrate that decreased cytosolic polyamine levels cause epigenetic reprogramming of midbrain astrocytes to an inflammatory state that is selectively cytotoxic to DNs. These results provide new mechanistic insights and highlight new therapeutic opportunities in early-onset and potentially late-onset PD.

Disclosures: E. Coccia: None. **G. Parfitt:** None. **T. Meimoun:** None. **S. Sohail:** None. **T. Ahfeldt:** None. **P. Vangheluwe:** None. **J. Blanchard:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.19/Q2

Topic: C.03. Parkinson’s Disease

Support: Bolashak Grant for D.Sharipov

Title: A vicious cycle of karyopherin abnormalities propagates neurodegenerative synucleinopathy in Parkinsonism and Dementia with Lewy Bodies

Authors: *D. SHARIPOV[1], E. BERECZKI[2], R. KILLICK[1], T. HORTOBAGYI[1], C. TROAKES[1], D. AARSLAND[1], F. HIRTH[1];
[1]King's Col. London, London, United Kingdom; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Parkinson’s Disease (PD), Dementia with Lewy Bodies (DLB), and Parkinson’s Disease Dementia (PDD) are synucleinopathies characterised by the accumulation and aggregation of alpha-synuclein (aSyn). aSyn is an intrinsically disordered protein prone to accumulation either via deposition of pathologic oligomeric and prefibrillar beta-sheets; or via condensation by liquid-liquid phase separation (LLPS), which eventually result in irreversible aggregates that are detectable as Lewy bodies and Lewy neurites. Previous studies showed that LLPS is associated with karyopherins, mediating nucleocytoplasmic cargo transport, and acting as disaggregases against misfolding proteins. Karyopherin abnormalities have also been implicated in synucleinopathies, but their role in disease formation remains enigmatic.
Using a multi-disciplinary approach, we analysed proteomics data of human post-mortem brain tissue of DLB, PDD and Alzheimer's Disease (AD) patients. Furthermore, we established new human cell culture and *Drosophila* models that accumulate aSyn in either wildtype or PD-related mutant A30P form to determine the levels, localisation, and effect on karyopherins.
Our analyses identified significant alterations in the expression levels of karyopherins in post-mortem brain tissue of PDD and DLB patients that were not detected in AD nor controls. We observed aSyn accumulation and aggregation in the cytoplasm and nucleus, and an altered nucleocytoplasmic ratio in post-mortem tissue of the investigated cases versus age-matched controls. Moreover, karyopherins were depleted from the cytoplasm and accumulated in the nucleus in PD, PDD and DLB, with abnormal nuclear colocalisation with monomeric aSyn. New *Drosophila* models of synucleinopathy demonstrated that accumulating aSyn caused alterations in levels and localisation of karyopherins and progressive motor impairment that was exacerbated by A30P mutant aSyn. In addition, human SH-SY5Y cell experiments revealed that both, accumulating wildtype and A30P mutant aSyn formed spontaneous intracellular aggregates that sequestered and mislocalised karyopherin. This pathogenic process was accelerated in the presence of prefibrillar aSyn.
Our findings demonstrate the pathological accumulation of nuclear aSyn accompanied by alterations of karyopherins in PD, PDD and DLB. Given their functions as nuclear transporter receptors and disaggregases, the observed karyopherin abnormalities suggest a vicious cycle of

protein alterations that propagate aSyn pathology during the onset and progression of neurodegenerative synucleinopathies.

Disclosures: **D. Sharipov:** None. **E. Bereczki:** None. **R. Killick:** None. **T. Hortobagyi:** None. **C. Troakes:** None. **D. Aarsland:** None. **F. Hirth:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.20/Q3

Topic: C.03. Parkinson's Disease

Support: 1R01NS124735-01A1

Title: The SATB1-MIR22-GBA axis mediates glucocerebroside accumulation inducing a cellular senescence-like phenotype in dopaminergic neurons

Authors: ***T. RUSSO**[1], B. KOLISNYK[2], A. BS[1], T. KIM[3], J. MARTIN[1], J. PLESSIS-BELAIR[1], J. NI[2], J. A. PEARSON[4], E. J. PARK[5], R. SHER[1], L. STUDER[3], M. RIESSLAND[1]; [1]Stony Brook University, Neurobio. & Behavior, Stony Brook, NY; [2]The Rockefeller Univ., New York, NY; [3]Mem. Sloan-Kettering Cancer Ctr., New York, NY; [4]Stony Brook University, Med. Scientist Training Program, Stony Brook, NY; [5]Baylor Col. of Med., Houston, TX

Abstract: Idiopathic Parkinson's Disease (PD) is characterized by the loss of dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc) of the midbrain, which is associated with neuroinflammation and reactive gliosis. The underlying cause of PD and the co-occurring neuroinflammation is not well understood. Previously, we identified transcription factor Special AT-Rich Sequence-Binding Protein 1 (SATB1) as a genetic master regulator as playing a neuroprotective role specifically in nigral DA neurons. Additionally, *SATB1* has been identified as a genetic risk factor for PD. We recently showed that knockout of SATB1 triggers p21-dependent cellular senescence specifically in post-mitotic DA neurons. Despite p21 elevation being sufficient to induce senescence, knockdown of p21 could not fully rescue the senescence phenotype in SATB1 knockout (KO) cells, suggesting the contribution of an additional pathway. Interestingly, we also observed a significant downregulation of the critical lysosomal membrane protein β;-glucocerebrosidase (GCase), which is encoded by *GBA*, in our SATB1-KO models. *GBA*, the most frequent genetic risk factor for PD, encodes the GCase enzyme that cleaves the β;-glucosidic linkage of glucosylceramides (GluCers). Both age- and PD-dependent downregulation of the levels and activity of GCase have been observed. Herein, we use human and mouse neuronal lines, stem cell-derived dopaminergic neurons, and mice to show that three previously identified genetic risk factors for PD, SATB1, MIR22HG, and GBA, are part of one gene regulatory pathway. We show that dysregulation of this pathway causes the upregulation of glucocerebrosides (GluCers), which triggers a cellular senescence-like phenotype in dopaminergic neurons. Our data reveal that downregulation of the transcriptional repressor

SATB1 de-represses the micro-RNA miR-22-3p which in turn decreases GBA expression and eventually leads to accumulation of GluCers. Importantly, we show that an increase of GluCers is sufficient to impair lysosomal and mitochondrial function and thereby induce S100A9- and stress-dependent cellular senescence. Our results show that dysregulation of the herein identified SATB1-MIR22-GBA pathway, as observed in PD patients as well as in normal aging, causes lysosomal and mitochondrial impairment via GluCers accumulation and induces a cellular senescence-like phenotype in dopaminergic neurons. Thus, our findings offer not only a novel pathway involving three genetic risk factors for PD, but also a potential mechanism for the observed senescence-triggered neuroinflammation and reactive gliosis seen in both PD and normal aging.

Disclosures: **T. Russo:** None. **B. Kolisnyk:** None. **A. Bs:** None. **T. Kim:** None. **J. Martin:** None. **J. Plessis-Belair:** None. **J. Ni:** None. **J.A. Pearson:** None. **E.J. Park:** None. **R. Sher:** None. **L. Studer:** Other; A scientific cofounder and paid consultant of BlueRock Therapeutics Inc., A scientific cofounder of DaCapo Brainscience.. **M. Riessland:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.21/Q4

Topic: C.03. Parkinson’s Disease

Support: 1R01NS124735-01A1

Title: Microglial deficiency of mitochondrial protein FAM49B as a model for inflammaging

Authors: *J. MARTIN[1], M. RIESSLAND[2];
[1]Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY; [2]Stony Brook Univ., Stony Brook University, Neurobio. & Behavior, Stony Brook, NY

Abstract: Mitochondrial dysfunction has been implicated as an integral player in the pathogenesis of Parkinson’s Disease (PD). One known genetic risk factor for PD is *Family with sequence similarity 49 member B (FAM49B)*, which encodes for the mitochondria-localized protein, FAM49B. Using a computational approach, we have found that PD-associated mutations in *FAM49B* are particularly vulnerable in microglia, cells that function as part of our innate immunity in the brain. We seek to investigate the role of FAM49B in mitochondria, and provide more insight as to how a genetic mutation in *FAM49B* can lead to mitochondrial dysfunction and downstream age-related inflammation (inflammaging) in order to understand it’s pathogenesis in PD. To do this, we ablate FAM49B in mouse and human microglia cells in culture and study its effects on mitochondrial dynamics using assays that directly measure parameters including energy production, accumulation of reactive oxygen species (ROS), and mitochondrial membrane potential. Doing so will allow us to pinpoint the exact localization of FAM49B in the

mitochondria, which in turn will shed light on its role and function in inflammaging, and may help lead to specialized treatments for patients with *FAM49B*-associated PD.

Disclosures: J. Martin: None. **M. Riessland:** None.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.22/Q5

Topic: C.03. Parkinson's Disease

Support: NINDS R01NS112203

Title: Hanging with the cool kids: how the 14-3-3 interactome changes in response to phosphorylation in neurodegenerative disorders

Authors: *S. PAIR[1], T. A. YACOUBIAN[2];
[1]Neurol., Univ. of Alabama, Birmingham, Birmingham, AL; [2]Neurol., Univ. of Alabama Birmingham, Birmingham, AL

Abstract: Our lab has shown a significant role for 14-3-3 proteins in Parkinson's Disease (PD) and Dementia with Lewy Bodies (DLB). 14-3-3 proteins are involved in many different neuronal functions including protein folding, axon growth, and apoptosis. Previous work from our lab demonstrated a protective role for 14-3-3s in several PD models, including neurotoxicant and alpha-synuclein (αsyn) models. A key gap in our understanding is why 14-3-3 proteins lose their protective effects in disease. When studying human PD and DLB brain samples, we found a significant increase in 14-3-3θ phosphorylation at S232 in PD and DLB insoluble brain fractions compared to age-matched controls. S232 phosphorylation was inversely correlated with cognitive function and positively correlated with pathologic severity. 14-3-3s mediate their cellular functions via protein-protein interactions (PPIs) and serve as major interactome hubs. In this study, we examined the effects of S232 phosphorylation on PPIs in the brain using affinity-purified mass spectrometry (AP-MS). We recently created novel conditional knock-in (KI) mice expressing either a phosphomimetic S232D or a non-phosphorylatable S232A 14-3-3θ. After immunoprecipitation of S232A or S232D 14-3-3θ from cortical brain lysates, we used LC mass spectrometry to determine changes in 14-3-3θ PPIs. After filtering for protein abundance, non-specific binding, and consistent sample presence there were over 190 proteins that bound differentially to our mutant 14-3-3 proteins. We found 20 proteins that showed a ≥50% reduction in pulldown with S232D compared to S232A. Among the top hits, we found the S232D mutant had reduced interactions with critical transport proteins, including cytoplasmic dynein, nuclear distribution protein nudE-like 1 (Ndel1), and Huntington Associated Protein 1 (HAP1). Pathway/network analysis revealed alterations in several major cellular networks, in particular, axonal and vesicular transport. To confirm our findings, we performed live-cell imaging of LysoTracker-positive acidic vesicles in primary hippocampal neurons from Cre control and

S232D mice. We observed a reduction in vesicular transport velocity in anterograde and retrograde directions. S232D neurons also showed increased pausing and direction switching in the retrograde direction. Impaired transport of degradative organelles due to 14-3-3θ phosphorylation could enhance αsyn pathology. We are currently examining the impact of 14-3-3θ phosphorylation on αsyn, lysosome, and autophagosome transport. Our findings suggest 14-3-3θ phosphorylation may be a promising target for future PD and DLB therapies.

Disclosures: S. Pair: None. **T.A. Yacoubian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patent NO7,919,262 on the use of 14-3-3s in neurodegeneration.

Poster

PSTR465. Cellular Mechanisms of Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR465.23/Q6

Topic: C.03. Parkinson’s Disease

Support: NINDS R01NS112203
Parkinson's Association of Alabama Scholar
Diversity Supplement R01NS112203

Title: Role of 14-3-3 phosphorylation in microglial activation

Authors: *W. J. STONE, H. AMARAL, T. YACOUBIAN;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Parkinson’s Disease (PD) is the second most common neurodegenerative disorder, affecting more than 6 million people worldwide. Our lab has generated data delineating the molecular hub protein, 14-3-3θ, as a key mediator of the pathologic mechanisms of PD, revealing aberrant phosphorylation of S232 on 14-3-3θ (pS232) in models of PD, and in human PD brains. We have shown 14-3-3θ pS232 blocks the protective effects of 14-3-3θ in PD models. *What promotes 14-3-3θ phosphorylation in PD is unclear*. A common pathologic feature of PD is chronic neuroinflammation marked by increased microglial activation. Microglia are brain resident phagocytes that uptake and are activated by alpha-synuclein (α-syn), a key protein in PD pathogenesis. When this process goes awry, α-syn accumulates, misfolds, and aggregates, resulting in microglial activation and dopaminergic neuron degeneration. Interestingly, 14-3-3s are known regulators of autophagosomal activities, as well as signaling pathways important to microglial inflammatory processes. Thus, **we hypothesize that 14-3-3θ S232 phosphorylation modulates microglial activation and neuroinflammation in PD**. Here, we stimulated immortalized murine microglial cells (BV2) and primary murine microglia with the pro-inflammatory molecules lipopolysaccharide (LPS) and interferon-gamma (IFN-γ) and assessed 14-3-3θ pS232 levels via western blotting. Stimulation with LPS and IFN-γ resulted in a significant time-dependent increase in 14-3-3θ pS232 levels in both BV2 cells and primary

microglia. Furthermore, visualization of 14-3-3s in primary microglia via confocal microscopy revealed altered immunostaining following stimulation with LPS, shifting localization of 14-3-3s from diffuse cytoplasmic staining to a more punctate form. Additionally, these puncta appear to colocalize with the cluster of differentiation molecule 11B (CD11b), suggesting possible involvement in adhesion processes and uptake of complement-coated molecules. Finally, we inhibited 14-3-3s in primary microglia with the non-peptide pan14-3-3 inhibitor, BV02, which binds all isoforms of 14-3-3s and prevents protein-protein interactions. We found that 14-3-3 inhibition results in a significant increase in lysosomal proteolysis, as measured by fluorescent intensity produced upon proteolytic cleavage of DQ-Red BSA. These data support our hypothesis that 14-3-3θ modulates the activation of microglial and resulting neuroinflammation in PD. Continuing to elucidate the mechanisms of 14-3-3 involvement in microglial activation will allow us to move closer toward understanding how to target novel avenues for disease-modifying therapies.

Disclosures: **W.J. Stone:** None. **H. Amaral:** None. **T. Yacoubian:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.01/Q7

Topic: C.03. Parkinson’s Disease

Support: County Council of Västerbotten
Lars Hierta memorial foundation
Åhlen-foundation
Research foundation for Clinical Neuroscience at Umeå university hospital
Umeå university medical faculty foundations (Insamlingsstiftelsen)

Title: N-acetylcysteine as a possible therapeutic treatment to restore dopamine levels and prevent neuroinflammation in Parkinson’s disease

Authors: R. EL-HABTA, P. LORENZON, S. AF BJERKÉN, ***A. VIREL**;
Umea Univ., Umea, Sweden

Abstract: Parkinson’s disease is a neurodegenerative disorder characterized by degeneration of the nigrostriatal dopamine system.The etiology of the disease is thought to be multifactorial and oxidative stress and chronic neuroinflammation has been proposed as one of the causes triggering dopamine-cell degeneration. Current treatments for Parkinson's disease aim to restore dopamine levels in the striatum or slow down the loss of dopamine cells, but they are not fully effective. Antioxidants have shown as a promising treatment to combat oxidative stress in neurological diseases. Recently, the antioxidant N-acetylcysteine (NAC), a precursor of glutathione, has been found to be beneficial in neurodegenerative diseases, including Parkinson's

disease. Previously, we had shown that NAC treatment promotes metabolic recovery, increases glutathione levels, and modulates the availability of dopamine transporter in the brains of rats with hemiparkinsonism induced by 6-hydroxydopamine (6-OHDA). However, the specific molecular targets and pathways through which NAC influences the damaged dopamine system are not yet well understood. To address this knowledge gap, we have examined the potential restorative effects of NAC on both the 6-OHDA hemiparkinsonian rat model and SH-SY5Y cells exposed to 6-OHDA. Our results demonstrate that NAC improves motor performance in hemiparkinsonian rats and prevents the toxic effects of 6-OHDA on key proteins involved in dopamine metabolism. Moreover, we observed that NAC increased dopamine synthesis and altered the kinetics of dopamine release. We also observed that NAC had an anti-inflammatory effect on the hemiparkinsonian rat brain. Overall, our study provides evidence for the beneficial effects of NAC on the injured dopamine system and sheds light on its potential molecular targets. These findings support further exploration of NAC as a potential therapeutic intervention for Parkinson's disease.

Disclosures: **R. El-Habta:** None. **P. Lorenzon:** None. **S. af Bjerkén:** None. **A. Virel:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.02/Q8

Topic: C.03. Parkinson’s Disease

Support: CarboCode Germany GmbH

Title: Neuroprotective effects of different gangliosides in cell culture and the MPTP mouse model of Parkinson's disease

Authors: *S. ZAFAR[1], W. XU[2], S. KORTAGERE[2], J. S. SCHNEIDER[1];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Parkinson's disease (PD) is a slowly progressive neurodegenerative disorder. Current therapies for PD improve motor symptoms but no treatment has been approved as a disease modifying therapeutic for PD. Previous work showed a deficiency in GM1 ganglioside as well as other brain gangliosides in the PD brain and that GM1 administration is neuroprotective in animal models of PD. There were also positive results in a GM1 clinical proof of concept disease modification trial in PD. All preclinical and clinical work with GM1 to date has been performed using either bovine (B-GM1) or porcine (P-GM1) brain-derived GM1. Naturally occurring GM1 exists as complex mixtures of molecular species, with the two most abundant forms of GM1 being the d18 and d20 forms (based on the sphingosine structure). In an effort to move away from use of animal brain-derived GM1, the current studies were performed using human-specific synthetic GM1d18 and GM1d20 to examine whether there are any differences in signaling properties *in vitro* or neuroprotective efficacy *in vivo* of these two main molecular species of

GM1 in comparison to either B-GM1 or P-GM1. Neuro2a cells (N2a; (ATCC® CCL-131™) were cultured and differentiated and following differentiation, gangliosides (50μM) or vehicle were added to the incubation medium (50μM) for 48 hrs. The presence of TrkA, p-TrkA, ERK1/2 and p-ERK1/2 (known signaling mechanisms influenced by GM1) were assessed by Western blotting and bands were quantified and normalized against GAPDH. All gangliosides showed an enhanced and similar ability to stimulate Erk phosphorylation compared to vehicle. B-GM1, P-GM1 and GM1d18 similarly increased TrkA phosphorylation while GM1d20 appeared slightly more effective but not significantly so. These same gangliosides were assessed for *in vivo* neuroprotective efficacy in the mouse MPTP model of PD. MPTP was administered to male C57Bl6 mice by subcutaneous injection twice daily, 4 hrs. apart, for 5 days. After the last MPTP injections, animals were randomly assigned to a ganglioside treatment, administered once daily for 14 days. All gangliosides showed neuroprotective efficacy (based on striatal dopamine levels and substantia nigra cell counts). P-GM1 was slightly more effective than B-GM1 or GM1d18, and GM1d20 was modestly more effective than GM1d18, but not significantly so. Studies with other brain gangliosides are in progress and will be discussed. However, data suggest that different molecular species of GM1 for the most part behave similarly in functional assays *in vitro* and *in vivo.* This information may have important implications for the continued development of synthetic GM1 for a variety of clinical applications.

Disclosures: S. Zafar: None. **W. Xu:** None. **S. Kortagere:** None. **J.S. Schneider:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.03/R1

Topic: C.03. Parkinson’s Disease

Title: Neuroprotective Effect of Methyl Jasmonate in MPP+ and SH-SY5Y Cellular Model of Parkinson’s disease

Authors: *J. ADEWALE ADEDIJI[1], D. E. BABATUNDE[2], G. O. OMOTOSO[3], O. A. OLAJIDE[4];
[1]Ctr. for Training Community Hlth. Officers, Univ. of Benin Teaching Hosp., Benin City, Nigeria; [2]Dept. of Anat., Bowen Univ., Iwo, Nigeria; [3]Dept. of Anat., Univ. of Ilorin, Ilorin, Nigeria; [4]Sch. of Applied Sci., Univ. of Huddersfield, Huddersfield, United Kingdom

Abstract: The most common neurodegenerative movement disorder is Parkinson’s disease (PD) which has neuroinflammation, oxidative stress and apoptosis as its hallmark. Compounds and mechanisms that regulate these hallmarks are great targets for PD therapy. Methyl Jasmonate (MJ) has been conferred with anti-inflammatory, antioxidant, and anti-apoptotic properties, but its role in the SH-SY5Y model of PD is unknown. This study investigates the neuroprotective role of methyl jasmonate on SH-SY5Y neuronal cells stimulated with l-methyl-4-

phenylpyridinium (MPP+) neurotoxin. The objectives of the study were to: (i) investigate the neuroprotective effects of MJ on l-methyl-4-phenylpyridinium (MPP+)-induced neurotoxicity in SH-SY5Y cells; and (ii) evaluate the mechanisms by which MJ exerts these neuroprotective effects. SH-SY5Y neuronal cells were pre-treated with MJ for 30 minutes and stimulated with MPP+ for 24 hours. The treatment groups are: (A) control (dimethyl sulfoxide); (B) MPP+ (100µM); (C) MJ (5µM) plus MPP+ (100µM); (D) MJ (10µM) plus MPP+ (100µM); (E) MJ (20µM) plus MPP+ (100µM); and (F) MJ (40µM) plus MPP+ (100µM). Cell viability and intracellular reactive oxygen species (ROS) assay was carried out. Levels of heme-oxygenase-1 (HO-1), NAD(P)H-oxidoreductase-quinone-1 (NQO-1), Caspase 3, B-cell lymphoma 2 (Bcl-2), bcl-2-associated protein X (BAX) were measured. DNA binding assay and Immunofluorescence assay were done to investigate the activation of nuclear factor-erythroid-2-related factor 2 (Nrf2). One-way analysis of variance and *post hoc* Tukey's test were used for statistical analysis at $p<0.05$. The findings of the study showed that MJ significantly ($p<0.05$): (i) inhibited MPP+-Induced neurotoxicity and improved viability of SH-SY5Y neuronal cells; (ii) reduced MPP+-Induced ROS production; (iii) inhibited MPP+-Induced HO-1 and NQO-1 suppression; (iv) inhibited MPP+-Induced suppression of Nrf2; (v) reduced MPP+-Induced Caspase 3 expression; (v) reduced MPP+-Induced BAX expression, but increased MPP+-Induced Bcl-2 suppression in SH-SY5Y cells. The study concluded that methyl jasmonate protected SH-SY5Y cells against (MPP+)-induced oxidative stress, and apoptosis via HO-1/NQO-1/Nrf2 pathway.

Disclosures: J. Adewale ADEDIJI: None. **D.E. Babatunde:** None. **G.O. Omotoso:** None. **O.A. Olajide:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.04/R2

Topic: C.03. Parkinson's Disease

Title: Neuroprotective effect of Emblica Officinalis extract on rotenone induced neurotoxicity in SHSY5Y cells and animal model

Authors: *R. K. AMBASTA;
Biotech., Delhi Technological Univ., Delhi, India

Abstract: Neuroprotective effect of *Emblica officinalis* extract on rotenone induced neurotoxicity in SH-SY5Y cells and animal model.Rashmi K. Ambasta[##]Guest Faculty, Department of Biotechnology, Molecular Neuroscience and Functional Genomics Laboratory, Delhi Technological University (*Former Delhi College of Engineering*), Delhi-110042
Background: Oxidative stress and mitochondrial dysfunction are the major cause of toxic events leading to movement disorders and has been identified as an objective for therapeutic intervention. This study investigates the neuroprotective effect of natural compounds against rotenone induced neurotoxicity.**Method:** SH-SY5Y neuroblastoma cell lines were treated with a

gradient of rotenone and neuroprotective effects of several natural products were screened. We have screened natural compounds for MTT assay, antioxidant assay and protein expression analysis. We further did an extensive analysis of various biomolecules on rotenone-induced Parkinson's rodent model and confirmed the efficacy of flavanones and other compounds including *Emblica officinalis*.**Result:** Rotenone treated cell death was reduced by *E. officinalis* extract in a dose and time dependent manner indicating potent neuroprotective effect and reversal in rotenone induced neurotoxicity. Rotenone treatment increases the levels of ROS which is reduced by *E. officinalis* extract and other biomolecules application. Rotenone treatment increases the apoptotic markers caspase 3 and 9 as well as decreases neuroprotective markers like Parkin, DJ1, Hsp70 while rotenone with *E. officinalis* and other plant extract application are proved to be neuroprotective under exposure of environmental toxin.**Conclusion:** Therefore, we summarize that *E. officinalis* and other biomolecules' extract has a neuroprotective effect on rotenone induced neurotoxicity in neuronal cells as well as in the *in vivo* model.Key words: Parkinson's disease; *Emblica officinalis*; Environmental toxin; neuroprotection; *in vivo* model; SH-SY5Y cells

Disclosures: R.K. Ambasta: None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.05/R3

Topic: C.03. Parkinson's Disease

Support: Innovative Areas JSPS KAKENHI (Grant No. JP19H05767A02)

Title: Analysis of disruption of intracellular iron homeostasis and mitochondrial dysfunction associated with ATPase Cation Transporting 13A2 deficiency

Authors: *T. MURAKAMI[1], K. OHUCHI[1], H. KURITA[1], K. KAWAI[1], T. HIRAYAMA[1], H. NAGASAWA[1], Z. WU[2], Y. MAEKAWA[2], I. HOZUMI[1], M. INDEN[1];
[1]Gifu Pharmaceut. Univ., Gifu-shi / Gifu-ken, Japan; [2]Gifu Univ., Gifu-shi/Gifu-ken, Japan

Abstract: Background and Purpose: ATP13A2 has been reported as a causative gene of familial Parkinson's disease, PARK9. ATP13A2 is an ATPase localized in lysosome and is thought to be involved in the membrane transport of cations such as polyamines, proton, and metal ions. Furthermore, it has been reported that iron accumulation is observed in the brains of PARK9 patients, suggesting that ATP13A2 contributes to the maintenance of intracellular iron homeostasis. However, the effects of ATP13A2 deficiency on iron homeostasis and the mechanism leading to neurodegeneration are still unknown. In this study, we attempted to elucidate the mechanism of intracellular iron increase. **Methods:** We generated PARK9 model cells by ATP13A2 knockdown in human neuroblastoma SH-SY5Y and analyzed iron levels, expression changes of iron-related genes, and function of cell organelles using PARK9 model

cells. Iron kinetics was examined using atomic absorption spectroscopy and iron probes. Real-time PCR and Western blotting were used to analyze changes in expression of iron-related genes and proteins. Extracellular flux analyzer was used to evaluate mitochondrial function. **Results and Discussion:** In PARK9 model cells, we observed iron uptake in mitochondria and lysosomes. Furthermore, expression of transport system of iron influx such as Transferrin Receptor (TfR) was increased, suggesting the disruption of iron homeostasis. The suppression of iron influx from TfR by competitive inhibition decreased iron levels and improved cell viability. We also analyzed mitochondrial function using an extracellular flux analyzer and confirmed a decrease in mitochondrial respiration. The ability to synthesize heme was also decreased in PARK9 model cells. Since heme is synthesized in mitochondria, it is assumed that mitochondrial disorders are involved in the reduction of heme synthesis capacity. Heme is known to play a role in regulating intracellular iron concentration. Therefore, it is possible that mitochondrial dysfunction may be a factor in the abnormal intracellular iron homeostasis in PARK9 model cells. These results may contribute to the elucidation of the pathogenesis of Parkinson's disease and other neurodegenerative diseases that exhibit iron deposition, as well as to the development of drug targets.

Disclosures: **T. Murakami:** None. **K. Ohuchi:** None. **H. Kurita:** None. **K. Kawai:** None. **T. Hirayama:** None. **H. Nagasawa:** None. **Z. Wu:** None. **Y. Maekawa:** None. **I. Hozumi:** None. **M. Inden:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.06/R4

Topic: C.03. Parkinson’s Disease

Support: Ministry of Science and Technology of Taiwan (MOST 111-2320-B-039-044-MY3)

Title: The neuroprotective mechanism of mesenchymal stem cells overexpressing FGF21 in the MPP^+-induced Parkinson’s disease cellular model

Authors: Y.-Y. LIN[1], D.-M. CHUANG[2], ***S.-Y. HUNG**[1];
[1]China Med. Univ., Taichung, Taiwan; [2]NIH, Bethesda, MD

Abstract: Parkinson's disease (PD) is the most prevalent movement disorder and the second most common neurodegenerative disorder following Alzheimer's disease. The underlying cause of PD is dopaminergic neuronal death in the substantial nigra of the midbrain, resulting in a dopamine deficiency in the striatum. Studies have indicated that both mesenchymal stem cells (MSCs) and fibroblast growth factor 21 (FGF21) hold therapeutic promise for treating PD. In the current investigation, we utilized the conditioned medium of mouse MSCs overexpressing FGF21 (MSCs-mCherry-FGF21) to treat a cellular model of PD induced by MPP^+. In the MPP^+-

induced PD cellular model, the conditioned medium of MSCs-mCherry-FGF21 demonstrated inhibition of mitochondrial reactive oxygen species accumulation compared to the control conditioned medium from MSCs-mCherry. Furthermore, the conditioned medium of MSCs-mCherry-FGF21 exhibited increased levels of phospho-Akt, Bcl-2, and BDNF, along with neuroprotective effects against MPP^+-induced neurotoxicity in dopaminergic cells SH-SY5Y. These findings suggest that MSCs-mCherry-FGF21 possess therapeutic potential for treating PD. However, further well-designed animal studies on PD are required to confirm and validate these results.

Disclosures: Y. Lin: None. **D. Chuang:** None. **S. Hung:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.07/R5

Topic: C.03. Parkinson’s Disease

Support: Brain Korea 21 FOUR Project for Medical Science, Yonsei University

Title: Neuroprotective effect of mesenchymal stem cell derived exosome through autophagy modulation in a parkinsonian model

Authors: *Y. KIM[1,2], J. SHIN[2], S. CHUNG[3], H. NA[2], H. SHIN[4], E. KIM[5], O. BANG[5], P. LEE[2]; [1]Yonsei Univ. Grad. Sch., Seoul, Korea, Republic of; [2]Dept. of Neurology, Yonsei Univ. Col. of Medicine, Seoul, South Korea, Seoul, Korea, Republic of; [3]Dept. of Neurology, Yongin Severance Hospital, Yonsei Univ. Hlth. System, Yongin, South Korea, yongin, Korea, Republic of; [4]Dept. of Neurology, Chung-Ang Univ. Col. of Med., Seoul, Korea, Republic of; [5]S&E bio Co., Ltd., Seoul, Korea, Seoul, Korea, Republic of

Abstract: Parkinson’s disease (PD) is chronic neurodegenerative disease characterized by selective loss of dopaminergic neurons and the presence of Lewy bodies composed mainly of α-synuclein in the substantia nigra. Although the initial triggering factors of PD remain unknown, ample evidence has shown that autophagy machinery is an important role in the pathogenesis of PD. Mesenchymal stem cell (MSC) is one of candidates for therapeutic strategy in PD considering their ability to modulate α-synuclein related microenvironments via pleiotropic effects. However, there are obstacles concerning about a low survival rate of transplanted cells, which would make it difficult for researchers to control for cell viability and differentiation. Recently, MSC-derived exosomes have been proposed as a promising therapeutic alternative due to therapeutic competence for regeneration and ability to maintain the therapeutic benefit of their origin cells without the risks associated with stem cell-based therapy. In the present study, we used preformed fibrils (PFFs) treated neuronal cell model and stereotaxic delivery of adeno-associated viral vectors encoding the human wild-type α-synuclein (AAV-α-synuclein) in the midbrain. Then, we investigated whether MSC-derived exosome would enhance autophagy and

exert a neuroprotective effect through the modulation of a-synuclein in parkinsonian models. As expected, coculture with MSCs increased cellular viability and attenuated expression of a-synuclein in PFFs treated neuronal cells. Similarly, treatment with exosomes increased neuronal viability and decreased expression of a-synuclein in PFFs treated neuronal cells, which was accompanied by increased the number of LC3-II positive autophagosomes compared with cells treatment with PFF-only group. In AAV-α-synuclein virus animal model, intraventricular administration of exosome reduced the expression of α-synuclein and increased LC3-II positive autophagosomes in the midbrain compared to the control group, which led to led to pro-survival effect on dopaminergic neurons. These results suggest that MSC-derived exosome treatment significantly enhances autophagy flux and may improve a-syn clearance in parkinsonian model, which may lead to increased neuronal survival in parkinsonian models.

Disclosures: Y. kim: None. **J. Shin:** None. **S. Chung:** None. **H. na:** None. **H. Shin:** None. **E. Kim:** None. **O. Bang:** None. **P. Lee:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.08/R6

Topic: C.03. Parkinson’s Disease

Support: NIA Grant F99/K00 - F99AG068509-02

Title: Investigating the mesenchymal stromal cell proteome response to synucleinopathy: A mutant methionyl-tRNA synthetase-based toolkit

Authors: *D. AMERNA[1], J. D. BURGESS[1], E. S. NORTON[2], T. M. PARSONS[1], R. B. PERKERSON[1], A. H. FAROQI[1], Z. K. WSZOLEK[3], H. GUERRERO CAZARES[2], T. KANEKIYO[1], M. DELENCLOS[1], P. J. MCLEAN[1];
[1]Neurosci., [2]Neurosurg., [3]Neurol., Mayo Clin., Jacksonville, FL

Abstract: Synucleinopathies are characterized by neuropathological aggregations of α-synuclein that disrupt neuronal networks and lead to neurodegeneration. However, no therapeutics currently address this issue and therefore do not provide disease-modifying benefits. Mesenchymal stromal cells (MSCs) demonstrate therapeutically promising neuroregenerative effects through bioactive factors secreted in response to their environment, yet current methods to characterize this dynamic proteome as it responds to specific disease states are limited. This study therefore aims to harness the regenerative capacity of MSCs towards synucleinopathy therapeutics by identifying factors that may target α-synuclein-induced pathology. Here we validate a mutant methionyl-tRNA synthetase ($MetRS^{L274G}$)-based toolkit to selectively profile secreted proteins from induced pluripotent stem cell (iPSC)-derived MSCs (iMSCs) when cultured with genetic and/or α-synuclein pre-formed fibril-induced iPSC-derived neuron synucleinopathy models. A bio-orthogonal non-canonical amino acid tagging-based pipeline

specifically isolates the proteome from iMSCs expressing MetRS^{L274G}, eliminating both proteins from co-cultured cells and confounding media components. Isolation of enriched factors in the conditioned media from this co-culture identified several differentially secreted factors between control vs pathological models, including decreased expression of heterogeneous nuclear ribonucleoprotein A1 (~0.4-fold change, p= .005) and increased expression of Aconitase 1 and Brain-specific Angiogenesis Inhibitor 1 (~1.3-fold, p= .038 and ~2.2-fold, p= .024 changes respectively) across escalating models of synuclein pathology. Further data analysis is ongoing and may identify additional key targets and pathways of interest. Overall, we demonstrate a MetRS^{L274G}-based toolkit for specifically isolating the MSC secretome from a complex, multicellular environment. This will enable future studies to better understand MSC-mediated neuroregenerative dynamics and support further work in developing secretome-based therapies that may target α-synuclein-induced deficits. This toolkit is further applicable to other co-culture combinations using cell types derived from iPSCs.

Disclosures: D. Amerna: None. **J.D. Burgess:** None. **E.S. Norton:** None. **T.M. Parsons:** None. **R.B. Perkerson:** None. **A.H. Faroqi:** None. **Z.K. Wszolek:** None. **H. Guerrero Cazares:** None. **T. Kanekiyo:** None. **M. Delenclos:** None. **P.J. McLean:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.09/R7

Topic: C.03. Parkinson’s Disease

Support: Smoking Research Foundation

Title: Kaempferol has potent protective effects for alpha-synuclein neurotoxicity in vitro

Authors: *M. INDEN, K. OHUCHI, H. KURITA;
Lab. of Med. Therapeut. and Mol. Therapeut., Gifu Pharmaceut. Univ., Gifu, Japan

Abstract: Aggregation of a-synuclein (α-Syn) is implicated in the pathogenesis of Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). The misfolding and aggregation of α-Syn in neurons, neuronal processes, or glial cells are thought to be critical pathogenic events in synucleinopathies. Therefore, the removal of α-Syn aggregation could lead to the development of many new therapeutic agents for neurodegenerative diseases. In this study, we succeeded in generating a new α-Syn stably expressing cell line using a piggyBac transposon system. Using this system, we investigated the neuroprotective effect of the flavonoid kaempferol on α-Syn toxicity. We found that kaempferol provided significant protection against α-Syn-related neurotoxicity. According to our previous study, kaempferol induced autophagy via AMP-activated protein kinase (AMPK), i.e., the mammalian target of the rapamycin (mTOR) pathway. To identify the downstream signaling of the mTOR-AMPK pathway mediated by kaempferol, we investigated TFEB, which is a transcription factor that is a well-known master

regulator of autophagy and lysosomal biogenesis processes. We found that CLEAR reporter expression was significantly activated by kaempferol treatment in a concentration-dependent manner. In addition, we used qRT-PCR to analyze the mRNA levels of autophagy-associated genes for which transcription could be regulated by TFEB. The mRNA levels of Tfeb, Lamp1, Lamp2, Ctsd, and Tpp1 were significantly upregulated in response to kaempferol treatment. In further analysis, we determined whether kaempferol increase lysosomal biogenesis using immunofluorescence staining with Lamp2 antibody. Kaempferol treatment accelerated lysosomal activation, as shown by the quantification of fluorescence intensity in Lamp2-stained cells. These results indicate that kaempferol promotes the function of autophagy via the nuclear transposition of TFEB. These results support the therapeutic potential of kaempferol in diseases such as synucleinopathies that are characterized by α-Syn aggregates.

Disclosures: **M. Inden:** None. **K. Ohuchi:** None. **H. Kurita:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.10/R8

Topic: C.03. Parkinson’s Disease

Support: NIH R01NS115809

Title: Development of a novel non-secretable form of s100band its relevance to parkinson’s disease

Authors: *C. RODRIGUEZ[1], E. BANCROFT[2], C. POLO[4], R. SRINIVASAN[3];
[1]Texas A&M Univ. Syst. Hlth. Sci. Ctr., Bryan, TX; [2]Texas A&M Univ., Bryan, TX; [3]Texas A&M Univ., College Station, TX; [4]Texas A&M Chapter, Bryan, TX

Abstract: Parkinson’s disease (PD) is a devastating neurodegenerative disorder with no known cure. Understanding pathogenic processes in early PD is therefore critical for developing neuroprotective treatments. Interestingly, cerebrospinal fluid from PD patients shows significantly increasing S100B, a protein ubiquitously expressed by astrocytes. Based on this finding, we used in vitro cultures of dopaminergic (DA) neurons and astrocytes isolated from the mouse embryonic midbrain to ask if acute exposure to extracellular S100B peptide alters DA neuron function. We showed that acute bath application of recombinant human S100B peptide inhibits A-type voltage-gated potassium channels in primary cultured DA neurons, thereby pathologically increasing L-type voltage-gated calcium channel-mediated calcium fluxes. To further test if the secretable form of S100B expressed by astrocytes is sufficient to induce DA dysfunction, we have now developed a novel adeno-associated virus (AAV) that expresses non-secretable S100B in astrocytes. To do this, we deleted a putative endoplasmic reticulum (ER) exit site motif in the C-terminus of human S100B. Both constructs contain the mCherry reporter followed by an internal ribosome entry sequence (IRES) at the N-terminus of S100B and are

driven by the astrocyte-specific GfaABC1D promoter. In this study, we validate our novel AAV that expresses a non-secretable form of S100B in midbrain DA neuron-astrocyte co-cultures. Midbrain neuron-astrocyte co-cultures were infected with either AAV5-GfaABC1D-mCherry-IRES-S100B-ERES-Del or control AAV5-GfaABC1D-mCherry-IRES-S100B viruses. When compared to full length AAV5-GfaABC1D-mCherry-IRES-S100B-infected control cultures, we found significantly decreased Sec13 labeled ER exit sites (ERES) in astrocytes from cultures infected with AAV5-GfaABC1D-mCherry-IRES-S100B-ERES-Del. This decrease in Sec13-ERES corresponded with a significant decrease in S100B from the culture media of AAV5-GfaABC1D-mCherry-IRES-S100B-ERES-Del-infected cells when compared to controls. Together, these results successfully validate our non-secretable S100B constructs. We are currently comparing the ability of AAV5-GfaABC1D-mCherry-IRES-S100B-ERES-Del versus AAV5-GfaABC1D-mCherry-IRES-S100B to accelerate DA loss in vivo, in a preclinical mouse model of PD.

Disclosures: **C. Rodriguez:** None. **E. Bancroft:** None. **C. Polo:** None. **R. Srinivasan:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.11/S1

Topic: C.03. Parkinson’s Disease

Support: NRF Grant 2021R1A2C1013180

Title: Modulating neuroinflammatory responses with Ursolic acid: potential therapeutic implications for neurodegenerative diseases

Authors: *S. MOON[1], H. CHOI[2];
[1]CHA Univ. - Bundang Campus, gyeonggi-do bundang-gu, Korea, Republic of; [2]CHA Univ. - Bundang Campus, Prefer Not to Answer, Korea, Republic of

Abstract: In the development of neurological disorders, inflammation has emerged as a key factor, exerting a substantial impact on the onset and advancement of the diseases. The disruption of normal inflammatory mechanisms in the central nervous system has been identified as a significant contributor to the occurrence and progression of diverse neurological disorders, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. In addition, the NLRP3, an intracellular pattern recognition receptor, is present primarily in immune and inflammatory cells and plays a crucial role in the activation of the inflammasome, a multiprotein complex that drives the production of pro-inflammatory cytokines IL-1β and IL-18. Therefore, the regulation of neuroinflammatory responses has emerged as a promising therapeutic strategy for these diseases. In this research, we asked whether ursolic acid has the anti-inflammatory effects on neuroinflammatory responses in microglial cells. We show that ursolic acid significantly reduced the expression of pro-inflammatory cytokines and inflammasome components in BV2 microglial

cells, as well as the inflammasome in HMC3 microglial cells and primary microglia cells. Ursolic acid induced a significant increase in the expression of BDNF, accompanied by the upregulation of FNDC5, as demonstrated not only in cellular models but also in animal models. These results underscore the critical importance of regulating neuroinflammatory responses, specifically targeting pro-inflammatory cytokines and inflammasome components, in the development of novel therapeutic approaches for neurological disorders. The results further demonstrate the significant impact of ursolic acid on mitigating neuroinflammation by effectively reducing pro-inflammatory cytokine levels and modulating inflammasome activity in microglial cells. Additionally, the observed upregulation of BDNF and FNDC5 in response to ursolic acid administration not only confirms its anti-inflammatory properties but also suggests its potential as a neuroprotective agent.

Disclosures: S. moon: None. **H. Choi:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.12/S2

Topic: C.03. Parkinson’s Disease

Support: VA BX003244-01A1
R01 NS119897
R21 NS118476-01A1
VA Seed Grants

Title: Investigating the role of GBA deficiency in propagation of Lewy pathology

Authors: *A. PARK, S. FISH;
Univ. of Washington, Seattle, WA

Abstract: Investigating the role of GBA deficiency in propagation of Lewy pathology
Anna Park[1], Arnav Khera[2], Jeremy Weiss[1], Sarah Fish[2], Selina Yu[2], Marie Davis[2,3]
[1]University of Washington, [2]VA Puget Sound Health Care System, [3]Department of Neurology, University of Washington
Parkinson’s Disease (PD) is a neurodegenerative disease characterized by progressive cognitive impairment and rigidity and slowness of motor movements. Mutations in the gene *glucosidase, beta acid 1* (*GBA*) are associated with an increased risk of PD, along with faster disease progression. Our previous work has led us to hypothesize that GBA may mediate faster spread of pathogenic protein aggregates through alteration of extracellular vesicles.
To test the hypothesis that *GBA* deficiency promotes propagation of Lewy pathology, we are using a *Drosophila* model of *GBA* deficiency (GBA^{del}) and a human induced pluripotent stem cells (iPSC)-derived neuronal culture model generated from an individual with PD heterozygous for GBA^{IVS2+1} (GBA^{IVS} PD). Exosomes isolated from GBA^{del} flies have altered protein cargo,

including increased levels of exosome-intrinsic proteins Rab11 and Rab7, and increased oligomerized Ref(2)p, the *Drosophila* ortholog for p62. Ectopic expression of the d*GBA1b* protein in flight muscle or glia of GBA^{del} flies reduced protein aggregation in the brain, and normalized levels of Rab11, Rab7 and Ref(2)p in exosomes.
Immunocytochemical staining of dopaminergic neurons differentiated from GBA^{IVS} PD iPSCs revealed an increased endocytic and lysosomal compartment in GBA^{IVS} PD neurons compared to dopaminergic neurons differentiated from age- and sex-matched healthy control and isogenic GBA^{WT} PD iPSCs. We are now investigating how *GBA* alters endolysosomal trafficking and exosome biogenesis, and whether exosomes isolated from GBA^{WT} PD neurons can propagate Lewy pathology from cell to cell more rapidly than exosomes isolated from control neurons. Elucidating how GBA influences the spread of Lewy pathology could reveal novel therapeutic targets for slowing PD progression.

Disclosures: **A. Park:** A. Employment/Salary (full or part-time):; SIBCR. **S. Fish:** A. Employment/Salary (full or part-time):; SIBCR.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.13/S3

Topic: C.03. Parkinson’s Disease

Support: VA BX003244-01A1
R01 NS119897
R21 NS118476-01A1
VA Seed

Title: Mechanisms Underlying Parkinson's Disease: Insights from the Genetic Risk Factor GBA

Authors: *S. FISH[1], C. KWON[2], J. WEISS[2], R. ESTES[4], A. KHERA[2], S. YU[2], M. DAVIS[3]; [1]Univ. of Washington, Seattle, Seattle, WA; [3]Neurol., [2]Univ. of Washington, Seattle, WA; [4]Neurosci. Grad. Program, Oregon Hlth. & Sci. Univ. Neurosci. Grad. Program, Portland, OR

Abstract: Mechanisms Underlying Parkinson’s Disease: Insights from the Genetic Risk Factor GBA
Sarah Fish[1], Caroline Kwon[3], Jeremy Weiss[3], Raja E. Estes[1,2], Arnav Khera[1], Selina Yu[1], Marie Y. Davis[1,4,5]
[1]VA Puget Sound Healthcare System, Seattle, WA, [2]Oregon Health Sciences University, Portland, OR, [3]University of Washington, [4]Institute for Stem Cell Regenerative Medicine, University of Washington, Seattle, WA, [5]Department of Neurology, University of Washington, Seattle, WA
The gene *glucosidase, beta acid I* (GBA) encodes for the lysosomal enzyme glucocerebrosidase (GCase), which breaks down glucosylceramide and glucosylsphingosine. Mutations in GBA are

the strongest genetic risk factor for Parkinson's Disease, increasing the risk of developing PD by 5-fold. GBA mutation carriers with PD also have faster motor symptom progression and accelerated cognitive decline. To understand how *GBA* deficiencies influence PD pathogenesis and progression, we are using two GBA deficient models: GBA^{del} *Drosophila melanogaster* and human induced pluripotent stem cell (iPSC)-derived neuronal and astrocyte cultures derived from a heterozygous $GBA^{IVS2+1G>A}$ PD (GBA^{IVS}) patient.
Previously, we found that accelerated non-cell autonomous propagation of protein aggregates in GBA mutant flies is associated with dysregulated extracellular vesicles (EVs). Furthermore, we observed that glial expression of wildtype *GBA* reduces protein aggregation in GBA^{del} flies. We hypothesize that GBA plays a neuroprotective role in astrocytes by reducing propagation of pathogenic aggregates via uptake of neuronal EVs. EVs are formed through the endolysosomal trafficking system, so we are utilizing immunocytochemistry (ICC) to quantify various endolysosomal marker expression in astrocytes and neurons differentiated from GBA^{IVS} iPSCs. ICC staining of GBA^{IVS} dopaminergic neurons revealed an increased endocytic and lysosomal compartment compared to both age- and sex-matched healthy control and isogenic GBA^{WT} PD dopaminergic neurons. We expect to see similar results with the corresponding astrocyte models. In the future, we plan to utilize an automated cell culture system to observe neuronal EV uptake in *GBA* deficient & control astrocytes as well as pathogenic protein aggregation in neurons co-cultured with astrocytes.

Disclosures: **S. Fish:** A. Employment/Salary (full or part-time):; SIBCR. **C. Kwon:** None. **J. Weiss:** None. **R. Estes:** None. **A. Khera:** A. Employment/Salary (full or part-time):; SIBCR. **S. Yu:** A. Employment/Salary (full or part-time):; SIBCR. **M. Davis:** A. Employment/Salary (full or part-time):; UW.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.14/S4

Topic: C.03. Parkinson's Disease

Support: P20NS123220 NINDS
PF-RC-936279 Parkinson's Foundation
T32MH087004 NIH T32

Title: Investigation of a novel midbrain neuron type with vulnerability in Parkinson's disease

Authors: *M. LIANG[1], X. ZHOU[1], I. CHOI[1], Q. WANG[1], M. WANG[1], W. WANG[2], L. SARRAFHA[1], L. HO[1], K. FARRELL[1], K. BEAUMONT[1], R. SEBRA[1,3], J. CRARY[1], T. AHFELDT[1], J. BLANCHARD[1], D. NEAVIN[4], J. POWELL[4,5], D. A. DAVIS[6], X. SUN[6], Z. WU[2], B. ZHANG[1], N. YANG[1], Z. YUE[1];
[1]Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Weill Cornell Med., New York, NY;

[3]Sema4, Stamford, CT; [4]Garvan Inst. for Med. Res., Sydney, Australia; [5]Univ. of New South Wales, Sydney, Australia; [6]Univ. of Miami, Miami, FL

Abstract: Parkinson's disease (PD) is the second leading neurodegenerative disorder characterized by degeneration of neuromelanin-containing dopaminergic (DA) neurons in the substantia nigra (SN). Whether neurons beyond DA neurons are vulnerable in PD is poorly understood. The cause of PD remains unclear, however single-nucleus RNA sequencing (snRNAseq) has significantly advanced our understanding of neurodegenerative diseases, but limited progress has been made in PD. We have generated snRNAseq data from human SN including 9 healthy controls and 23 idiopathic PD. A combination of immunostaining and validation against datasets from independent cohorts resulted in the identification of molecularly distinct subtypes of DA-related neurons, including a RIT2-enriched population in aged human SN. RIT2 variants have previously been linked to PD. Validation in mouse and human SN identifies a RIT2 population that partially overlaps with TH, a marker for DA neurons, and the subpopulation (RIT2+/TH-) was found to be vulnerable in PD. Using mouse models and human midbrain neurons derived from pluripotent stem cells, we investigate distinct RIT2 neuronal subpopulations with PD-associated pathology. Characterization of distinct neuronal RIT2 subpopulations in the SN will provide mechanistic insights into the complexity of PD and identify pathways warranting further exploration for therapeutic studies in PD.

Disclosures: M. Liang: None. **X. Zhou:** None. **I. Choi:** None. **Q. Wang:** None. **M. Wang:** None. **W. Wang:** None. **L. Sarrafha:** None. **L. Ho:** None. **K. Farrell:** None. **K. Beaumont:** None. **R. Sebra:** None. **J. Crary:** None. **T. Ahfeldt:** None. **J. Blanchard:** None. **D. Neavin:** None. **J. Powell:** None. **D.A. Davis:** None. **X. Sun:** None. **Z. Wu:** None. **B. Zhang:** None. **N. Yang:** None. **Z. Yue:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.15/S5

Topic: C.03. Parkinson's Disease

Support: Mayo Clinic Center for Biomedical Discoveries Career Development Award for Under-Represented Minorities
Mayo Clinic CCaTS-Fink Family Career Development Award in Neurology

Title: Stn dbs modulates multiple mechanisms associated with neuronal degeneration in alpha-synuclein model of parkinson's disease

Authors: *M. J. LIM[1], M. J. PRIMITIVO[2], S. JOHN[4], J. SILVERNAIL[3], W. D. LUJAN[3], X. HOU[1], W. SPRINGER[1], L. LUJAN[3], P. J. MCLEAN[1], S. L. BOSCHEN DE SOUZA[1];

[1]Mayo Clin., Jacksonville, FL; [2]Dept. of Comparative Med., [3]Dept. of Neurologic Surgery, Mayo Clin., Rochester, MN; [4]Hamilton Col., Clinton, NY

Abstract: Although the etiology of Parkinson's disease (PD) remains unknown, aggregation of alpha-synuclein (αsyn) and chronic neuroinflammation may be exacerbated and induce glial senescence, creating a feed-forward mechanism of neuroinflammation, senescence, and cell death. STN DBS in multiple animal models have shown a potential neuroprotective effect although clinical evidence is still very conflicting. Therefore, it is critical to evaluate the potential neuroprotective mechanisms of STN DBS from a holistic perspective in order to further understand why clinical trials have been inconclusive and steer new studies towards a more effective manner of delivering DBS. Here, our overall goal is to determine whether STN DBS may exert neuroprotective effects by modulating different pathophysiological mechanisms associated with PD. We evaluated the asymmetrical use of paws, dopaminergic neuronal survival, levels of microglia and astrocytes, and presence of senescence markers in the brains of a rat model of PD overexpressing αsyn and receiving daily STN DBS starting at an early (1 week after αsyn injection) or a late (4 weeks after αsyn injection) stage of dopaminergic degeneration. Late STN DBS improved motor function, reduced αsyn accumulation in the striatum, and loss of dopaminergic neurons in the substantia nigra of rats overexpressing αsyn. Additionally, late STN DBS reduced presence of microglia in the midbrain, but did not affect astrocytes levels. Finally, late STN DBS seems to reduce phospho-ubiquitin and lamin B1 compared to non-stimulated rats. Therefore, there seems to be an optimal time point during disease progression in that STN DBS is neuroprotective by modulating multiple cellular and molecular pathways.

Disclosures: M.J. Lim: None. **M.J. Primitivo:** None. **S. John:** None. **J. Silvernail:** None. **W.D. Lujan:** None. **X. Hou:** None. **W. Springer:** None. **L. Lujan:** None. **P.J. McLean:** None. **S.L. Boschen De Souza:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.16/S7

Topic: C.03. Parkinson's Disease

Support: NINDS R01NS086778

Title: New Drosophila models of Dentatorubral-pallidoluysian atrophy

Authors: *N. PATEL, M. V. PRIFTI, K. LIBOHOVA, S. V. TODI;
Pharmacol., Wayne State Univ., Detroit, MI

Abstract: Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant, progressively degenerative disorder that is predominantly distinguished by ataxia, myoclonus, epilepsy, choreoathetosis, dementia, and psychiatric disturbances. DRPLA is a member of the polyglutamine (polyQ) family of diseases, which comprises nine members and is caused by

abnormal expansion of a polyQ tract repeat in specific proteins. The gene at the core of DRPLA is ATROPHIN-1 (ATN1). ATN1 is a transcriptional co-repressor that is expressed in the central nervous system and other organs. When the polyQ repeat of ATN1 is expanded beyond normal ranges, it causes neuronal malfunction and death. The precise molecular mechanisms underlying DRPLA are unclear. The pathogenic CAG repeat length of ATN1 ranges from 48 to 93. To assist with the understanding of the biological processes at the core of DRPLA, and with the hope of supporting the identification and development of therapeutics for this incurable disease, we recently generated isogenic Drosophila melanogaster transgenic lines that express full-length, human ATN1 with a normal (Q7) or pathogenic (Q88) repeat. We are in the process of characterizing these new models and will present a full phenotypic and biochemical characterization of these new lines. We look forward to sharing our findings.

Disclosures: **N. Patel:** None. **M.V. Prifti:** None. **K. Libohova:** None. **S.V. Todi:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.17/S8

Topic: C.03. Parkinson’s Disease

Support: Sastry Foundation Parkinson’s Disease Research

Title: Exploring the Potential of Akt Modulator A-443654 as a Therapeutic Approach for Parkinson's Disease: Insights from Drosophila melanogaster Models

Authors: Z. BANAGSH[1], ***Z. CHBIHI**[1], B. RANXHI[1], S. V. TODI[1], P. A. LEWITT[2], W.-L. TSOU[1];
[1]Dept. of Pharmacol., [2]Dept. of Neurol., Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting more than 10 million individuals worldwide. Its impact on the nervous system manifests in a variety of physical and cognitive symptoms, including tremors, muscle rigidity, slow movements, balance impairment, and psychiatric symptoms. Despite intensive research, the etiology of PD remains elusive; it might be a multifactorial condition influenced by genetic, environmental, and age-related factors. An α-synucleinopathy, characterized by the accumulation of misfolded and toxic α-synuclein protein, is recognized as a common final pathway underlying PD. The buildup of α-synuclein in dopamine-producing neurons within the substantia nigra region leads to their degeneration and dysfunction.
Recent investigations suggest that the Akt modulator A-443654 holds promise as a therapeutic agent for modulating α-synucleinopathy in PD. Studies have demonstrated that A-443654 reduced both α-synuclein mRNA and its protein production. In our study, we investigate A-443654 as a therapeutic approach for PD using *Drosophila melanogaster* as a model organism. We employed two experimental models: one involved the overexpression of wild-type α-

synuclein in neurons, while the other involved feeding flies with 500 uM rotenone. In both models, flies exhibited shortened lifespan and impaired motility compared to the control group. However, upon administration of A-443654, we observed a significant extension of lifespan in both models. Notably, no significant changes were observed in climbing ability of the fly.Our findings provide evidence that the Akt modulator A-443654 may hold potential as a treatment for PD, as demonstrated in the enhanced longevity in the fly models subjected to α-synuclein toxicity. These results serve as a pilot study for further exploration in mouse models of PD, paving the way for future investigation of a promising direction of therapeutic intervention for PD.

Disclosures: Z. Banagsh: None. **Z. Chbihi:** None. **B. Ranxhi:** None. **S.V. Todi:** None. **P.A. LeWitt:** None. **W. Tsou:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.18/S9

Topic: C.03. Parkinson’s Disease

Support: Sastry Foundation Parkinson’s Disease Research

Title: Decoding the Roles of Polyamine Metabolism in Parkinson's Disease

Authors: B. RANXHI[1], Z. M. CHBIHI[1], Z. BANGASH[1], Z. QADRI[1], S. V. TODI[1], P. A. LEWITT[2], *W.-L. TSOU[1];
[1]Dept. of Pharmacol., [2]Dept. of Neurol., Wayne State Univ., Detroit, MI

Abstract: Polyamines play essential and conserved roles in organisms, interacting with physiological functions such as cell growth, survival, and other biological processes such as the synthesis of proteins and nucleic acids, stabilizing the structure of chromatin, regulating apoptosis, and protecting cells against oxidative damage. Polyamine metabolism has also been investigated for yielding disease-specific biomarkers. Our recent study found major increase in serum concentrations of three L-ornithine-derived polyamines (putrescine, spermine, and spermidine), each of which showed correlation to Parkinson’s disease (PD) progression and its clinical subtypes. Given the key physiological roles of polyamines and their tight homeostatic regulation, we investigated whether the biomarker findings might offer biochemical insights into the neurodegeneration of PD (and possibly other neurodegenerative diseases involving proteinopathy). To further investigate the relationship between polyamine metabolism and PD, we engineered experimental changes in polyamine metabolism (knocking down critical polyamine interconversion enzymes) in *Drosophila* synucleinopathy models that overexpress neuronal α-synuclein.
We observed substantial alterations in the lifespan and motility of *Drosophila* after suppressing key enzymes of polyamine metabolism (spermine synthase [SMS], spermidine/spermine N1-

acetyltransferase 1 [SAT1], spermine oxidase [SMOX], or polyamine oxidase [PAOX]. As polyamine metabolism is vital for maintaining neuronal integrity, we found the functional involvement of each polyamine in neuronal homeostasis and their regulation in relation to α-synuclein. Additionally, we explore whether transformations in specific polyamine enzymes within our α-synuclein overexpression fly model would result in a decrease in the extent of α-synuclein aggregation.
Our analysis provides insights into the origins of neuronal and systemic changes in polyamine metabolism. The *Drosophila* model of altering polyamine compounds and their metabolites may offer insights into the neurodegenerative process in PD vis-à-vis α-synuclein aggregation in PD. These findings may also help to guide the development of clinical trials using therapeutic interventions targeting polyamine pathways.

Disclosures: **B. Ranxhi:** None. **Z.M. Chbihi:** None. **Z. Bangash:** None. **Z. Qadri:** None. **S.V. Todi:** None. **P.A. LeWitt:** None. **W. Tsou:** None.

Poster

PSTR466. Neuroprotective Mechanisms in Parkinson's Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR466.19/S10

Topic: C.03. Parkinson’s Disease

Support: NINDS R01NS086778 (ST)
Wayne State University Graduate School Dean’s Diversity Fellowship (MP_

Title: Metformin's effect on drosophila models of polyglutamine diseases

Authors: *M. V. PRIFTI[1], S. V. TODI[2];
[1]Pharmacol., [2]Pharmacol. and Neurol., Wayne State Univ., Detroit, MI

Abstract: Repurposing old drugs or creating new drugs to slow down the natural process of aging has the potential to vastly improve quality of life for patients suffering from non-curable diseases. Targets for these drugs usually involve major communication nodes such as mTOR, AMPK, or SIRT1. Among drugs candidate for repurposing is Metformin, a medication typically prescribed to combat insulin-sensitivity deficiencies in type 2 diabetic patients. Metformin is a potent AMPK activator and an agonist of SIRT1 that mimics metabolic responses to caloric restriction; caloric restriction itself is considered the gold standard as the best way to delay and ‘treat’ aging since the discovery of its longevity-increasing effects in mice roughly 80 years ago. Polyglutamine (polyQ) mutations are a group of nine distinct genetic abnormalities characterized by the toxic expansion of a CAG/A triplet nucleotide repeat in their protein coding regions. The diseases include Huntington’s, Spinocerebellar Ataxias Type 1, 2, 3, 6, 7, 17, Spinal and Bulbar Muscular Atrophy, and Dentato-rubral Pallidoylusian Atrophy. The effect of these mutations is strongly related to the length of the polyQ expansion, which increases every time it is passed on

to the next generation. Following translation, polyQ proteins often aggregate, change cellular sub-localization, cause altered gene expression, and lead to various cell pathway perturbation, eventually leading to cell death in different regions of the brain. There are currently no cures for polyQ diseases. AMPK activation has neuroprotective effects in mouse models of the polyQ disease Huntington's Disease (HD), potentially by reducing aggregates that may be interfering with regular cell functions. In this study, I expressed two different polyQ disease proteins, ones that cause Spinocerebellar Ataxia Type 3 (Atxn3) and Spinal and Bulbar Muscular Atrophy (androgen receptor) in *Drosophila* neurons and examined the effect of varying concentrations of Metformin had on overall fly lifespan. This work is a preview of examining the effect of metformin more widely among all polyQ disease models in the fly.

Disclosures: M.V. Prifti: None. **S.V. Todi:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.01/T1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: ABCD Charitable Trust

Title: Comparison of the Huntington's Disease-Like 2 (HDL2) and Huntington's Disease (HD) Insoluble Proteomes

Authors: *S. G. DOLL[1], ***S. DOLL**[2], N. M. EL DEMERDASH[3], T. RYU[5], C. NA[6], L. G. NUCIFORA[4], R. L. MARGOLIS[7];
[1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Psychiatry, Johns Hopkins Med. Institutions, Lafayette Hill, PA; [4]Psychiatry and Behavioral Sci., [3]Sch. of Med., Baltimore, MD; [5]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [6]Dept. of Biol. Chem., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [7]Psychiatry, Johns Hopkins Univ., Baltimore, MD

Abstract: ABSTACT Huntington's Disease-Like 2 (HDL2) is an adult-onset autosomal dominant neurodegenerative disorder with clinical, neuroimaging, and pathological features that are remarkably similar to those of Huntington's Disease (HD). Neuronal inclusion bodies that stain with anti-ubiquitin and anti-expanded polyglutamine antibodies and have a fibral appearance by EM are prominent features of both disorders. These inclusion bodies are exclusively nuclear in HDL2, and both nuclear and cytoplasmic in HD. Aggregate formation in HD is nucleated by the expanded polyglutamine tracts of mutant huntingtin protein (Htt). In HDL2, the initiating factor is less clear. On the sense strand the repeat occurs in the CTG orientation within a differentially spliced exon of *junctophilin-3* (*JPH3*) on chromosome 16q24, variably falling within the 3' UTR or in-frame to encode poly-leucine or poly-alanine. On the antisense strand, the repeat is contained within a cryptic transcript in-frame to encode polyglutamine. Whether this polyglutamine tract is expressed at sufficient levels to account for

HDL2 inclusions is unknown. In an effort to elucidate the composition of nuclear inclusion bodies in HDL2, we isolated nuclei from the superior frontal gyrus from HD, HDL2 and control patients (N =3 each). Nuclei were lysed, and the detergent-resistant fraction purified through sequential ultracentrifugation. Aggregated protein from this fraction was then fragmented and separated by liquid chromatography, and peptide fragments were identified through mass spectrometry (LC-MS). Our preliminary results indicate that the HDL2 nuclear insoluble proteome overlaps with that of HD. From the HD nuclear insoluble proteome, we found that 45 proteins were uniquely enriched relative to control, compared with 195 uniquely enriched proteins from HDL2 brain. Western blot with 1C2 antibody revealed the presence of an expanded poly-glutamine protein that corresponded to mutant Htt in insoluble protein from HD brain, but a similar signal corresponding to the length of JPH3-antisense protein with a polyglutamine expansion was not detected in insoluble protein from HDL2 brain. Gene ontology (GO) functional annotation indicated that over 20% of enriched proteins in the insoluble HD fraction, and over 25% of enriched proteins in insoluble HDL2 fraction, were related to mitochrondrial function. These data leave the question of the nucleation of inclusions in HDL2 unresolved; future experiments will further investigate the formation of HDL2 aggregates, and the implication of mitochondrial dysfunction in HDL2.

Disclosures: **S.G. Doll:** None. **S. Doll:** None. **N.M. El Demerdash:** None. **T. Ryu:** None. **C. Na:** None. **L.G. Nucifora:** None. **R.L. Margolis:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.02/Web Only

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Huntingtin plays a role in Drosophila ethanol response

Authors: *E. CLABOUGH[1], C. ASPILI[1], W. FUSSY[2], J. INGERSOLL[2], A. KISLYAKOV[1], E. LI[1], M.-J. SU[1], D. WILES[2], T. WATSON[2], A. WILLY[2], T. VINYARD[2], P. MOLLICA[2], J. TAYLOR[2], C. SMITH[2], D. ROARK[2], Z. TABRANI[2], H. THOMAS[2], M. SHIN[1], J. VENTON[1], D. HAYES[3], C. SIPE[3];
[1]Univ. of Virginia, Charlottesville, VA; [2]Hampden-Sydney Col., Hampden-Sydney, VA; [3]Shepherd Univ., Shepherdstown, WV

Abstract: Huntingtin (htt) protein is an essential regulator of nervous system function due to its various neuroprotective and pro-survival functions. Loss of wild-type htt function is implicated in the etiology of Huntington's Disease. While its pathological role is typically understood as a toxic gain-of-function, some neuronal phenotypes result from a loss of routine htt function. Thus, it is important to understand possible roles for htt in other physiological contexts. Here, we investigated htt function in mediating ethanol sensitivity, tolerance, preference, and recovery using Drosophila. We found that dhtt-null flies are both less sensitive and more tolerant to

ethanol exposure in adulthood. Moreover, flies that lack dhtt are more averse to alcohol in capillary feeding assays compared to controls, and they recover mobility faster following acute ethanol intoxication. We showed that dhtt mediates these effects at least in part through the dopaminergic system, as dhtt is required to maintain normal levels of dopamine neurotransmitter in the brain, as well as normal numbers of dopaminergic cells in the adult protocerebrum. Taken together, our results demonstrate that htt is a regulator of the physiological response to ethanol and indicate a novel neuroprotective role for htt in the dopaminergic system, raising the possibility that it may be involved more generally in the response to toxic stimuli.

Disclosures: **E. Clabough:** None. **C. Aspili:** None. **W. Fussy:** None. **J. Ingersoll:** None. **A. Kislyakov:** None. **E. Li:** None. **M. Su:** None. **D. Wiles:** None. **T. Watson:** None. **A. Willy:** None. **T. Vinyard:** None. **P. Mollica:** None. **J. Taylor:** None. **C. Smith:** None. **D. Roark:** None. **Z. Tabrani:** None. **H. Thomas:** None. **M. Shin:** None. **J. Venton:** None. **D. Hayes:** None. **C. Sipe:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.03/T2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CHDI Foundation contract
Maryland Stem Cell Research Fund Discovery Award

Title: A single-cell multi-omic atlas for transcriptomic and chromatin accessibility changes in a mouse model of the Huntington’s disease mutation and the effects of an Htt-lowering gene therapy

Authors: *E. WILDERMUTH;
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Title: A single-cell multi-omic atlas for transcriptomic and chromatin accessibility changes in a mouse model of the Huntington’s disease mutation and the effects of an Htt-lowering gene therapy
Erin Wildermuth [1,2], Marcia Cortés-Gutiérrez [2], Robert Bragg [3], Jeff Cantle [3], Sonia Malaiya [4], Jeffrey B. Carroll [3,5], Seth A. Ament [2,6]
1. Medical Scientist Training Program, University of Maryland School of Medicine, Baltimore, MD2. Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD3. Department of Psychology, Western Washington University, Bellingham, WA 4. Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD5. Department of Neurology, University of Washington, Seattle, WA6. Department of Psychiatry, School of Medicine University of Maryland School of Medicine, Baltimore, MD
Abstract:

Huntington’s disease (HD) is a fatal and incurable neurodegenerative disorder caused by dominant inheritance of trinucleotide repeat expansions in the *HTT* gene. One challenge to clinical innovation is that we lack sufficient information about how the HD mutation and its potential therapeutics impact the brain. We performed single-nucleus RNA sequencing (snRNA-seq) and single-nucleus Assay for Transposase-Accessible Chromatin using sequencing (snATAC-seq) to characterize cell type-specific transcriptomic and epigenomic effects of the HD mutation in the striatum of a knock-in mouse model, $Htt^{Q111/+}$. We found substantial transcriptional and epigenomic effects of the HD mutation, most prominently in spiny projection neurons, the cell type most vulnerable to HD neurodegeneration. Differentially expressed and differentially accessible peak regions were enriched for targets of the polycomb repressive complex (PRC2), providing new evidence for the hypothesis that PRC2 is partially non-functional in HD and may constitute a potential therapy target. We also evaluated transcriptional changes associated with an Htt-lowering ASO therapy. Surprisingly, ASO treatment exacerbated HD-associated transcriptional changes. Understanding these cell type-specific effects of ASO treatment could aid in the interpretation of Htt-lowering toxicity that has recently been observed in human patients.

Disclosures: E. Wildermuth: None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.04/T3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CHDI Foundation, Inc.

Title: Large-scale Genetic Perturbation of Mutant Huntingtin Induced Transcriptomes and CAG-length-Dependent Molecular Networks

Authors: *N. WANG[1,2], P. LANGFELDER[2], L. RAMANATHAN[2], M. PLASCENCIA[2], F. GAO[2], M. STRICOS[2], R. VACA[2], X. GU[2], S. ZHANG[2], J. RICHMAN[2], C. PARK[2], C. CHOI[2], A. SHAMBAYATE LOPEZ[2], T. VOGT[4], J. AARONSON[4], J. ROSINSKI[4], S. HORVATH[3], X. YANG[2];
[2]Ctr. for Neurobehavioral Genetics, Semel Inst. of Neurosci. and Human Behavior, [3]Departments of Human Genet. and Biostatistics, David Geffen Sch. of Med. at UCLA, [1]UCLA, Los Angeles, CA; [4]CHDI Foundation/CHDI Mgmt. Inc., Princeton, NJ

Abstract: Huntington’s disease (HD) causes striatum-selective neurodegeneration. Such brain region selectivity is partially recapitulated in mouse models as a mutant Huntingtin (mHtt) CAG-length-dependent dysregulation of striatal transcriptomes and weighted gene coexpression networks (WGCNA; Langfelder et al., *Nature Neuroscience*, 2016, PMID: 26900923). In this study, we performed a systematic large-scale genetic perturbation analysis of HD striatal

transcriptomes to address whether heterozygous knockout of the HD network hub genes could significantly modify mHtt-dysregulated transcriptomes. We selected 115 genes based on their hub gene status in the HD gene network, crossed each heterozygous knockout (KO-Het) to mHtt Q140 knockin mice, aged the mice to 6-months and then performed striatal RNA-sequencing. Our study produced an experimentally validated causal gene expression network consisting of 115 perturbations and over 6000 differentially expressed genes in the striata of wildtype versus Q140 mice. We implemented a bioinformatic pipeline to analyze each Q140/KO-Het cross for the rescue or exacerbation of mHtt-induced transcriptome at both the transcriptome and module levels. We also compared all the KO-Het perturbations to rank their HD transcriptomic or module modifying effects. The latter analysis revealed interesting clustering of KO-Het perturbations that have similar molecular effects in wildtype and Q140 backgrounds, and novel relationships among the mHtt CAG-length dependent WGCNA modules. Finally, we performed validation studies on the major transcriptomic modifier genes to provide insights into disease pathogenesis. Together, our study demonstrated a novel, unbiased and scalable in vivo platform, using transcriptome readouts, to yield unique insights into the disease-modifying roles of candidate molecular targets for HD.

Disclosures: **N. Wang:** None. **P. Langfelder:** None. **L. Ramanathan:** None. **M. Plascencia:** None. **F. Gao:** None. **M. Stricos:** None. **R. Vaca:** None. **X. Gu:** None. **S. Zhang:** None. **J. Richman:** None. **C. Park:** None. **C. Choi:** None. **A. Shambayate Lopez:** None. **T. Vogt:** None. **J. Aaronson:** None. **J. Rosinski:** None. **S. Horvath:** None. **X. Yang:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.05/T4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS110694
Biomedical research award in interdisciplinary science (BRAINS)

Title: Cognitive effects of Heat Shock Transcription Factor 1 depletion in patwhay specific excitatory synapse regulation in the striatum

Authors: *N. B. ROZEMA[1], N. ZARATE[1], R. MANSKY[3], N. BLOMME[1], J. SAWYER[1], E. LIND[2], R. GOMEZ-PASTOR[1];
[1]Neurosci., [2]Mouse Behavior Core, Univ. of Minnesota, Minneapolis, MN; [3]Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Heat Shock Transcription Factor 1 (HSF1) is a stress protective transcription factor canonically known for its role in the regulation of protein quality control systems that declines during aging and neurodegeneration. However, recent evidence has demonstrated that HSF1 also participates in the regulation of synaptic genes within different contexts and brain regions,

highlighting non-canonical roles for HSF1. Here, we showed that HSF1 regulates memory consolidation by directly controlling the expression of the postsynaptic scaffolding protein PSD-95, essential in maintaining excitatory synapse stability and neurotransmission within the striatum. Striatal PSD-95 declines during aging and contributes to alterations in synaptic dysfunction and behavioral deficits, but whether HSF1 is responsible for age-dependent loss of PSD-95 and associated cognitive deficits has remained elusive. We have utilized i) a model of chronic HSF1 reduction across the mouse lifespan through genetic ablation of a single *Hsf1* allele (*Hsf1*$^{+/-}$ mice) and ii) a model of acute HSF1 reduction via adeno-associated viruses (AAVs) to spatially and temporally restrict HSF1 reduction to the striatum, a brain region that controls movement and some forms of cognition. We demonstrated that both chronic and acute reduction of HSF1 decreased the levels of PSD-95 in the striatum. Decreased PSD-95 paralleled a specific reduction in excitatory thalamo-striatal (T-S) synapses, an important synaptic circuit involved in cognitive functions such as goal-directed learning, action selection, and flexible control of behavior. This suggests that depletion of HSF1 may contribute to memory-related deficits associated with aging and that HSF1 may exert its effects on synaptic health differentially based on synaptic population. In support of this hypothesis, acute reduction of HSF1 in the striatum via AAVs resulted in apparent disruption of spatial working memory and cognitive inflexibility of associative learning. Additionally, we are currently making strides towards understanding the role of HSF1 at the synapse within the context of individual excitatory pathways within the striatum. These results demonstrate an emerging role for HSF1 in synaptic gene regulation that has important implications in synapse maintenance and memory during aging and that can result in effective therapeutic interventions.

Disclosures: **N.B. Rozema:** None. **N. Zarate:** None. **R. Mansky:** None. **N. Blomme:** None. **J. Sawyer:** None. **E. Lind:** None. **R. Gomez-Pastor:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.06/T5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Natural Sciences and Engineering Research Council RGPIN-2022-03513
Canadian Institutes of Health Research Canada Graduate Scholarships-Master’s
University of British Columbia Four-Year Fellowship

Title: Synaptic modulation of glutamate release in striatum of the YAC128 mouse model of Huntington disease

Authors: *J. CHENG, E. T. KOCH, L. A. RAYMOND;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Studies have suggested that altered balance between striatal direct and indirect pathway activity contributes to early motor symptoms in Huntington disease (HD). Degeneration of striatal D2 dopaminergic receptor-expressing indirect pathway medium spiny neurons (iMSNs) precedes that of D1-expressing direct pathway ones (dMSNs), promoting involuntary movements. However, altered corticostriatal synaptic function precedes degeneration. In addition to iMSNs, D2 receptors are expressed on projections to striatum, including dopaminergic terminals from substantia nigra and glutamatergic cortical terminals, as well as on striatal cholinergic interneurons. In addition to reducing their excitability, D2 receptor-mediated signaling on iMSNs also promotes endocannabinoid (eCB) synthesis and consequent inhibition of glutamate release from cortical afferents. Altered dopaminergic, eCB and cholinergic signaling may contribute to early striatal dysfunction in HD. We used acute cortico-striatal brain slice and optogenetic probes iGluSnFR and dLight to monitor glutamate and dopamine release, respectively, comparing the transgenic YAC128 HD mouse model with wild-type (WT) controls. We found that 0.5uM and 5uM of the D2 agonist quinpirole significantly reduced cortically-evoked glutamate release in striatum of YAC128 slices, whereas only 5uM quinpirole reduced glutamate release in WT. We are testing whether increased D2 receptor sensitivity in HD mice is due to: 1) increased expression of cortical terminal D2 receptors; 2) reduced D2 signaling on dopamine terminals, facilitating more dopamine release; 3) amplified eCB release from D2 activation on MSNs; or 4) upregulated D2 signaling on cholinergic interneurons. Our results show no genotype difference in cortically-evoked dopamine release in striatum. Blocking type 1 cannabinoid receptors (CB1R) or α7 nicotinic acetylcholine receptors (α7nAChR) also did not diminish YAC128 slices' increased sensitivity to quinpirole. This suggests that increased D2 sensitivity at the YAC128 cortico-striatal synapse does not occur due to differences in dopamine release, CB1R activation, or α7nAChR function, and we are currently testing other mechanisms using pharmacological and immunostaining approaches. Our findings will provide a greater understanding of the interplay between key neuromodulators that regulate glutamate transmission and potentially give rise to motor symptoms in HD.

Disclosures: **J. Cheng:** None. **E.T. Koch:** None. **L.A. Raymond:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.07/T6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CBET-1464686
NeuroNex-1707352
CMU PECP

Title: Targeted circuit manipulation for ameliorating Huntington's Disease pathogenesis

Authors: *E. IKEFUAMA[1], R. SCHALAU[3], A. UPRETY[3], M. TREE[4], G. L. DUNBAR, Sr.[5], J. ROSSIGNOL[4], U. HOCHGESCHWENDER[2];
[1]Neurosci., [2]Col. of Med., Central Michigan Univ., Mount Pleasant, MI; [3]Neurosci., [4]Col. of Med., [5]Psychology, Cental Michigan Univ., Mount Pleasant, MI

Abstract: Almost 30 years after identifying the genetic mutation underlying Huntington's disease (HD), treatments remain limited to managing late-stage symptoms of motoric, psychiatric, and cognitive deficits. Findings from patients and mouse models of HD point to pre-symptomatic imbalances in neuronal circuit activity, well before any overt symptoms are observed. Our central hypothesis is that manipulating the firing activity within selected microcircuits before the onset of symptoms by chemogenetic inhibition and/or excitation of key target populations will slow HD disease progression. A crucial early event in HD is the pathological increase in the overall excitatory output from cortex onto striatum. The enhanced excitability of cortical pyramidal neurons (CPNs) in pre-symptomatic HD is one key target for correctional intervention. The window before the onset of symptoms presents an opportunity to inhibit the firing rate of CPNs projecting to the striatum with the prospect of preventing or slowing disease progression. For manipulation of neuronal activity, we utilized bioluminescent optogenetics (BL-OG) that employs light-emitting luciferases to activate light-sensing opsins. We are testing the effects of circuit manipulation on preventing or delaying behavioral deficits in the R6/2 transgenic mouse model of HD. To selectively target CPNs, an AAV vector carrying a Cre-inducible inhibitory LMO (AAV-Ef1a-DIO-NCS3-hGtACR1) was injected into the cortex of 3-week-old mice. Two weeks later, luciferin or vehicle were administered once every day for 2 weeks to decrease CPN firing. Rotarod, open field, and CatWalk were used to assess motor coordination, exploratory behavior, and gait function. We assessed cognitive behavior through water T-maze, novel object recognition test, and passive avoidance test. Our studies will contribute to understanding how microcircuit manipulation influences motor and cognitive behavior in HD and will drive translational progress toward novel therapeutic purposes.

Disclosures: E. Ikefuama: None. **R. Schalau:** None. **A. Uprety:** None. **M. Tree:** None. **G.L. Dunbar:** None. **J. Rossignol:** None. **U. Hochgeschwender:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.08/T7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NINDS R21/R33 1R21NS104320-01A1/R33
NIH R01 NS086452-06
HD PMCOE

Title: Inhibition of PKC a&β1 kinase activity protects Huntington's disease human striatal neurons

Authors: *M. JIANG, R. MIRYALA, T. SHI, R. WANG, M. RODRIGUEZ, K. BELKAS, R. SULTANIA, L. GUTTMAN, K. MAE BOCKLEY, Y. LI, A. CUI, Y. XUE, Y. UM, A. YUAN, C. HOLLAND, J. JIN, J. TRONCOSO, W. DUAN, T. RATOVITSKI, W. SMITH, C. ROSS; Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin (HTT) gene. The repeat expansion leads to the pathogenic expansion of a polyglutamine tract in the huntingtin protein. HD is characterized by the loss of striatal medium spiny neurons and the presence of cellular inclusion bodies containing mutant HTT proteins. Currently, there is no disease-modifying treatment available for HD. In order to identify disease modifying treatment, we established a human neuronal model by immortalizing and differentiating HD patient iPSCs into highly homogeneous striatal precursor neurons (SPN), which recapitulated HD-like phenotypes of the parental iPSCs including expression of MAP2/DARPP32 (Akimov et al, Hum Mol Genet. 2021 Nov 30;30(24): 2469-2487). Further, we developed a 96-well plate screening platform using CellTiter-Glo luminescent cell viability assay in the SPNs and screened a kinase inhibitor library (ApexBio) containing 765 compounds in HD SPNs expressing 180 CAG repeats (180Q-SPNs). We identified approximately 20 compounds that exhibited protection to HD SPNs against stress-induced neuronal toxicity.
Among the hits, there was a small molecule PKC-a and ß1 inhibitor GO6976, which we validated and prioritized. We found that GO6976 dose-dependently rescued HD SPNs from stress-induced toxicity. Furthermore, we examined PKCa / ß1 activity and protein levels in HD conditions. The activity of PKCa and PKCß1 was significantly increased in HD ISPNs, mouse, and human brains. Moreover, PKCa and PKCß1 interacted with both wild-type and mutant HTT, and their overexpression was toxic to HD SPNs. These findings suggest that PKCa/ß1 may play important roles in HD neurodegeneration and that inhibition of PKCa/ß1 activity may attenuate mutant HTT toxicity and provide novel therapeutic targets for developing neuroprotective HD treatments.

Disclosures: M. Jiang: None. **R. Miryala:** None. **T. Shi:** None. **R. Wang:** None. **M. Rodriguez:** None. **K. Belkas:** None. **R. Sultania:** None. **L. Guttman:** None. **K. Mae Bockley:** None. **Y. Li:** None. **A. Cui:** None. **Y. Xue:** None. **Y. Um:** None. **A. Yuan:** None. **C. Holland:** None. **J. Jin:** None. **J. Troncoso:** None. **W. Duan:** None. **T. Ratovitski:** None. **W. Smith:** None. **C. Ross:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.09/T8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Dake Family Fund
CHDI Grant to MDF and KK-G

Title: Mutant Huntingtin lowering averts changes in lipids important for synapse function and white matter maintenance in the LacQ140 mouse model.

Authors: K. SHING[1], E. SAPP[1], A. BOUDI[1], S. LIU[1], C. SEELEY[1], D. MARCHIONINI[2], M. DIFIGLIA[1], *K. KEGEL-GLEASON[1];
[1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]CHDI Fndn., New York, NY

Abstract: Huntington's disease (HD) is an inherited neurodegenerative disease driven by a CAG expansion in the huntingtin gene (*HTT*), resulting in a polyglutamine expansion in the protein product (HTT). Changes in white matter are among the first detectable symptoms through brain imaging in HD patients. Approaches that lower mutant HTT (mHTT) are promising treatments for HD, but it is still unknown which pathologic changes can be prevented or reversed by reducing mHTT burden. To address this question, we leveraged a HD mouse model bearing a regulatable promoter preceding the endogenous m*Htt* allele containing 140 CAGs (LacQ140). Cohorts of mice (n=6) with m*Htt* transcript repression initiated at different ages and sustained for different time-periods were examined at 6, 9, and 12-months of age together with wild-type (WT) mice. We used a high resolution liquid chromatography and tandem mass spectrometry (LC-MS/MS) lipidomic platform to survey for changes in LacQ140 caudate-putamen and the effects of lowering m*Htt* for different durations. Statistical significance was determined by one-way ANOVA for each lipid subclass and by species among the treatment groups and genotypes. To control for multiple testing a two staged linear step-up procedure by Benjamini, Krieger and Yekutieli was applied with a false discovery rate of 5%. Total lipids were reduced in 9-month-old LacQ140 mice and preserved by early m*Htt* lowering. Detailed analysis of lipid composition revealed reductions in ceramide (Cer), sphingomyelin (SM), monogalactosyl diacylglycerol (MGDG), and hexosylceramide (Hex1Cer) species which are known to be important for myelin stabilization and maintenance. Increases in the membrane phospholipids phosphatidylinositol (PI), phosphatidylserine (PS), and in bismethyl phosphatidic acid (BisMePA) were also measured. Critically, many of these alterations were prevented with m*Htt* lowering. The RNA-sequencing dataset (GSE156236) in this model was re-analyzed to determine if our results were driven by transcriptional alterations for enzymes involved in lipid biosynthesis or metabolism. Pathway enrichment analysis demonstrated extensive dysregulation of pathways related to lipid metabolism/biosynthesis and myelination. Lowering m*Htt* prevented these transcriptional alterations in 6-month-old mice, however, most benefits were attenuated in 12-month-old mice. Together, our findings suggest that the presence of white matter pathology analogous to that seen in HD patients is detectable using a lipidomic approach. Further, early and sustained reduction in m*Htt* is sufficient to prevent changes in lipids enriched in myelin and important for synaptic function.

Disclosures: K. Shing: None. **E. Sapp:** None. **A. Boudi:** None. **S. Liu:** None. **C. Seeley:** A. Employment/Salary (full or part-time):; Biogen. **D. Marchionini:** A. Employment/Salary (full or part-time):; CHDI Foundation. **M. DiFiglia:** None. **K. Kegel-Gleason:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.10/T9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CHDI
HDF
Dake family

Title: Early impairments of oligodendrocyte lineage in Huntington’s disease

Authors: *A. BOUDI, E. SAPP, K. L. SHING, Y. LI, M. DIFIGLIA, X. LI;
Massachusetts Gen. Hosp. - Harvard Med. Sch., Charlestown, MA

Abstract: White matter alterations are a recurring phenomenon observed in various neurodegenerative diseases. Determining whether it contributes to neuronal loss or arises as a consequence remains a hypothesis that requires further investigation for most of these pathologies.
Despite being an autosomal dominant disorder, the precise sequence of events leading to the fatal outcome in Huntington's disease (HD) remains unclear. While the susceptibility of medium spiny neurons in the striatum and glutamatergic neurons in the cortex has been extensively studies, comprehensive research focusing on glial cells, including the oligodendroglial lineage, is needed. This poster presents our findings obtained from an HD mouse model (HDQ140/Q140). Our protein analysis reveals a progressive decline in myelin-related proteins within the striatum and cortex. Notably, some of these impairments are observable as early as 3 months. We have identified abnormalities at the ultrastructural level through transmission electron microscopy. Additionally, our *in vitro* analysis of oligodendrocyte progenitor cells from neonatal brains suggests that mutant huntingtin directly contributes to intrinsic defects.
Overall, our data underscore the early and progressive dysfunctions of the oligodendrocyte lineage in Huntington's disease.

Disclosures: A. Boudi: None. **E. Sapp:** None. **K.L. Shing:** None. **Y. Li:** None. **M. Difiglia:** None. **X. Li:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.11/T10

Topic: C.01. Brain Wellness and Aging

Support: Université Grenoble Alpes - EUR 2021
Fondation Recherche Médicale - Team Humbert

Title: Premature Aging of Neural Stem Cells in Huntington's Disease

Authors: *C. LAFAGE[1], L. RATIÉ[1], F. AGASSE[1], S. HUMBERT[1,2];
[1]Grenoble Inst. Neurosciences, La Tronche, France; [2]Paris Brain Inst., Paris, France

Abstract: An overall decline in neurogenesis is observed during physiological aging as well as in neurodegenerative disorders and Huntington's disease (HD) is no exception to the rule. While neural stem cells (NSCs) in the adult Rodent subventricular zone (SVZ) generate new neurons throughout life, this neurogenesis persists in the adult Human brain with new interneurons, probably born in the nearby SVZ, addressed to the striatum. Interestingly, this particular striatal neurogenesis decreases in HD patients (Ernst *et al*. Cell *156* (2014)). This fatal neurodegenerative disorder with an adult onset is caused by an autosomal dominant mutation in the huntingtin gene. The mutation is an abnormal expansion of a polymorphic CAG repeat leading to an extended polyglutamine stretch at the N-terminus of the huntingtin protein (HTT). This decline in neurogenesis, both in physiological and pathological conditions, can be attributed to the exhaustion of the NSCs pool, here from the SVZ, which represents one of the hallmarks of aging. Through the study of a knock-in mouse model of HD, the *Hdh*^{Q111} strain, we were able to show that mutant HTT decreases SVZ neurogenesis by altering NSCs properties. *In vitro* NSCs demonstrate restricted potency including a limited capacity to self-renew and proliferate suggesting a decreased NSCs pool. This result is further supported by the decreased number of SVZ NSCs and progenitors in 6 months old HD brains as compared to WT counterparts. Thus, it would appear that markers of aging emerge from the study of SVZ stem cells. Therefore, we further hypothesize that this reduced neurogenesis may be due to accelerated aging features observed in HD, triggering stem cells entry in a senescence-like state. Preliminary experiments indeed show higher proportions of SA-β-galactosidase positive cells in HD stem/progenitor cells cultures. Ongoing genetic and epigenetic analysis aim to screen changes in gene expression associated with stemness and aging, thereby validating a set of aging hallmarks. Understanding the regulation of NSCs in the SVZ may help to design new therapies to sustain neurogenesis in the old and diseased brain.

Disclosures: C. Lafage: None. **L. Ratié:** None. **F. Agasse:** None. **S. Humbert:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.12/U1

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Establishing a hPSC derived Huntington’s disease neuronal model suitable for phenotypic drug screening and to identify small molecule modulators of mutant HTT

Authors: L. BUTLER[1], M. STEBBEDS[1], D. MAGNANI[1], M. BROWN[1], G. LANGLEY[1], J. ANTON[1], G. GILBAHACE[1], C. MANSAT-BHATTACHARYYA[1], P. MITCHELL[1], ***M. HERVA MOYANO**[1], M. IOVINO[1], T. OOSTERVEEN[2], O. DOVEY[2], T. FROLOV[2], F.

PATELL-SOCHA[2];
[1]Charles River Labs., Saffron Walden, United Kingdom; [2]bit.bio, Cambridge, United Kingdom

Abstract: Lowering of the pathogenic mutant huntingtin (mHTT) protein in Huntington's disease (HD) patients is one of the leading approaches to ameliorate the fatal neurodegeneration caused by the poly-CAG expansion in the Htt gene. Current therapeutics in development involve small molecule splicing modulators or use of novel biological agents such as ASOs, RNAi, ZFTR and CRISPR/Cas9. Therefore, physiologically relevant and scalable models of HD are needed to improve outcomes and efficiency of drug development. The use of human induced Pluripotent Stem Cell (iPSC)-derived neurons would offer disease relevant in vitro system however can be hindered by low scalability, and long complex protocols. The cell reprogramming technology, opti-oxTM , in combination with CRISPR-Cas9 gene editing has been used to develop iPSC-derived ioGlutamatergic Neurons carrying a HTT allele with an abnormal 50 CAG repeat expansion. We have used high content imaging analysis and branched DNA assay to characterise ioGlutamatergic Neurons HTT50CAG/WT together with their isogenic control. Moreover, we have used Multi Electrode Array (MEA) platform to study functional activity of the ioGlutamatergic Neurons HTT50CAG/WT compared to the isogenic control. Preliminary functional data showed that ioGlutamatergic Neurons HTT50CAG/WT demonstrated formation of synchronous activity at late stage of maturation, suggesting these neurons are electrophysiologically active and amenable to functional studies. Additionally, we have tested Branaplan, a splicing modulator small molecule that lowers HTT levels, and shown the expected effects on the ioGlutamatergic HTT50CAG/WT neurons.

Disclosures: **L. Butler:** None. **M. Stebbeds:** None. **D. Magnani:** None. **M. Brown:** None. **G. Langley:** None. **J. Anton:** None. **G. Gilbahace:** None. **C. Mansat-Bhattacharyya:** None. **P. Mitchell:** None. **M. Herva Moyano:** None. **M. Iovino:** None. **T. Oosterveen:** None. **O. Dovey:** None. **T. Frolov:** None. **F. Patell-Socha:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.13/U2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NS105709
NS119471
NS091161
NS049206
CHDI Foundation

Title: Base editing strategies to convert CAG to CAA in huntington's disease

Authors: *D. CHOI, J. SHIN, S. ZENG, E. HONG, J. JANG, J. LOUPE, V. WHEELER, H. STUTZMAN, B. KLEINSTIVER, J.-M. LEE;
Massachusetts Gen. Hospital/ Harvard Med. Sch., Boston, MA

Abstract: An expanded CAG repeats in huntingtin gene (*HTT)* results in changes in various neurological domains in Huntington's disease (HD). Recent large-scale Genome-wide association studies (GWASs) of HD subjects revealed naturally occurring variations that eliminate and generate CAA-CAG interruption at the end of the *HTT* CAG repeat are associated with significantly hastened and delayed age at onset respectively compared to the canonical repeat of the same polyglutamine length, supporting the importance of the size of the uninterrupted CAG repeat. Since the length of uninterrupted CAG repeat, not polyglutamine, determines HD onset, base editing (BE) strategies to convert CAG to CAA may delay onset by shortening uninterrupted CAG repeat. Here, we evaluated the use of cytosine base editors (CBEs) to convert CAG-to-CAA, and determined its feasibility, molecular outcomes, and effects on relevant disease phenotypes using HD patient-derived iPSCs, differentiated neurons, and HD HEK293-51 CAG cells . Combinations of four cytosine base editors (CBE) and eight therapeutic strategies efficiently converted *HTT* CAG repeat at various sites to CAA without generating significant indels in the MiSeq data, off-target conversions, or alternations of transcription profile from RNA-seq data, demonstrating the feasibility and on-target specificity. Base editing strategies were able to decrease the size of the uninterrupted CAG repeat without altering polyglutamine length, aiming at delaying age-at-onset. A candidate CBE converted CAG to CAA on both expanded and non-expanded *HTT* CAG repeats without altering the levels of *HTT* mRNA and protein. Importantly, the CAG-to-CAA conversion strategy significantly decreased somatic CAG repeat expansion in HttQ111 HD knock-in mice, AAV9-CBE-gRNA delivered by retro-orbital injection to 6-week-old mice, supporting the therapeutic potential of CAG-to-CAA conversion in HD.

Disclosures: D. Choi: None. **J. Shin:** None. **S. Zeng:** None. **E. Hong:** None. **J. Jang:** None. **J. Loupe:** None. **V. Wheeler:** F. Consulting Fees (e.g., advisory boards); Acadia Pharmaceuticals Inc., Biogen Inc. and Passage Bio. **H. Stutzman:** None. **B. Kleinstiver:** F. Consulting Fees (e.g., advisory boards); Acrigen Biosciences, Life Edit Therapeutics, and Prime Medicine. **J. Lee:** F. Consulting Fees (e.g., advisory boards); GenEdit Inc..

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.14/U3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH RO1 grant NS113612
Hereditary Disease Foundation

Title: Fan1/FAN1 genetic mutants bidirectionally modify mutant huntingtin CAG-repeat instability and neuropathology in Q140 knockin mouse model of HD

Authors: *L. DENG[1,2], J. RICHMAN[2], K. TAMAI[1], N. WANG[2], P. LANGFELDER[2], X. GU[1,2], F. GAO[1,2], G. COPPOLA[3], C. S. COLWELL[1], X. YANG[1,2];
[1]Psychiatry and Biobehavioral Sci., [2]Semel Inst. of Neurosci. and Human Behavior, [3]Neurol., UCLA, Los Angeles, CA

Abstract: In Huntington's Disease (HD), the mutant Huntingtin (mHtt) CAG repeat length is inversely correlated with the age of onset of motor and cognitive symptoms. Recent Genome-Wide Association Studies (GWAS) revealed significant loci that modify the age of onset and/or progression landmarks in HD (McAllister et al., 2022, PMID: 35379994). Among the genes linked to the HD GWAS modifier loci, four alleles near FAN1 (a DNA repair gene) either accelerate or delay the age of onset. Here we undertook a mouse genetic approach to study three Fan1 genetic mutations in a HD mouse model, a knockdown allele of Fan1 (about 70% reduction of Fan1 expression; Fan1-KD allele), a novel Fan1-R510H knockin allele (similar to disease hastening FAN1-R507H allele; Fan1-KI allele), and a human BAC transgenic FAN1 overexpression allele (resulting in elevated FAN1 expression and delaying disease onset; BAC-FAN1 allele). We crossed these three Fan1/FAN1 mutant alleles to Q140 murine mutant Huntingtin (mHtt) knockin mice to assess disease modifications. We found that Fan1-KD allele shows a significant increase in mHtt CAG repeat somatic instability in the striatum and liver while BAC-FAN1 shows repeat stabilization. Both Fan1-KD and Fan1-KI alleles enhance mHtt aggregates, while BAC-FAN1 reduces mHtt aggregates in the striatum of Q140 mice. Additionally, Fan1-KI exacerbates locomotor behavioral deficits while BAC-FAN1 ameliorates such deficits in HD mice. We also showed that genetic overexpression of human FAN1 continues to rescue mHtt deficits in Q140 mice up to 12-months of age. We conclude that Fan1 mutant mice, mimicking the molecular genotypes of HD modifier alleles, exert bi-directional effects on mHtt somatic repeat instability and aggregation. Furthermore, the disease-reducing effects of human FAN1 genomic overexpression are long lasting. Our study provides novel insights into the genetic modifier effects of FAN1 with the potential to develop human FAN1-targeted therapy for HD.

Disclosures: L. Deng: None. **J. Richman:** None. **K. Tamai:** None. **N. Wang:** None. **P. Langfelder:** None. **X. Gu:** None. **F. Gao:** None. **G. Coppola:** None. **C.S. Colwell:** None. **X. Yang:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.15/U4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH/NINDS Grant RO1-NS110943
NIH/NIA Grant AG057509

Title: Investigating HAP40's Role in Regulating Huntingtin: A Structural-Functional Analysis

Authors: *A. SOLBACH[1], S. XU[2], X. YE[2], S. FARMER[1], Y. YU[1], L. YE[2], S. ZHANG[2]; [1]Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [2]The Brown Fndn. Inst. of Mol. Med., Houston, TX

Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by an abnormal expansion of polyglutamine (polyQ) repeats in Huntingtin (HTT). Characterized by movement disorders, psychiatric symptoms, and cognitive deficits, HD primarily affects specific neurons in the striatum and cortex. The precise etiology of HD remains unclear, but it is believed to involve both gain-of-function toxicity caused by long polyQ repeats and disruption of HTT's normal functions. As a result, "HTT-lowering" strategies, such as antisense oligonucleotide (ASO) drugs to downregulate HTT expression, are being explored. However, permanently reducing HTT levels may have adverse effects due to its essential role as a scaffold protein in various cellular processes, from autophagy to transcription, throughout brain development and maintenance. When studying the *Drosophila* HTT homolog (dHtt), we identified Htt-associated protein 40 (HAP40) as one of the strongest binding partners of HTT, and demonstrated that the fly ortholog of HAP40 shares loss-of-function phenotypes with dHtt and acts as its potent modulator. In both flies and human cells, HAP40 affects HTT's protein stability, normal physiological functions and the toxicity of mutant HTT. Notably, loss of HAP40 leads to significantly reduced levels of endogenous HTT in both *Drosophila* and human cells, establishing HAP40 as a conserved central regulator of HTT. Further investigation of HAP40 identified a unique structural motif at the N-terminus of HAP40, termed "BΦ," which we hypothesize does not directly bind to HTT but plays a crucial regulatory role in the HTT/HAP40 complex. Supporting evidence suggests that the BΦ motif modulates the functions of the HTT/HAP40 complex by interacting with upstream regulators and/or downstream effectors of HTT. To explore the functional significance of the BΦ motif, we have conducted deletional analyses on both *Drosophila* and human HAP40 proteins. These analyses involved replacing the BΦ domain with small HA or Myc epitopes and expressing the BΦ domain alone with double tags (e.g., "BΦ-3XMyc-TagRFP"). Through *in vitro* biochemical studies using cultured mammalian cells and *in vivo* functional evaluations with transgenic flies, we have been systematically examining the effect of the BΦ motif on the stability, subcellular localization, and physiological activities of HAP40 and HTT proteins. Additionally, we have been investigating its potential influence on the neuronal toxicity of polyQ-expanded mutant HTT. These findings will contribute to our understanding of the molecular mechanisms through which HAP40 regulates HTT and may unveil novel therapeutic targets to treat HD.

Disclosures: A. Solbach: None. **S. Xu:** None. **X. Ye:** None. **S. Farmer:** None. **Y. Yu:** None. **L. Ye:** None. **S. Zhang:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.16/U5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH/NINDS Grant RO1-NS110943
NIH/NIA Grant AG057509

Title: Huntingtin-hap40 core complex regulates endolysosomal trafficking in huntington's disease

Authors: *S. M. FARMER[1], Y. YU[1], S. XU[2], X. YE[2], A. SOLBACH[1], B. RIOS[1], D. COVARRUBIAS[3], T. I. MOORE[4], S. ZHANG[2];
[1]Grad. Sch. of Biomed. Sci., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Ctr. for Metabolic and Degenerative Dis., Univ. of Texas Hlth. Sci. Ctr. at Houston, The Brown Fndn. Inst. of Mol. Med., Houston, TX; [3]Rice Univ., Houston, TX; [4]Dept. of Integrative Biol. and Pharmacol., Univ. of Texas Hlth. Sci. Ctr. at Houston, McGovern Med. Sch., Houston, TX

Abstract: With no effective treatments and cures, ageing-related neurodegenerative diseases (NDs) present a pressing threat to our society. Increasing evidence links NDs to endolysosomal trafficking, a major homeostasis process that maintains cellular tidiness by regulating protein-cargo recycling and degradation. Dysfunction in this biological process often causes abnormal accumulation of misfolded proteins in the brain leading to NDs. One representative ND is Huntington's disease (HD), a dominantly transmitted disease caused by a single type of mutation (CAG amplification) that lead to an expanded poly-glutamine tract (polyQ) in the Huntingtin (HTT) protein. Converging evidence from biochemical, structural, and genetic studies, including that from our lab, revealed that full-length HTT exists primarily in a complex with HAP40, and HAP40 regulates HTT's protein stability and cellular functions. Both proteins are conserved in *Drosophila* (fruit flies), a powerful model for studying genes involved in human diseases including HD. While analyzing HTT and HAP40 homologs in *Drosophila,* we found that their depletion causes similar abnormal lysosomal phenotypes. Functional experiments in flies demonstrated that HTT and HAP40 bind Rab7-positive late endosomes, and that their depletion causes specific endosomal defects, a phenotype that was further validated in cultured mammalian cells using structured illumination microscopy (SIM). Moreover, HTT was found enriched in regions immuno- positive for Rab7-late endosomes in neurons (Elav+) rather than Glia (Repo+). Lastly, we observed abnormal dendritic structures in multi-dendritic neurons from both HTT- and HAP40-knockout flies. Together, these data support a potentially novel functional role of the HTT/HAP40 complex in endosomal trafficking with important implication in neuronal development and function. Our current works aim to elucidate the mechanism of HTT/HAP40's involvement in endosomal pathways and to address how this process becomes pathogenic upon polyQ expansion, and new therapeutic targets for HD.

Disclosures: S.M. Farmer: None. **Y. Yu:** None. **S. Xu:** None. **X. Ye:** None. **A. Solbach:** None. **B. Rios:** None. **D. Covarrubias:** None. **T.I. Moore:** None. **S. Zhang:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.17/U6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Dake Family Foundation

Title: Ww domain-containing oxidoreductase is increased in huntington's disease and contributes to dna damage

Authors: *S. S. HUNTRESS[1], T. PETROZZIELLO[1], A. L. CASTILLO-TORRES[1], S. LIU[1], F. MAHMOOD[1], A. BOUDI[1], M. WU[1], E. SAPP[1], M. A. POULADI[2], M. DIFIGLIA[1], K. KEGEL-GLEASON[1], R. MOURO PINTO[1], G. SADRI-VAKILI[1];
[1]Neurol., Massachusetts Gen. Brigham, Boston, MA; [2]Univ. of British Columbia, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The specific mechanisms underlying neuronal loss in Huntington’s disease (HD) are not fully elucidated, despite the genetic cause of the disease being well characterized. Here, we investigated the potential role exerted by the WW domain-containing oxidoreductase (WWOX) in HD. WWOX, widely involved in other neurodegenerative diseases, plays a crucial role in several cellular functions, such as the DNA damage response (DDR), a mechanism involved in HD pathogenesis. Our results revealed a significant increase in WWOX levels in HD post-mortem prefrontal cortex (PFC) compared to controls as well as in HD embryonic stem cell (ESC)-derived cortical neurons compared to isogenic control cells. Additionally, our findings demonstrated that the treatment of human neuroblastoma SH-SY5Y cells with high concentrations of a recombinant human WWOX protein (rWWOX) induced an increase in histone H2AX phosphorylation at serine 139 (γ-H2AX), a marker of DNA double stranded breaks (DSBs), as measured by immunofluorescence. A similar increase in γ-H2AX was observed in cells following treatment with post-mortem HD PFC. Importantly, γ-H2AX levels were significantly increased in cells treated with HD PFC fractions that were enriched for endogenous WWOX while there was no effect on γ-H2AX levels following treatment with HD PFC that was depleted of WWOX. Taken together, these findings reveal a role for WWOX in activating histone H2AX suggesting that increases in WWOX may induce DNA damage in HD.

Disclosures: S.S. Huntress: None. **T. Petrozziello:** None. **A.L. Castillo-Torres:** None. **S. Liu:** None. **F. Mahmood:** None. **A. Boudi:** None. **M. Wu:** None. **E. Sapp:** None. **M.A. Pouladi:** None. **M. Difiglia:** None. **K. Kegel-Gleason:** None. **R. Mouro Pinto:** None. **G. Sadri-Vakili:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.18/U7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NRF-2016M3C7A1905074
NRF-2017R1A2A2A05069493
NRF-2021R1H1A2010055
NRF-2022R1A2C2009817

Title: Micrandilactone C, a Nortriterpenoid Isolated from Roots of Schisandra chinensis, Ameliorates Huntington's Disease by Inhibiting Microglial STAT3 Pathways

Authors: *H. JO[1], M. JANG[2], J. CHOI[2], I. CHO[2];
[1]Dept. of Convergence Med. Science, Col. of Korean Medicine, Kyung Hee Univ., Seoul, Korea, Republic of; [2]Dept. of Convergence Med. Science, Col. of Korean Medicine, Kyung Hee Univ., seoul, Korea, Republic of

Abstract: Huntington’s disease (HD) is a neurodegenerative disease that affects the motor control system of the brain. Its pathological mechanism and therapeutic strategies have not been fully elucidated yet. The neuroprotective value of micrandilactone C (MC), a new schiartane nortriterpenoid isolated from the roots of Schisandra chinensis, is not well-known either. Here, the neuroprotective effects of MC were demonstrated in 3-nitropropionic acid (3-NPA)-treated animal and cell culture models of HD. MC mitigated neurological scores and lethality following 3-NPA treatment, which is associated with decreases in the formation of a lesion area, neuronal death/apoptosis, microglial migration/activation, and mRNA or protein expression of inflammatory mediators in the striatum. MC also inhibited the activation of the signal transducer and activator of transcription 3 (STAT3) in the striatum and microglia after 3-NPA treatment. As expected, decreases in inflammation and STAT3-activation were reproduced in a conditioned medium of lipopolysaccharide-stimulated BV2 cells pretreated with MC. The conditioned medium blocked the reduction in NeuN expression and the enhancement of mutant huntingtin expression in STHdhQ111/Q111 cells. Taken together, MC might alleviate behavioral dysfunction, striatal degeneration, and immune response by inhibiting microglial STAT3 signaling in animal and cell culture models for HD. Thus, MC may be a potential therapeutic strategy for HD.

Disclosures: H. Jo: None. **M. Jang:** None. **J. Choi:** None. **I. Cho:** None.

Poster

PSTR467. Huntington's Disease: Molecular and Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR467.19/U8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: JSPS

Title: Repetitive Transcranial Magnetic Stimulation (rTMS) induced NET (norepinephrine transporter) and NET b regulate the expression of caspases mRNA and protein activities on PC12 cells

Authors: *T. IKEDA;
Aomori-University, Aomori-City, Japan

Abstract: rTMS is a noninvasive technique to induce electric current in the brain and is supposed to be beneficial for the treatment of patients with depression, schizophrenia and neurodegenerative disorders. We reported previously that rTMS modulates norepinephrine transporter (NET) on PC12 cells. On the other hand, we reported that NETb is dominant negative type of NET and its function after rTMS is unclear. So we analyzed the changes in mRNA expression of NET and NETb after rTMS with real time PCR. Following 15 days of rTMS, NET and NETb mRNA were increased in PC12 cells. So, to confirm the function of expression of NET and NETb, we transiently expressed both NET and NETb on PC12cells. The expression of NET and NETb regulated caspaces mRNA expression levels. Down-regulation of caspase-2, -3, -4, -8 and -11 mRNA expression levels were observed. And more, up-regulation of caspase-1 and -9 mRNA expression levels were observed. So we confirmed the effects of NET and NETb co-expression on N2a cells expressing 150Q (cell model of Huntington's disease). Compare to control, NET and NETb co-expression decreased the caspase-3 protein activity and cell death on N2a cells expressing 150Q. These results indicated that rTMS induced NET and NET b may regulate the expression of caspases and protect from cell death on N2a cells expressing 150Q, may be involved in the therapeutic mechanisms of rTMS for patients with neurodegenerative disorders.

Disclosures: T. Ikeda: None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.01/U9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: German Federal Ministry of Education and Research (BMBF, E-Rare project SCA-CYP, grant no. 01GM1803)
German Federal Ministry of Education and Research (BMBF, EuSAge project, grant no. 01DN18020)

Title: Common genetic polymorphisms as disease modifying factors in Spinocerebellar Ataxia Type 3 (SCA3) / Machado-Joseph disease (MJD)

Authors: L. CZISCH[1], J. J. WEBER[1,2], R.-M. BURGER[1], J. JUNG[1], C. MEYER[1], P. PEREIRA SENA[1], H. HAN[1], S. MARTINS[3], L. BANNACH JARDIM[4], M. L. SARAIVA-PEREIRA[5], M. C. FRANÇA, Jr,[6], C. R. GORDON[7], M. R. CORNEJO-OLIVAS[8], ***T. SCHMIDT**[1];

[1]Univ. Tuebingen, Med. Genet., Tuebingen, Germany; [2]Ruhr-Univ Bochum, Dept. of Human Genet., Bochum, Germany; [3]Univ. Porto, Inst. of Mol. Pathology and Immunol., Porto, Portugal; [4]Hosp. de Clinicas de Porto Alegre, Porto Alegre, Brazil; [5]Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil; [6]Univ. Estadual de Campinas (UNICAMP), Campinas, Brazil; [7]Tel Aviv Univ., Tel Aviv, Israel; [8]Inst. Nacional de Ciencias Neurológicas, Lima, Peru

Abstract: Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited, neurodegenerative disorder caused by the expansion of a CAG repeat within the *ATXN3* gene resulting in an expanded polyglutamine repeat in the encoded ataxin-3 protein. SCA3/MJD, therefore, belongs to the group of polyglutamine diseases comprised of nine neurodegenerative diseases including Huntington's disease. Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists and patients with more CAG repeats have an earlier onset of symptoms. However, this statistical correlation is not perfect and the number of CAG repeats contributes only about 55% to the age at onset. Therefore, the remaining 45% are influenced by other factors, which we aim to identify in this study. In order to identify modifiers of the disease progression, we genotyped in a combined European and South American approach more than 500 SCA3/MJD patients for promising polymorphisms in candidate genes. Candidate genes included *ATXN3* itself, genes coding for known interaction partners of ataxin-3, functional modifiers identified in previous studies as well as genes with known relevance for the pathophysiology of SCA3/MJD including genes involved in the nucleocytoplasmic transport. We selected polymorphisms with a high likelihood of having a functional relevance i.e. polymorphisms in the promoter regions as well as polymorphisms leading to amino acid changes. While controlling for ethnic origin we assessed the contribution of the respective polymorphism to the age at onset in addition to the already known modifying factor, the length of the expanded CAG repeat within *ATXN3*. We indeed identified interesting polymorphisms contributing to the age at onset including certain haplotypes within *ATXN3* itself and could validate their functional impact on pathogenic mechanisms in SCA3/MJD. Our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3/MJD.

Disclosures: L. Czisch: None. **J.J. Weber:** None. **R. Burger:** None. **J. Jung:** None. **C. Meyer:** None. **P. Pereira Sena:** None. **H. Han:** None. **S. Martins:** None. **L. Bannach Jardim:** None. **M.L. Saraiva-Pereira:** None. **M.C. França:** None. **C.R. Gordon:** None. **M.R. Cornejo-Olivas:** None. **T. Schmidt:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.02/U10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: KRIBB KGM4562222

Title: Ataxin-3 overexperession via AAV viral vector injection in the primate cerebellum

Authors: *K. KEONWOO[1,2], P. JUNGHYUNG[1], W. JINYOUNG[1], S. JINCHEOL[1], J. CHANG-YEOP[1], L. KYUNG SEOB[3], G. LEE WHA[1,4], P. SUNG-HYUN[1], C. WON SEOK[1], L. YOUNGJEON[1], L. DONG-SEOK[5];
[1]KRIBB Natl. Primates Res. Ctr., Cheongju-si, Korea, Republic of; [2]Sch. of Life Sci. and Biotechnology, BK21 FOUR KNU Creative BioResearch Group,, Kyungpook Natl. Univ., Daegu, Korea, Republic of; [3]KRIBB Futuristic Animal Resource and Res. Ctr., Cheongju-si, Korea, Republic of; [4]Univ. of Sci. and Technol., Daejeon, Korea, Republic of; [5]Sch. of Lifescience, KyungpookNational Univ., Daegu, Korea, Republic of

Abstract: Ataxin-3 is a well-established gene associated with spinal cerebellar ataxia 3 (SCA3), a known neurodegenerative disorder. Ataxin-3 have of PolyQ repeats in its exon 10. When these PolyQ repeats are abnormally expanded, mutant Ataxin-3 protein is generated and aggregated in the brain, resulting in SCA3 disorder. Transgenic or KI (knock-in) SCA3 model animals have been developed, but the onset and progression of the disease is rapid, as opposed to human patients. Thus, these models cannot reproduce all pathogenic aspects of the disease in humans. So, we tried to develop a new SCA3 model using viral vector delivery. Previous studies have established methods to transfer and overexpress genetic material into animal tissues using viral vectors resulting in disease model development as well as gene therapy. AAV vectors are currently well-known as most effective carriers for intra-tissue transfer due to their non-pathogenicity and low immunogenicity. For this study, we constructed an AAV-viral vector loaded with mutant Ataxin-3 with PolyQ 84. The AAV vector loaded with mutant Ataxin-3 was injected into the cerebellum of the monkey while AAV vector loaded with GFP was injected into the cerebellum of the control monkey. After 8 weeks of injection, immunostaining using Ataxin-3 antibody, myc antibody and PolyQ inclusion antibody was performed on the cerebellar tissue of both monkeys to verify the expression of the viral vector and the number of purkinje cells expressing Ataxin-3 was counted. The number of purkinje cells expressing Ataxin-3 was found to be higher in the test monkey injected with the viral vector loaded with Ataxin-3. These results suggest that the injected vector moved into the cerebellar tissue and expressed Ataxin-3. However, we could not confirm the behavioral symptoms caused by the overexpressed mutant ataxin-3 and whether neurodegeneration occurred.

Disclosures: K. Keonwoo: None. **P. Junghyung:** None. **W. Jinyoung:** None. **S. Jincheol:** None. **J. Chang-Yeop:** None. **L. Kyung Seob:** None. **G. Lee Wha:** None. **P. Sung-Hyun:** None. **C. Won Seok:** None. **L. Youngjeon:** None. **L. Dong-Seok:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.03/V1

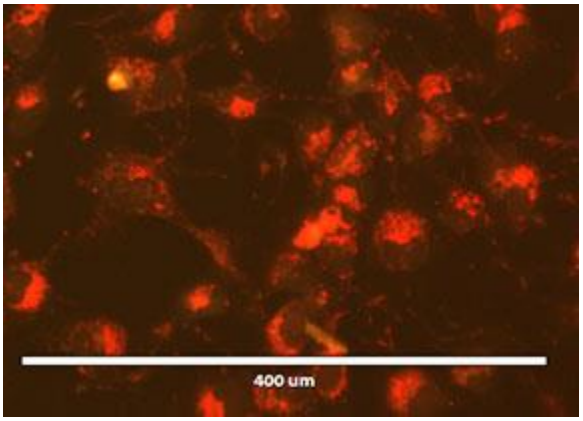
Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NSF 2018952

Title: Imaging physiological deficits in a Spinocerebellar ataxia type 1 cell model

Authors: *A. U. GLYNN, S. LAGALWAR;
Neurosci., Skidmore Col., Saratoga Springs, NY

Abstract: Spinocerebellar Ataxia Type 1 (SCA1) is a progressive neurodegenerative disease primarily affecting cerebellar Purkinje neurons, characterized by an abnormal expansion of CAG repeats within the coding region of the ataxin-1 (ATXN1) gene. Recent published work supports the interaction between mutant polyQ-expanded ATXN1 and mitochondrial proteins involved in apoptosis, oxidative phosphorylation (OXPHOS), membrane composition, and mitochondrial gene transcription. Work in our lab has further found that mitochondrial dysfunction is associated with SCA1 in mice models and in vivo application of the OXPHOS substrate succinic acid ameliorates Purkinje cell neurodegeneration and cerebellar behavioral deficits. Human cerebellar-derived cellular models of SCA1 reveal gross mitochondrial morphological, locational and compositional abnormalities, along with increased oxidative stress and metabolism. In these models, succinic acid treatment and mitochondrial-specific antioxidants reduce the effects of oxidative stress. Here we characterize in vitro physiological deficits in our cellular models through live cell imaging of mitochondrial membrane potential and calcium signaling. Since high energy-demanding cerebellar Purkinje cells bear the brunt force of the pathology, mitochondria emerge as potential targets for therapeutic intervention to alleviate the symptoms and pathology of the disease.



Disclosures: A.U. Glynn: None. **S. Lagalwar:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.04/V2

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: 2R01NS027699-34

Title: Exploring the Effects of Wildtype Ataxin-1 Dosage in Spinocerebellar Ataxia Type 1

Authors: *E. XHAKO[1,2,8], L. NITSCHKE[3,8], S. COFFIN[4,8], M. DURHAM[5,6,8], N. A. BYRON[1,8], H. Y. ZOGHBI[2,7,8,9];
[2]Mol. and Human Genet., [3]3Program in Integrative Mol. and Biomed. Sci., [4]Dept. of Mol. and Human Genet., [5]Med. Scientist Training Program, [6]Program in Developmental Biol., [7]Pediatrics, [1]Baylor Col. of Med., Houston, TX; [8]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [9]Howard Hughes Med. Inst., Houston, TX

Abstract: Spinocerebellar Ataxia Type 1 (SCA1) is an adult-onset neurodegenerative disease characterized by motor incoordination, mild cognitive decline, respiratory dysfunction, and early lethality. These symptoms are due to the progressive degeneration of neurons in the cerebellum, hippocampus, and brainstem. SCA1 is caused by the expansion of a CAG repeat encoding the polyglutamine (polyQ) tract in ATAXIN-1 (ATXN1). Previous studies from our lab have shown that the polyQ-expansion causes a gain-of-function of ATXN1, leading to toxicity in cerebellar Purkinje cells. As such, decrease of the polyQ-expanded ATXN1 improves the cerebellar SCA1 phenotypes, and most therapeutic work has since focused on developing methods to decrease ATXN1 levels. Interestingly, in a recent study, we found that combined reduction of both expanded and wildtype (WT) ATXN1 drastically decreases the phenotypic rescue. We thus hypothesize that the ratio of WT to expanded ATXN1 is important to the phenotypic severity in SCA1, and that WT ATXN1 might have a protective role in SCA1. To test this hypothesis and investigate the importance of WT ATXN1 in all affected brain regions, we first crossed our *Atxn1*$^{154Q/+}$ (SCA1) mice to *Atxn1*$^{+/-}$ heterozygous knockout mice to generate SCA1 mice that do not contain the WT *Atxn1* allele (*Atxn1*$^{154Q/-}$). We found that across all behavioral and molecular tests performed *Atxn1*$^{154Q/-}$ were more severe than *Atxn1*$^{154Q/+}$ animals, indicating that WT ATXN1 is important for the severity of disease phenotypes in SCA1. Following up on these results, we have created an ATXN1 overexpression mouse model that conditionally increased WT ATXN1 levels. Characterization of this animal will allow us to determine the consequences of increased WT ATXN1 in our SCA1 model. While the cerebellum is the most affected area in SCA1, brainstem and hippocampal phenotypes will also be characterized. This study will allow us to gain insights into brain-region specific disease mechanisms in SCA1, understand the mechanism of the protective role of WT ATXN1 and provide a framework by which other region-specific neurodegenerative diseases can be studied.

Disclosures: E. Xhako: None. **L. Nitschke:** None. **S. Coffin:** None. **M. Durham:** None. **N.A. Byron:** None. **H.Y. Zoghbi:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.05/V3

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NS082351

Title: Jnk driven Bergmann glial inflammation plays a central role in the pathology of spinocerebellar ataxias

Authors: V. MOHAN[1], C. R. EDAMAKANTI[1], ***P. OPAL**[2];
[1]Northwestern, Chicago, IL; [2]northwestern, Chicago, IL

Abstract: Background and Objective: Bergman glial cells are unipolar astrocytes (only radial astrocyte of cerebellum) play important role in cerebellar development, physiology. Like other astroglial cells, Bergmann glia cells also respond to neuronal damage with morphological and functional changes, hence become reactive or activated glial cells. Despite the widespread expression of mutant proteins throughout nervous system, including glial cells, most of autosomal dominant Spinocerebellar ataxias characterized by predominant loss of Purkinje cells in cerebellum. Understanding the role of glia has been difficult to decipher because of the variety of glial subtypes each with their potential distinct contribution to neuronal function. While many studies focused on the significant contribution of glial cells (including microglia and astrocytes) in selective vulnerability of neurons and disease progression, there is lack of evidence for the contribution of Bergmann glial specific gliosis in Purkinje cell pathology and its associated cerebellar ataxia phenotype. In this study we unraveled the evidence and mechanism for the Bergmann glial-driven toxicity of Purkinje cells in Spinocerebellar ataxia disorders. **Results:** We discovered that the c-Jun N-terminal kinase (JNK)- dependent c-Jun transactivation is essential for Bergmann glial reactive state in both SCA patients and mice cerebellum. The release of Bergmann glial specific inflammatory factor Interleukin 1 beta (IL-1β) in cerebellum requires the JNK dependent c-Jun phosphorylation of Bergmann glial cells. Treatment of SCA1 mice with JNK inhibitor abrogates the c-Jun phosphorylation thereby reduces the inflammation in cerebellum. Following JNK inhibitor treatment for 8 weeks, SCA1 mice show significant improvement in cerebellar associated phenotype including motor coordination function and Purkinje cell pathology. Together, these findings support the evidence for the role of c-Jun in Bergmann glial-mediated neuroinflammatory dysfunction of the cerebellum in SCA1 disorder.**Conclusion:** To our knowledge, this is the first study to address the contribution of Bergmann glia-specific inflammation in neurodegenerative disease condition. Overall, our findings support the fact that the JNK/c-Jun pathway is critical for the release of IL-1β from Bergmann glia in SCA1 disease condition and by targeting either JNK/c-Jun pathway or IL-1β could serve as therapeutic approach in treating the disease. This unique pharmacological intervention in tamping down the Bergmann glial-specific inflammation could be applicable to other type of Spinocerebellar ataxias other than SCA1.

Disclosures: V. Mohan: None. **C.R. Edamakanti:** None. **P. Opal:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.06/V4

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R15NS116511

Title: Molecular consequences of spinocerebellar ataxia type 5 mutations in the spectrin-repeat domains of β-III-spectrin

Authors: *S. A. DENHA, N. R. DELAET, A. W. ABUKAMIL, A. N. ALEXOPOULOS, A. E. ATANG, A. W. AVERY;
Chem., Oakland Univ., Rochester, MI

Abstract: Spinocerebellar ataxia type 5 (SCA5) is a neurodegenerative disease caused by autosomal dominant mutations in *SPTBN2* gene encoding the cytoskeletal protein β-III-spectrin. SCA5 mutations target multiple domains of β-III-spectrin including the N-terminal actin-binding domain (ABD) and a central region containing 17 spectrin-repeat domains (SRDs). We previously showed that increased actin-binding affinity is a common molecular consequence of many ABD-localized SCA5 mutations. However, little is known about how SRD-localized SCA5 mutations disrupt β-III-spectrin at the molecular level. Here, we report the impact of R480W and L532_M544del mutations in SRD2 and SRD3, respectively, on β-III-spectrin actin binding and dimerization with α-II-spectrin. Previous biochemical studies in the spectrin family of proteins reported that SRDs either directly or allosterically enhance ABD affinity to actin. Using actin co-sedimentation assay, we show that the E532_M544del mutant has increased actin-binding affinity relative to wild-type. In contrast, R480W mutant β-III-spectrin has a comparable actin-binding affinity to wild-type. By analogy to other spectrin isoforms, β-III-spectrin SRD1 and 2 are thought to bind SRD21 and 20 of α-II-spectrin to nucleate α/β heterodimerization. Significantly, both R480W in SRD2 and E532_M544del in SRD3 are predicted to localize to the α/β interface. Using co-immunoprecipitation and FRET assays in HEK293T cells, we show that both R480W and E532_M544del destabilize the α/β dimer. These data, together with Alphafold protein-protein binding predictions, indicate that E532_M544del disrupts the coupling of adjacent α/β SRDs C-terminal to the position of the mutation in SRD3. In conclusion, our data show that SCA5 SRD-localized mutations can increase actin binding, like the ABD-localized mutations. In addition, the data indicate destabilization of the spectrin dimer may be a common consequence of the SRD-localized mutations.

Disclosures: S.A. Denha: None. **N.R. DeLaet:** None. **A.W. Abukamil:** None. **A.N. Alexopoulos:** None. **A.E. Atang:** None. **A.W. Avery:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.07/V5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R15NS116511

Title: Increased actin-binding affinity is a common molecular consequence of SCA5 mutations in β-III-spectrin

Authors: *A. E. ATANG[1], A. R. KELLER[2], S. A. DENHA[2], A. W. AVERY[2];
[1]Chem., Oakland Univ., Rochester Hills, MI; [2]Oakland Univ., Rochester, MI

Abstract: Spinocerebellar ataxia type 5 (SCA5) is a neurodegenerative disease caused by mutations in the *SPTBN2* gene encoding the cytoskeletal protein β-III-spectrin. Previously, we demonstrated that a L253P missense mutation, localizing to the β-III-spectrin actin-binding domain (ABD), causes increased actin-binding affinity. Here we investigate the molecular consequences of nine additional ABD-localized, SCA5 missense mutations: V58M, K61E, T62I, K65E, F160C, D255G, T271I, Y272H, and H278R. We show that all of the mutations, similar to L253P, are positioned at or near the interface of the two calponin homology subdomains (CH1 and CH2) comprising the ABD. Using biochemical and biophysical approaches, we demonstrate that the mutant ABD proteins can attain a well-folded state. However, thermal denaturation studies show that all nine mutations are destabilizing, suggesting a structural disruption at the CH1-CH2 interface. Importantly, all nine mutations cause increased actin binding. The mutant actin-binding affinities vary greatly, and none of the nine mutations increase actin-binding affinity as much as L253P. ABD mutations causing high-affinity actin binding, with the notable exception of L253P, appear to be associated with early age of symptom onset. More specifically, many of the newly characterized mutations were reported as infantile or adolescent onset, below the age range reported for L253P patients. Additionally, some early onset mutations are accompanied with developmental delays, a phenotype not seen with L253P. Altogether, the data indicate increased actin-binding affinity is a shared molecular consequence of numerous SCA5 mutations, which has important therapeutic implications.

Disclosures: **A.E. Atang:** None. **A.R. Keller:** None. **S.A. Denha:** None. **A.W. Avery:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.08/V6

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: ARSACS Foundation - Charting the neurodevelopmental stage of ARSACS

Title: Identification of mRNA Trafficking Alterations in Sacsin Deficient Primary Neurons

Authors: *S. BOESHORE[1], J. WOLTER[2];
[1]Genet., Univ. of Wisconsin, Madison, Madison, WI; [2]Genet., Univ. of Wisconsin Madison, Madison, WI

Abstract: The establishment and maintenance of the complex architecture of neurons relies on the transport of mRNAs from the cell body to distal processes. mRNAs are shuttled along axons and dendrites with the help of cytoskeletal and RNA binding proteins until they reach a designated location for local translation. Local protein synthesis is necessary for synaptic maturation, and abnormal synaptic structure can induce cascades of molecular events that ultimately trigger neuronal death. The causal deficits in these events are varied, but can include the availability of trophic signaling factors that support neuronal health, the balance of excitatory and inhibitory inputs to neurons, and the abnormal accumulation of proteins. Each of these distinct mechanisms represent a potential therapeutic strategy that could be targeted to slow disease progression. We are focused on the neurodegenerative cerebellar ataxia named Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS). ARSACS neurons have deficits in microtubule dynamics, protein mislocalization, and synaptic structure. The precise molecular deficits in ARSACS are unknown, but we hypothesize that a change in molecular trafficking is the underlying cause of synaptic disorganization. To test this hypothesis, we employed a primary cell culture system that creates the spatial separation of cell bodies from axons, and used this system to profile the populations of mRNAs in each compartment. Future studies will build on this experiment by characterizing localized synaptic translation and proteomics to map out the molecular landscape of ARSACS synapses. These experiments may help inform therapeutic strategies aimed at alleviating the underlying molecular deficit in ARSACS.

Disclosures: **S. Boeshore:** A. Employment/Salary (full or part-time):; University of Wisconsin Madison. **J. Wolter:** A. Employment/Salary (full or part-time):; University of Wisconsin Madison.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.09/V7

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01DK1200047
NIH R01DK120330
NIH R35GM130292
Michigan Protein Folding Disease Initiative

Title: Purkinje cell SEL1L-HRD1 ERAD deficiency leads to progressive cerebellar ataxia

Authors: *H. WANG, M. TORRES, P. PEDERSON, A. BUGARIN-LAPUZ, L. QI;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Purkinje cell SEL1L-HRD1 ERAD deficiency leads to progressive cerebellar ataxia
Hui Wang, Mauricio Torres, Brent Pederson, Amara Bugarin-Lapuz, and Ling Qi.(contact

information: mtorresg@med.umich.edu; lingq@med.umich.edu) Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48105, USA
Disclosures: The authors declare no conflict of interest. Cerebellar ataxia, characterized by a progressive decline in motor coordination and balance, represents a heterogeneous group of neurodegenerative disorders with limited treatment options. Endoplasmic reticulum-associated protein degradation (ERAD) plays a vital role in maintaining protein homeostasis by identifying and eliminating misfolded or damaged proteins within endoplasmic reticulum. Recent studies have implicated the crucial role of ERAD in the pathogenesis of neurodegenerative diseases. SEL1L, an essential ERAD component, acts as an adapter protein facilitating the recognition and degradation of aberrant proteins. Studies revealed that mutations or dysregulation of SEL1L can lead to ER stress and impaired protein degradation, subsequently triggering neuronal dysfunction, which are closely associated with cerebellar ataxia. Here, we established a Purkinje cell-specific Sel1L-deficient mouse model ($Sel1L^{Pcp2Cre}$) to investigate the role of SEL1L in cerebellar ataxia. Loss of function of ERAD in Purkinje cells resulted in a progressive loss of Purkinje cells in the cerebellum, starting between 9 and 12 weeks of age and reaching nearly 90% loss by 20 weeks of age. Transmission electron microscopy (TEM) analysis revealed dilated endoplasmic reticulum and fragmented nuclei in dying Purkinje cells, further supporting the role of SEL1L in maintaining the ER-homeostasis. $Sel1L^{Pcp2Cre}$ mice showed that they initially grew comparably to their wild-type littermates and appeared indistinguishable in gait and balance beam tests at 6 weeks of age. However, as they aged, $Sel1L^{Pcp2Cre}$ mice exhibited deteriorating motor function, including asymmetric gait, loss of balance, and coordination deficits around 20 weeks of age. These motor impairments were consistent with the progressive cerebellar ataxia observed in the mice. The findings from this study highlight the pathophysiological importance of SEL1L in Purkinje cells in the development of cerebellar ataxia. These insights into the role of SEL1L in disease pathogenesis may guide the development of novel therapeutic strategies targeting the ERAD pathway to alleviate the debilitating effects of cerebellar ataxia.

Disclosures: H. Wang: None. **M. Torres:** None. **P. Pederson:** None. **A. Bugarin-Lapuz:** None. **L. Qi:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.10/V8

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS121130

Title: Mutation of the mitochondrial intermembrane space protein Mix23 results in a progressive ataxia and neurodegenerative phenotype in mice

Authors: A. HOBBINS[1], J. RANDOLPH[1], N. SILVER[1], A. STOUT[1], ***J. HAMMOND**[2]; [1]Univ. of Rochester, Rochester, NY; [2]Univ. of Rochester, Penfield, NY

Abstract: Mix23 is a poorly characterized protein residing in the mitochondrial intermembrane space that may play a role in protein import into the mitochondria. Using whole exon sequencing, we identified a 4.5kb deletion that eliminates exon 4 from the Mix23 gene as the most likely cause of a late-onset, autosomal recessive ataxia phenotype that originated spontaneously in our Sez6L2 knockout mouse colony. Affected mice develop a progressive wobbly and slow gait affecting their hindlimbs that becomes visibly obvious between 4-10 months of age. However, symptom onset is likely much earlier as Mix23 mutant mice weigh less, travel shorter distances in open field assays, and perform poorly on rotarod at 2 months of age. Cerebellums from Mix23 mutant mice show significant astrogliosis and microgliosis that is concentrated in the white matter arbor vitae, deep cerebellar nuclei, and granular layers of the cerebellum. Although the Purkinje cell density is not altered in Mix23 mutant mice, many Purkinje cells have axonal swellings called “axonal torpedoes” within the granular layer. Purkinje cell axonal torpedoes have been previously observed in human patients and mouse models of spinocerebellar ataxias, autosomal-recessive cerebellar ataxias, and essential tremor and are associated with cerebellar damage and degeneration. Future studies will seek to elucidate how mutant Mix23 affects mitochondrial protein import and other mitochondrial functions and whether Mix23 mutations cause similar autosomal-recessive cerebellar ataxias in humans.

Disclosures: A. Hobbins: None. **J. Randolph:** None. **N. Silver:** None. **A. Stout:** None. **J. Hammond:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.11/V9

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant 1R35NS116868

Title: Voltage gated calcium channel genes are bicistronic, producing C-terminal proteins independent of the gene's canonical start site. CACNA1A produces $Ca_v2.1$ and a transcription factor with an important role in gene expression in the cerebellum.

Authors: *T. THAXTON[1], X. DU[2], C. GOMEZ[2];
[1]Neurobio., [2]Neurol., Univ. of Chicago, Chicago, IL

Abstract: Expansion of the polyglutamine (polyQ) tract in the gene encoding the pore-forming subunit of voltage-gated calcium channel (VGCC) $Ca_v2.1$ (*CACNA1A)* leads to spinocerebellar ataxia type 6 (SCA6), an adult onset, progressive cerebellar ataxia. We have identified a second gene product of *CACNA1A*, α1ACT, a novel transcription factor (TF) that is responsible for SCA6 when bearing the mutation. The lab has also confirmed that at least two other pore-forming subunit VGCC genes, *CACNA1C*, and *CACNA1H*, produce additional C-terminal proteins (CTP) from the same VGCC mRNA through a second open reading frame in the coding

sequence, suggesting the possibility that bicistronic expression may be a general property of VGCC family. It is important to understand how pervasive this expression system is in the rest of the VGCC genes since we have shown that the CTP, α1ACT, is a TF that drives gene expression in Purkinje cells and is implicated in causing symptoms of SCA6 when the polyQ tract of the protein is expanded. The mechanisms behind α1ACT's function need further study but motif searches reveal an AT-Hook motif, a TF region that preferentially binds to the minor groove of A/T-rich DNA; and a His-tract motif, thought to serve as a localization signal to transcriptional hubs called nuclear speckles. This study first assessed the bicistronic capability of the VGCC family by building constructs of each VGCC gene containing a 3xFLAG tag on the 3' end of each coding region. Similar vectors were built but contain a STOP codon just downstream of the start site of the coding region to halt expression of full-length product, but allow CTPs to persist, since they will contain their own start site. Indeed, all 10 VGCCs have CTPs that persist after addition of a 5' STOP codon. Moreover, microscopy confirmed presence of CTPs in transfected H293T cells, with all CTPs entering the nucleus, albeit at varying percentages. We then focused on one CTP, α1ACT, to assess TF function. Preliminary qRT-PCR studies on H293T cells transfected with α1ACT with a mutated AT-Hook (α1ACTΔAT-Hook) suggest differences in gene expression among known gene targets when compared to wild-type (WT) α1ACT. Given these results, we will perform RNA analyses on a neuronal cell line expressing either α1ACT-WT or α1ACTΔAT-Hook, providing insight into how α1ACT regulates gene expression. Confocal microscopy will be used to assess co-localization of α1ACT-WT, and α1ACT-null His-tract, with nuclear speckles, providing insight into the His-tract's function within α1ACT. Since there are many diseases associated with VGCC genes, understanding the potential bicistronic products, like α1ACT, of VGCCs may lead to new discoveries and therapies.

Disclosures: T. Thaxton: None. **X. Du:** None. **C. Gomez:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.12/V10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: FRQS
CIHR

Title: Disrupted mitochondria function and mitophagy contribute to disease progression in a mouse model of spinocerebellar ataxia type 6 (SCA6)

Authors: *S. LEUNG[1], E. FIELDS[2], N. RANA[4], L. SHEN[5], A. E. BERNSTEIN[3], D. E. PHILLIPS[1], A. A. COOK[1], A. J. WATT[1];
[1]Dept. of Biol., [2]Integrated Program in Neurosci., [3]Fac. of Arts and Sci., McGill Univ., Montreal, QC, Canada; [4]Global Hlth. Grad. Programs Dept., McMaster Univ., Hamilton, ON, Canada; [5]Univ. of Toronto, Toronto, ON, Canada

Abstract: Spinocerebellar ataxia type 6 (SCA6) is a rare late-onset disease characterized by progressive ataxia and eventual cerebellar Purkinje cell loss. SCA6 is caused by a CAG-repeat expansion mutation in the gene *CACNA1A*, which is highly expressed in Purkinje cells. The molecular underpinnings of disease remain incompletely understood for SCA6, and treatment options are limited. To gain deeper insight into the molecular changes contributing to SCA6, we performed RNA sequencing at disease onset to explore genes that are differentially expressed in litter-matched SCA6 and wildtype (WT) controls. We identified over 500 significant differentially-expressed genes. Gene Ontology enrichment analysis revealed that multiple mitochondria-related gene sets were downregulated.Mitochondria provide energy for cells and are particularly important in fast-spiking neurons like cerebellar Purkinje cells. To determine whether mitochondria function was altered in SCA6, we used the potentiometric fluorescent dye tetramethylrhodamine, ethyl ester (TMRE) to assay mitochondrial membrane potential. We found that Purkinje cell mitochondria had normal membrane potential at disease onset, but as disease progressed, mitochondrial membrane potential reduced. This argues that Purkinje cells have reduced capacity to produce ATP and impaired function during disease progression in SCA6. We next investigated mitochondria ultrastructure using electron microscopy and discovered that Purkinje cell mitochondria showed loss of cristae and swelling in SCA6, which worsened as disease progressed. Damaged mitochondria can lead to the elevation of reactive oxygen species in cells. We next used immunostaining against an oxidative damage marker 8-hydroxy-2-deoxyguanosine (8-OHdG) and found that oxidative stress in SCA6 Purkinje cells was normal at disease onset but elevated as disease progressed. The accumulation of damaged mitochondria in SCA6 Purkinje cells suggests that the degradation of mitochondria through mitophagy may be disrupted. To address this, we measured the expression of LC3, an autophagy marker, in Purkinje cells and found a significant reduction in SCA6 compared to WT. The final step of mitophagy is the fusion of autophagosome with lysosome. We quantified the colocalization of lysosomes and mitochondria-containing autophagosomes with CoxIV and Lamp1 immunoreactivity. We discovered that there was significant reduction in colocalization in SCA6 Purkinje cells, suggesting that mitophagy was reduced. Taken together, our data demonstrates a hitherto unappreciated role for accumulative mitochondrial dysfunction in the progression of SCA6.

Disclosures: S. Leung: None. **E. Fields:** None. **N. Rana:** None. **L. Shen:** None. **A.E. Bernstein:** None. **D.E. Phillips:** None. **A.A. Cook:** None. **A.J. Watt:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.13/V12

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Peripheral nerve excitability abnormality in spinocerebellar ataxia type 6

Authors: *Y. OSAKI[1], H. NODERA[4], R. MIYAMOTO[1], H. MORINO[2], M. CHAN[5], R. KAJI[3], Y. IZUMI[1];
[1]Dept. of Neurol., [2]Dept. of Med. Genet., [3]Ctr. for Res. Admin. & Collaboration, Tokushima Univ. Grad. Sch., Tokushima-shi, Japan; [4]Dept. of Neurol., Tenri Hosp., Tenri-shi, Japan; [5]Div. of Physical Med. and Rehabil., Univ. of Alberta, Edmonton, AB, Canada

Abstract: [Background] Spinocerebellar ataxia type 6 (SCA6) is an autosomal-dominant disorder caused by expanded CAG repeats in the CACNA1A gene encoding α1 subunit of P/Q type voltage-gated calcium channel (VGCC) embedded in Purkinje cells and motor nerve terminals. However, the function of the mutant VGCC reported in cultured cells and mutant mice is controversial. The aim of this study is to clarify whether and how the mutant VGCC affects motor nerve excitability in SCA6 patients. [Methods] We tested the motor nerve excitability of the median nerve in 16 SCA6 patients compared to 28 age-matched controls by doing threshold tracking using the TROND protocol in the Qtrac program. Properties studied included strength-duration time constant (SDTC), threshold electrotonus (TE), current-threshold relationship (IV), and recovery cycle (RC). The results were compared with mathematical modeling simulations using MEMFIT to detect which ion channels might affect the excitability differences between SCA6 patients and controls. In addition, we studied the motor nerve excitability in wild-type mice that were given ω-agatoxinIVA, a VGCC blocker. Finally, we tested motor nerve excitability in two Lambert-Eaton syndrome (LEMS) patients with an autoimmune P/Q type VGCC dysfunction.[Results] Two abnormalities were observed in the SCA6 patients. There was an increased S2 accommodation to depolarizing conditioning current in TE ($p < 0.001$) due to outward rectifying current via slowly activated potassium channels (Ks), Kv7.2/3. The second abnormality is increased late subexcitability in the RC ($p < 0.001$), also due to increased activation of Ks. These conjectures are supported by mathematical modeling of alternations in the activation of Ks. Mice that received P/Q type VGCC-blockade also showed excitability changes similar to those seen in the SCA6 patients. Lastly, in two LEMS patients treated with 3,4-aminopyridine (DAP), a potassium channel blocker, S2 accommodation in depolarizing TE was normalized. [Discussion] The present study showed that SCA6 patients had abnormal motor nerve excitability due to hyperactivation of Ks, which was likely a secondary result of VGCC dysfunction supported by the VGCC-blockade experiments. Further, this is corroborated by results from the LEMS patients after the administration of a potassium blocker. These findings reveal the presence of motor axonal dysfunction in SCA6 that potentially could be reversed through pharmacological intervention.

Disclosures: Y. Osaki: None. **H. Nodera:** None. **R. Miyamoto:** None. **H. Morino:** None. **M. Chan:** None. **R. Kaji:** None. **Y. Izumi:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.14/V13

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Friedreich's ataxia research alliance grant

Title: Downregulation of Acyl-CoA thioesterase 2 in a mouse model of Friedreich's ataxia

Authors: *Y. DONG, M. ADESHINA, L. NGABA, A. CAMURA, D. LYNCH;
The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Friedreich ataxia (FRDA) is an autosomal recessive neurodegenerative disease characterized by progressive gait and limb ataxia, cerebellar, pyramidal and dorsal column involvement, vision loss, scoliosis and cardiomyopathy. Cardiomyopathy is the main cause of death in FRDA, but the etiology of this condition is less clear. Here, we report that the acyl-CoA thioesterase 2 (Acot2), an enzyme involved in fatty acid metabolism and peroxisomal lipid metabolism, is downregulated in the heart of FRDA animal model. Acot2 physically interacts with frataxin in cardiac homogenates. In human fibroblasts cultured *in vitro* and in frataxin knockdown mice, siRNA-mediated frataxin knockdown reduces the levels of Acot2 protein. Acot2 protein levels are also decreased in a mouse model of chronic frataxin depletion, suggesting that frataxin regulates Acot2. This regulation is not mediated by changes in Acot2 mRNA transcripts but frataxin-dependent suppression of ubiquitin-proteasome system-dependent Acot2 degradation. Our results show that frataxin regulates Acot2 turnover. The significance of Acot2 deficiency in the pathophysiology of FRDA remains to be elucidated.

Disclosures: Y. Dong: None. **M. Adeshina:** None. **L. Ngaba:** None. **A. Camura:** None. **D. Lynch:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.15/V14

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: JSPS KAKENHI Grant JP19H05729

Title: Characteristics of Structural Changes in the Cerebrum of Patients with Spinocerebellar Degeneration

Authors: *H. HAMADA[1], Y. KIKUCHI[2], W. WEN[1,3], Q. AN[1], A. YAMASHITA[1], H. ASAMA[1];
[1]The Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan; [2]Mihara Mem. Hosp., Isesaki city, Gunma, Japan; [3]Rikkyo Univ., Tokorozawa city, Saitama, Japan

Abstract: Spinocerebellar degeneration (SCD) is a neurodegenerative disease of the cerebellum and its input-output pathways, causing ataxia. Although degeneration of the cerebellum, brainstem, and spinal cord was conventionally thought to be the main pathophysiology, an increase in cerebral volume has been reported, suggesting that the cerebral cortex compensates

for the cerebellar dysfunction. However, the relationship between the characteristics of the volume changes and cerebellar syndromes has not been fully elucidated. Hence, we aimed to statistically classify the characteristics of structural changes in the cerebrum in patients with SCD in order to understand compensation by the cerebrum. A total 50 patients with SCD and 11 healthy adults were included. Cerebellar volume was calculated by Voxel-based morphometry and cerebral volume (68 areas) was calculated by Surface-based morphometry from structural T1 images acquired using a 3T MR Scanner. We used an unpaired t-test to compare cerebellar volume between the SCD and healthy groups, and Spearman's rank correlation coefficient for the relationship between cerebellar volume, disease duration, and the Scale for the Assessment and Rating of Ataxia (SARA) score. Furthermore, the volume of each region of the cerebrum of each patient was subjected to principal component analysis (PCA) to extract components whose cumulative contribution ratio exceeded 80%, and the scores of the components were classified by hierarchical clustering (Ward's method). The results showed a significant decrease in cerebellar volume in the SCD group compared to the healthy group ($p < 0.05$), and a significant negative correlation between the disease duration and cerebellar volume in the SCD group ($p < 0.01$), but no correlation between cerebellar volume and SARA score, or between the disease duration and the SARA score. Nine components were extracted from the volume of each cerebral region in the SCD group by PCA, and the scores of each component were classified into four characteristics by clustering. The first cluster, characterized by increased left and right superior frontal, temporal, and parietal regions, showed a significantly lower SARA score than the third cluster ($p < 0.05$), but there were no significant differences in cerebellar volume and the disease duration. These results indicate that cerebellar volume changes in SCD patients can be classified into multiple characteristics. Increased volumes of motor- and sensory-related areas may be associated with the cerebellar syndromes expressed in the SARA.

Disclosures: H. Hamada: None. **Y. Kikuchi:** None. **W. Wen:** None. **Q. An:** None. **A. Yamashita:** None. **H. Asama:** None.

Poster

PSTR468. Ataxias

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR468.16/V15

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: A novel approach using neurofeedback learning to enhance cerebellar braininhibition in spinocerebellar degeneration patients

Authors: E. OMAE[1], A. SHIMA[1], K. TANAKA[1], M. YAMADA[1], C. YEDI[1], *T. MIMA[2], T. ISA[1], S. KOGANEMARU[1];
[1]Kyoto university, Kyoto, Japan; [2]Ritsumeikan Univ., Kyoto, Japan

Abstract: Spinocerebellar degeneration (SCD) shows progressive cerebellar dysfunction as well as impairments in brain stem, spinal cord, and basal ganglia, leading to various symptoms such

as limb and truncal ataxia, gait and balance disorders, oculomotor disturbance, dysarthria, and dysphagia. However, there has been no effective therapeutic medication. Cerebellar function can be evaluated by cerebellar brain inhibition (CBI) using a paired transcranial magnetic stimulation (TMS) technique; one to the cerebellar cortex and one to the motor cortex for observation of motor evoked potentials. As for the mechanism of CBI, it is hypothesized that stimulation of the cerebellar cortex activates Purkinje cells, which inhibit dentate nucleus and indirectly suppress the motor cortex through the cerebello-thalamo-cortical pathway. CBI is impaired in SCD patients. On the other hand, neurofeedback (NFB) learning is a method to self-regulate the neural activities to reinforce desired activity patterns presented to the subjects in real-time by feedback interfaces. Now, in this study, we investigated whether impaired CBI in SCD patients could be recovered by the neurofeedback learning using CBI (NFB-CBI learning). 12 SCD patients participated in this study. The randomized cross-over study design was used. The CBI was evaluated before and after both NFB-CBI learning and control conditions. In the NFB-CBI learning, the patients were given the real feedback of their CBI as the size of the circle on the monitor in front of them. If CBI was enhanced, the size of the circle became smaller. They were asked to make the circle as small as possible by using any kind of mental strategy. In the control condition, they were given the false feedback by using the other patient's data. They also tried to reduce the size of the circle. During the intervention, the NFB-CBI learning or control condition were executed for 10 min per session and two sessions were performed with 5 min-break of intersession. The paired TMS were conducted 100 times in each session. As a result, all the patients showed the impaired CBI before the intervention, and their CBI were recovered after the NFB-CBI learning, not after the control condition. It suggests that the SCD patients could recover their cerebellar function by the NFB-CBI learning and that NFB-CBI learning would be a possible therapeutic approach based on physiological activity of the cerebellum for SCD patients. Further evaluation of systemic ataxia and motor behavior would be necessary.

Disclosures: **E. Omae:** None. **A. Shima:** None. **K. Tanaka:** None. **M. Yamada:** None. **C. Yedi:** None. **T. Mima:** None. **T. Isa:** None. **S. Koganemaru:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.01/V16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH087332
NIH MH104131
NIH MH105330
NIH DA052209
NIH DA026306

Title: Treatment with low dose methamphetamine modulates genes associated with aging, potentially interfering with pathways contributing to AD/HAND

Authors: *I. S. HARAHAP-CARRILLO[1,2], R. MAUNG[2,3,4], D. OJEDA-JUÁREZ[3,2], P. SANCHEZ-PAVON[3], A. B. SANCHEZ[3,4], A. ROBERTS[5], M. KAUL[2,4,3], T. TMARC GROUP[4];
[1]UCR, Riverside, CA; [2]Biomed. Sci., Univ. of California, Riverside, Riverside, CA; [3]Ctr. for Infectious and Inflammatory Dis., Sanford Burnham Prebys Med. Discovery Inst., La Jolla, CA; [4]Translational Methamphetamine AIDS Res. Ctr. (TMARC), UCSD, San Diego, CA; [5]Dept. of Mol. and Integrative Neurosci., The Scripps Res. Inst., La Jolla, CA

Abstract: Despite the success of combined antiretroviral therapy (cART), up to 50% of people living with HIV (PLWH) continue to develop HIV-associated neurocognitive disorders (HAND). Interestingly, increased amyloid-beta (Aβ)1-42 and phosphorylated Tau (pTau) has been observed in the cerebrospinal fluid of HAND patients but are considered hallmarks of Alzheimer's disease (AD). This suggests the possibility of a common therapeutic target for both HAND and AD, as they share pathways of injury in the central nervous system (CNS). Methamphetamine (METH) is widely known as a strong and addictive psychostimulant that is frequently abused. PLWH who use METH reportedly have increased neuronal injury, cognitive impairment and viral load. However, low concentration of METH has an approved clinical use as a treatment for patients diagnosed with attention-deficit/hyperactivity disorder (ADHD). Recent studies have also reported potential points of neuroprotection via low-dose METH exposure, where improved learning and memory, and decreased neuronal injury were observed. This suggest that the dosage of METH plays a key role in determining its effects. This study explored in vivo the effects of a long-term, low-dose METH regimen (12 weeks) in the HAND animal model with inducible expression of HIV-1 transactivator of transcription (Tat). We observed METH driven decrease in the expression of cytokines and signaling factors involved in neuronal injury in HAND and AD. Interestingly, genes involved in the amyloid precursor protein (APP) processing pathway seems to be affected by the presence of METH. In addition, in the male cortex, reduced expression of synaptophysin (pre-synaptic) is ameliorated in Tat+METH animals compared to Tat and METH independently. Overall, low dose of METH has an effect that may ameliorate AD-like pathology and in turn improve HAND. The observations from this study creates the framework for future identification of potential targets for the treatment of neuroHIV/HAND.

Disclosures: I.S. Harahap-Carrillo: None. **R. Maung:** None. **D. Ojeda-Juárez:** None. **P. Sanchez-Pavon:** None. **A.B. Sanchez:** None. **A. Roberts:** None. **M. Kaul:** None. **T. TMARC Group:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.02/V17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant K08NS119882

Title: Mutations in HEXB increase risk for virally induced prenatal brain injury

Authors: *A. R. HORVATH, S. MATHESWARAN, B. FASIL, C. ABDELMALEK, Y. A. KOUSA;
Ctr. for Genet. Med., Children's Natl. Med. Ctr., WASHINGTON, DC

Abstract: Prenatal viral infections can have serious consequences on the developing brain. Zika virus exemplifies this pathophysiology, causing severe microcephaly, cortical visual impairment, epilepsy, and intellectual disability in its most severe presentation. Despite its epidemiological and clinical significance, virally induced prenatal brain injury is not yet predictable, treatable or preventable. Varied outcomes among affected individuals is suggestive of distinct vulnerability within the host. In the following research we aim to uncover this potential genetic susceptibility to Zika virus and other prenatal viral infections to inform future preventative therapies.
We first performed whole exome sequencing on prenatally infected infants with (N=22) or without (N=17) brain injury. Rigorous filtering of 1,400+ variants revealed a loss of function mutation in a neuroviral candidate gene, HEXB, in affected individuals. Consistent with our prior application in cellular models, we predict that loss of function mutation in HEXB increases susceptibility to prenatal brain injury through roles in neuronal survival and viral processing. We developed an immunocompetent mouse model of prenatal Zika virus infection to further study this phenomenon *in vivo*. Humanized mice with various titrations of HEXB were infected with Zika virus at timed gestational ages translatable to the first trimester in human pregnancy, when neural stem cells are expanding. In the first arm of the study, we harvested brain and placental tissue for gross measurement, histology, and immunostaining to evaluate molecular markers of neuronal dysregulation after infection. Tissue, blood, and saliva samples were also collected to quantify viral replication *in vivo*. In the second arm, we perform neurobehavioral testing from postnatal day 3 to 30 to evaluate disruption of cognitive, social, and motor developmental attainment. Thus far, humanized embryos show higher levels of viral replication in placental tissue, with the maternal decidua containing significantly more virus. Cortical volume of infected embryos decreases with viral infection as well. This finding is exacerbated with the loss of the HEXB allele, where we also observe pronounced deep gray injury. Together, these data suggest that HEXB mediates vulnerability in virally induced prenatal brain injury.

Disclosures: A.R. Horvath: None. **S. Matheswaran:** None. **B. Fasil:** None. **C. Abdelmalek:** None. **Y.A. Kousa:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.03/V18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grants R01 NS084817
NIH Grant R01 DA044552

NIH Grant R01 DA044552-03S1
NIH Grant R01 DA033966
NIH Grant R01 NS060632
NIH Grant MH122241
NIH Grant R01 DA057197

Title: Methamphetamine exacerbates neuroHIV-induced K_v channel dysfunction in live cortical astrocytes through TAAR-1 signaling pathway

Authors: *L. CHEN, S. L. CASSODAY, A. DONNER, D. IMERI, L. AL-HARTHI, X.-T. HU; Rush Univ. Med. Ctr., Chicago, IL

Abstract: Methamphetamine (Meth) is a highly addictive and widely abused psychostimulant. There is no FDA-approved medicine for treating people with Meth use disorders (MUD). Chronic exposure to Meth decreases neuronal activity in certain brain regions, including the medial prefrontal cortex (mPFC, one of the critical regulators of cognition and addiction), which may contribute to the mechanism underlying Meth addiction. The mPFC is profoundly altered by Meth and HIV. However, little is known whether such neuronal dysfunction results from the alterations in the synaptic/intrinsic excitability of neurons *per se*, dysfunction of astrocytes that disturbs extracellular homeostasis of glutamate and K^+ levels, or both. To determine the effects of Meth and/or neuroHIV on extracellular K^+ homeostasis mediated by astrocytes, we assessed the functional activity of voltage-gated K^+ channels (K_v) in live astrocytes using brain slices containing the mPFC from adolescent HIV-1 transgenic (HIV-1 Tg) rats, a rodent model of neuroHIV after cART with the absence of HIV but the expression of multiple viral proteins, at the age of 5~7-week (wk). Age matched F344 non-Tg rats (non-Tg) were used as control. Whole-cell patch-clamping approaches were used to assess dysfunction of live mPFC astrocytes in rat brain slices. For assessing acute Meth effects, brain slices were treated with vehicle or Meth (20, 100 μM) for 10 min in a continuing perfusion system. For evaluating chronic Meth effects, rats received daily repetitive s.c. injection of Meth (5 mg/kg/day) for 5 days followed by a 3-day withdrawal. We found that acute Meth exposure suppresses the functional activity of K_v channels in conducting outflowing I_K in mPFC astrocytes from both non-Tg and Tg rats in a dose-dependent manner. Chronic Meth exposure, as well as neuroHIV, also significantly decreases the efflux of K^+ currents in mPFC astrocytes, regardless of genotype, while the greatest reduction occurs with combination of chronic Meth abuse/neuroHIV. However, blockade of trace amine-associated receptor 1 (TAAR-1)-mediated signaling pathway reverses the effects of chronic Meth and neuroHIV on suppressing voltage-gated K^+ efflux in astrocytes of both genotypes. In summary, both chronic Meth abuse and neuroHIV decrease the functional activity of live mPFC astrocytes in the brain by reducing the activity of K_v channels mediated by TARR1 signaling. The comorbidity of Meth abuse and neuroHIV exacerbates astrocyte dysfunction caused by either one alone. Such dysfunction of astrocytic K_v channels could cause an abnormal reduction in extracellular K^+ levels, thereby reducing firing of surrounding cortical neurons.

Disclosures: L. Chen: None. **S.L. Cassoday:** None. **A. Donner:** None. **D. Imeri:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.04/V19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Investigating the Neurological Immune Response to SARS-CoV-2 Infection: Activation of Microglia and Astrocytes in K18-hACE2 Mice

Authors: A. ISLAM[1], D. THEOBALD[2], H. SHELTON[4], S. M. AKULA[3], S. SRIRAMULA[2], *J. EELLS[5];
[1]Anat. & Cell Biol., [2]Pharmacol. & Toxicology, [3]Immunol. & Microbiology, East Carolina Univ. Brody Sch. of Med., Greenville, NC; [4]East Carolina Univ., Greenville, NC; [5]Anat. & Cell Biol., East Carolina Univ. Sch. of Med., Greenville, NC

Abstract: Since the onset of the coronavirus pandemic, extensive research to explore the impact of SARS-CoV-2 infection on the human immune system and the brain has been conducted. Specifically, research has focused on understanding the effects of this viral infection on the brain's immune response; thus, a higher emphasis has been placed on looking at microglia and astrocytes. Microglia, the resident immune cells in the brain, actively respond to pathogens, while astrocytes, regulate homeostasis and carry out neuroprotective functions. By analyzing these glial cells, we aim to determine whether there is a neurological immune response to inflammation caused by SARS-CoV-2. Our hypothesis is that infection with SARS-CoV-2 would result in distinct differences in glial cell activation. To investigate this, our study used K18-hACE2 mice infected with the virus or a control group given saline and euthanized 30 days post-infection following a mild infection. Brain tissue samples were collected, paraffin-embedded, and sections were labeled using immunofluorescence for Iba1, GFAP, and DAPI to visualize microglia, astrocytes, and nuclei, respectively. Subsequently, the hippocampus and amygdala regions were imaged at 20X magnification using a Keyence microscope. Image J/Fiji software was optimized to calculate glial cell density. The software enabled skeletal analysis on randomly selected cells. Based on t-test to determine statistical significance at an alpha of 0.05, preliminary findings indicate no difference between glial cell density. However, differences were found in other parameters, particularly in the hippocampus, between the infected and control groups. Notably, both astrocytes ($p = 0.017$) and microglia ($p = 0.031$) exhibited significantly fewer branches in the infected group compared to the control in the hippocampus. Factors such as number of junctions ($p = 0.016$, $p = 0.027$), slab voxels ($p = 0.002$, $p = 0.028$), and number of triple points ($p = 0.012$, $p = 0.029$) were all significantly reduce for both astrocytes and microglia in hippocampus, respectively. These observations suggest a potential neurological immune response with more activated astrocytes and microglia. The ongoing analysis aims to validate these findings and explore whether activated microglia induce inflammation or possess anti-inflammatory properties as M1 or M2 subtypes. Our previous research found elevated kinin B1 receptors, therefore, future research will expand this investigation by exploring the role of B1R in mediating these changes in glial activation. We will involve similar analytical methods to gain further insights into these intricate mechanisms.

Disclosures: **A. Islam:** None. **D. Theobald:** None. **H. Shelton:** None. **S.M. Akula:** None. **S. Sriramula:** None. **J. Eells:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.05/V20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR Team Grant - HAL-157984

Title: Perinatal exposure to HIV protease-inhibitor-based antiretroviral therapy leads to hippocampal-dependent memory deficits and hyperactivity in long-term

Authors: *S. H. DHUME[1,3,2], K. BALOGUN[2], A. SARKAR[2], S. ACOSTA[3], H. MOUNT[4], L. SERGHIDES[2,3];
[1]Toronto Gen. Hosp. Res. Inst., [2]Univ. Hlth. Network, Toronto, ON, Canada; [4]Inst. of Med. Sci., [3]Univ. of Toronto, Toronto, ON, Canada

Abstract: Introduction: Treatment of HIV using antiretrovirals (ARVs) has been pivotal in reducing perinatal transmission of the disease from mother to child. While most children who are HIV-exposed but uninfected (cHEU) remain in good health, previous studies showed that these children are at higher risk for growth impairments, lower IQ levels, language and cognitive delays, and other neurological deficits. In this study, we developed mice models of in-utero ARV exposure to understand the consequence of clinically recommended ARV regimens on the fetal brain and subsequent neurodevelopmental and behavioral outcomes.
Methods: Plugged C57BL/6 female mice (n=40) were randomly assigned to one of the three treatment arms and administered therapeutic doses of either abacavir/lamivudine + ritonavir-boosted atazanavir (ABC/3TC+ATV/r) or tenofovir/emtricitabine + ATV/r (TDF/FTC+ATV/r), or water (control) by oral gavage throughout gestation. Offspring (both sexes) were used for behavioral tests (rotarod, open field maze, zero maze, light/dark box, and contextual fear conditioning). The mice were further used for morphological analysis using magnetic resonance imaging (MRI) and immunohistochemistry. The hippocampus and striatum were extracted and used to study gene expression using real-time quantitative PCR. Sex-specific differences were analyzed in all experiments.
Results: Compared to the controls, mice exposed to TDF/FTC+ATV/r showed significantly increased hyperactivity in 4 independent behavioral tests. Further, we observed antiretroviral drug class-based differences in hippocampal-dependent behavioral tests, where ABC/3TC+ATV/r exposed mice showed deficits in working memory while TDF/FTC+ATV/r exposed mice showed 77.4% decreased freezing in contextual fear memory. Interestingly, MRI studies showed substantial volumetric changes including decreased gray matter volumes in ABC/3TC+ATV/r exposed mice. Gene expression analysis revealed changes in the expression of the neurotrophic factor BDNF and its receptor TrkB, and the glutamate receptors, NMDAR and

AMPAR, in the striatum and hippocampus for both treatment groups.
Conclusion: In-utero exposure to HIV antiretroviral regimens caused deficits in the working and contextual memory of antiviral-exposed neonates as well as hyperactivity reminiscent of autism spectrum disorders. This phenotype was supported by our gene expression analysis and MRI findings which showed molecular and morphological changes in neurons and brain volume. Thus, our findings highlight the importance of optimizing the ARV treatment regimens to reduce the risk of neurodevelopmental disorders in cHEU.

Disclosures: **S.H. Dhume:** None. **K. Balogun:** None. **A. Sarkar:** None. **S. Acosta:** None. **H. Mount:** None. **L. Serghides:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.06/V21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DFG, German Research Foundation (EXC 2145 SyNergy, ID 390857198)

Title: Sars-cov-2 spike protein accumulation in the skull-meninges-brain axis: potential implications for long-term neurological complications in post-COVID-19

Authors: *Z. RONG, H. MAI, A. ERTURK;
Helmholtz Munich, Munich, Germany

Abstract: Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), has been associated with a range of neurological symptoms, including brain fog and brain tissue loss, raising concerns about the virus's acute and potential chronic impact on the central nervous system. In this study, we utilized mouse models and human post-mortem tissues to investigate the presence and distribution of the SARS-CoV-2 spike protein in the skull-meninges-brain axis. Our results revealed the accumulation of the spike protein in the skull marrow, brain meninges, and brain parenchyma. The injection of the spike protein alone caused cell death in the brain, highlighting a direct effect on brain tissue. Furthermore, we observed the presence of spike protein in the skull of deceased long after their COVID-19 infection, suggesting that the spike's persistence may contribute to long-term neurological symptoms. The spike protein was associated with neutrophil-related pathways and dysregulation of the proteins involved in the PI3K-AKT as well as complement and coagulation pathway. Overall, our findings suggest that SARS-CoV-2 spike protein trafficking from CNS borders into the brain parenchyma and identified differentially regulated pathways may present insights into mechanisms underlying immediate and long-term consequences of SARS-CoV-2 and present diagnostic and therapeutic opportunities.

Disclosures: **Z. Rong:** None. **H. Mai:** None. **A. Erturk:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.07/V22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 DA057197
R01 DA044552
R01 DA044552-03S1
DA033966
NS060632
MH122241

Title: Ace2 levels in the brain are reduced in the context of neurohiv modeled in hiv-1 transgenic rats.

Authors: *D. IMERI, L. CHEN, S. CASSODAY, A. DONNER, L. AL-HARTHI, X.-T. HU; Rush Univ., Chicago, IL

Abstract: HIV-associated neurocognitive disorders (HAND) are a well-documented phenomenon that occurs in ~50% of people living with HIV (PLWH), even after combined antiretroviral therapy (cART). Coronavirus disease-19 (COVID-19) has also been shown to induce neurocognitive dysfunction. It is unknown whether, how, and to what extent these two diseases synergistically exacerbate neurocognitive impairments. With roughly 38 million PLWH globally, and in the wake of the recent COVID-19 pandemic, it is critical for us to understand the effects of these two diseases, and the potential comorbidity of which on the neurocognitive function. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The spike protein of SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2), a receptor that is located on the surface of many cell types and acts to modulate the levels of angiotensin II, while also facilitating the entry of the virus into neurons/cells, which is the basis for the infection and replication of SARS-CoV-2. While there are numerous studies revealing the expression of ACE2 in epithelial cells in the peripheral systems such as the lung and gastrointestinal (GI) tract, there are very few studies that report the ACE2 expression in the brain, especially in those regions regulating neurocognition. To our knowledge, there is no published data showing whether the expression of ACE2 is altered by neuroHIV. Thus, this study is designed to initially assess the expression of ACE2 protein in the brain regions that regulate neurocognition, including, but not limited to, the mPFC, in the context of neuroHIV modelled in HIV-1 transgenic (HIV-1 Tg) rats at age of ~6 months. Age-matched non-transgenic (non-Tg) rats were used as control. We assessed the expression of ACE2 receptors in the brain, including the olfactory bulbs, mPFC, frontal cortex, and cerebellum via western blot. We also evaluated potential changes in the ACE2 protein levels in the lung. We found that ACE2 protein was expressed in the olfactory bulbs, mPFC, cerebellum, and lung tissues from both non-Tg and HIV-1 Tg rats. However, the ACE levels were significantly reduced in the olfactory bulbs,

mPFC, and lung in HIV-1 Tg rats compared to non-Tg rats (all $p<0.05$). There was no significant change in the cerebellum. These novel findings suggest that neuroHIV downregulates the expression of ACE2 receptors in the brain of PLWH in a region-specific manner. We expect to confirm this reduction in our next study using immunofluorescence. Further, we will also determine how SARS-CoV-2 affects the expression of ACE2 in the brain.

Disclosures: D. Imeri: None. **L. Chen:** None. **S. Cassoday:** None. **A. Donner:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.08/V23

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Bdnf-trkb signaling in neuroprotection for HIV-associated neurocognitive disorders (HAND)

Authors: J. BRYANT[1], T. MAKAR[2], H. DAVIS[1], S. WILLIAMS[4], S. BOSUNG[5], K. KELEDJIAN[5], I. GHOSH[3], S. MAKAR[3], L. MALCOLM[6], V. GERZANICH[5], I. MERCHENTHALER[6], *F. DENARO[4];
[1]Animal Core, IHV Univ. of Maryland, Baltimore, MD; [2]Animal Core, IHV Univ. of Maryland, BALTIMORE, MD; [3]IHV Univ. of Maryland, Baltimore, MD; [4]Morgan State Univ., Baltimore, MD; [5]Dept. of Surgery, Sch. of Med., [6]Epidemiology and Publ. Hlth., Univ. of Maryland, Baltimore, MD

Abstract: HIV-Associated Neurocognitive Disorder (HAND) is a significant clinical problem associated with HIV infection despite treatment with cART. There is a need to develop therapeutics targeted at preventing neurocognitive decline. The testing of neuroprotective therapies is now an important step to eventually treating this HIV comorbidity. Brain derived neurotrophic factor (BDNF) promotes the growth, differentiation, maintenance, and survival of neurons. However, application of BDNF in clinical treatment still has problems. Intravenous injection of BDNF is safer but BDNF cannot penetrate the blood-brain barrier (BBB). Furthermore, direct intracerebral injection of BDNF is too invasive. A small molecule 7,8-dihydroxyflavone (DHF) is an alternative that can mimic BDNF function and crosses the BBB. DHF displays neuromodulator, neuroprotective, antipsychotic, anti-obesity, anticancer, antioxidative and anti-inflammatory activities in different preclinical studies. We assessed the role of DHF on the brain of TG26 mice, an animal model of HAND. The mice were divided into 3 groups: wild type, untreated-Tg26 (3-month-old male), and DHF-treated (5 mg/kg i.p. injection for 30 days) Tg26 (Tg+DHF). Mice were sacrificed after 30 days and 7 µm thick paraffin sections of the brain of each mouse were prepared. Tissues were immunohistochemically stained to detect the expressions of GFAP, SUR1, TRPM4, AQP4, Sunapsine1, Synaptophysin, NAMPT, iNOS, HO-1, STAT3 and NRF-2 in the hippocampal and cortex regions. We also

previously measured the expressions of TrkB, AKT and NF-kB from the same group of mice (Bryant et.al.2021,SCI Report11:18519.) Our results show, that in these regions of Tg26 mice, GFAP, SUR1, TRPM4, SIRT1 expressions were increased. On the other hand, Synapsine 1 Synaptophysin, NAMPT, STAT-3 and Nrf-2 expressions were downregulated. But DHF treatment reverse those parameters. As a result, astrogliosis and ionic imbalance were normalized. Furthermore, we also observed a reduction in oxidative stress along with an increase in NAD metabolism resulting in synaptogenesis and neuroprotection. Moreover, our findings demonstrate that DHF increases TrkB activation, providing new insights into the role of TrkB-Akt-NFkB, or STAT-3, and or NRF-2 signaling pathways in mediating HAND associated neuroprotection. These data implicate crosstalk among TrkB, Akt and transcription factors including NF-κB, STAT3 and NRF-2 in neuroprotection of HAND. This research explores the future of DHF which shows promise as a TrkB agonist treatment for HAND patients in conjunction with current antiviral therapies.

Disclosures: **J. Bryant:** None. **T. Makar:** None. **H. Davis:** None. **S. Williams:** None. **S. Bosung:** None. **K. Keledjian:** None. **I. Ghosh:** None. **S. Makar:** None. **L. Malcolm:** None. **V. Gerzanich:** None. **I. Merchenthaler:** None. **F. Denaro:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.09/V24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: K22NS118975
5R21MH095524
R01NS099036
5R25NS094093
U54MD007600
5P20GM103475
U54GM133807

Title: Dysregulated interferon type 1 signaling in HIV-1 neuropathogenesis

Authors: *Y. CANTRES-ROSARIO[1], E. MEDINA COLON[1], B. COLLAZO[3], J. LOPEZ MORALES[2], K. HERNANDEZ[2], E. RODRIGUEZ[1], B. DIAZ[1], M. MATOS[1], V. SEPULVEDA[1], Y. GERENA[1], V. WOJNA[1];
[1]Univ. of Puerto Rico, San Juan, PR; [2]Univ. of Puerto Rico, Bayamon, PR; [3]Univ. Central del Caribe, Bayamon, PR

Abstract: People living with HIV (PWH) develop neurocognitive disorders associated with the infection, driven by infiltrated monocytes, neuroinflammation, and neuronal dysfunction. Despite the effectiveness of antiretroviral therapy, there are no therapies available for cognitive

decline. Type 1 interferon (IFN-1) signaling is a potent immune antiviral response, with known functions in essential processes such as microglia activation, synaptic plasticity, and cognitive function. We hypothesized that disrupted IFN-1 signaling triggers monocyte infiltration, neuronal dysfunction, and cognitive decline in PWH. We measured Interferon alpha (IFN-a) and beta (IFN-b) in the plasma of age-matched HIV-negative controls, HIV-positive normal cognitive, HIV-positive cognitive impaired, and Alzheimer's disease (AD) patients, by ELISA. Then, we measured interferon alpha receptor 1 (IFNAR1) in CD14+ monocytes (n=24), in peripheral blood mononuclear cells, by flow cytometry. Finally, we co-cultured monocytes from patients with human cortical brain organoids to uncover mechanisms driving neuronal dysfunction *in vitro*. IFN-a1 levels, but not IFN-b, were slightly higher in the plasma of cognitive impaired PWH and significantly higher AD patients (p=0.03), compared to HIV-negative. Flow cytometry revealed a lower percentage of IFNAR1+ monocytes from PWH (p=0.02) and AD patients (p=0.01) compared to HIV-negative. IFNAR1 levels significantly decreased in monocytes from males (p=0.03), but not from females. Brain organoids co-cultured with monocytes showed infiltration of CD14+ cells in the brain organoid lysates, with no significant differences between the groups. Decreased IFNAR1 and increased activation of interferon regulatory factors 3 and 7 were also observed, with different levels when comparing between males and females as well as between cognitive groups. Finally, the cytokines IFN-a, IFN-b, and macrophage chemoattractant protein 1 were elevated in the supernatants of brain organoids co-cultured with monocytes from cognitive impaired patients, compared to the other groups. Results suggest that IFN-a and IFN-b respond differently during HIV infection and disrupted IFN-1 signaling in monocytes may contribute to HIV neuropathogenesis. Biological sex differences observed in IFN-1 responses warrant further characterization.

Disclosures: **Y. Cantres-Rosario:** None. **E. Medina Colon:** None. **B. Collazo:** None. **J. Lopez Morales:** None. **K. Hernandez:** None. **E. Rodriguez:** None. **B. Diaz:** None. **M. Matos:** None. **V. Sepulveda:** None. **Y. Gerena:** None. **V. Wojna:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.10/V25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NINDS 5R01NS109529-01A1
NIH NCATS TL1TR003106

Title: Long-covid's impact on the brain: exploring the neuroinflammation nexus

Authors: *I. A. JORDAN, M. L. MCDANIEL, S. N. FOX, J. W. YOUNGER; Psychology, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Background: Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) has long been suspected to be a post-viral illness, as viral infections can stimulate inflammatory cytokine production. Millions of coronavirus disease 2019 (COVID-19) patients are also predicted to develop persistent symptoms that mimic ME/CFS symptomology after disease resolution, referred to as Post-Acute Sequelae of COVID-19 (PASC). Inflammatory mediators produced by sustained microglia activation have been shown to lead to illness response symptoms, such as pain sensitivity, fatigue, cognitive impairment, and mood disturbances - all symptoms associated with ME/CFS and PASC. Previous magnetic resonance spectroscopy (MRS) research suggests that ME/CFS exhibit markers of neuroinflammation, but no studies have investigated neuroinflammation in PASC patients using this technique. By collecting multi-voxel MRS information across the brain, we can expand our knowledge of PASC. This study aimed to evaluate if PASC patients exhibit markers of neuroinflammation similar to ME/CFS patients. **Methods:** Four women with PASC, forty-eight women with ME/CFS and twenty-four age-matched healthy women completed whole-brain MRSI and fatigue questionnaires. Lactate (LAC) and N-acetylaspartate (NAA) were evaluated in 47 regions of interest (ROIs) as ratios over creatine (Cr). A One-Way ANOVA compared PASC patients, ME/CFS patients and healthy controls. **Results:** Our preliminary findings indicated significant metabolite variations between groups in 11 of 47 ROIs at $p < .05$. There was a statistically significant difference in LAC/Cr between groups as determined by one-way ANOVA in the left hippocampus, left calcarine gyrus, left lingual gyrus, and left fusiform gyrus. A Tukey HSD post hoc test revealed that PASC patients have higher LAC/Cr compared to both healthy controls and ME/CFS patients in these regions. We also found a significant difference in NAA/Cr between groups in the bilateral operculum, right occipital cortex, right fusiform gyrus, left postcentral gyrus, left parietal cortex, and the left precuneus. The Tukey HSD further found that PASC patients have significantly lower NAA/Cr compared to ME/CFS, but no significant differences with the healthy controls. **Conclusions:** We report neuroinflammatory metabolite abnormalities suggesting PASC involves a generalized/widespread inflammatory process. These results should be replicated in future studies with larger samples to further establish the profile of pathophysiological abnormalities in the brains of PASC patients.

Disclosures: I.A. Jordan: None. **M.L. McDaniel:** None. **S.N. Fox:** None. **J.W. Younger:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.11/W1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 DA059197
R01 DA044552
R01 DA044552-03S1
DA033966

NS060632
MH122241

Title: Sars-cov-2 induces hyperactivity of medial prefrontal cortex pyramidal neurons in non-tg and hiv-1 tg rats that self-administer cocaine

Authors: *S. L. CASSODAY[1], L. CHEN[2], A. DONNER[2], D. IMERI[2], T. L. SHULL[2], J. SCHNEIDER[2], L. AL-HARTHI[3], X.-T. HU[2];
[1]Microbial Pathogens and Immunity, Rush Univ., Chicago, IL; [2]Microbial Pathogens and Immunity, [3]Dept. Immunology/Microbiology, Rush Univ. Med. Ctr., Chicago, IL

Abstract: Despite global vaccine and masking mandates to reduce the spread of SARS-CoV-2, COVID-19 remains pervasive. Persistent neurological manifestations, like neurocognitive deficits, are common indications of COVID-19 infection. Neurocognitive function is vital for everyday life and is controlled by various brain regions, including the prefrontal cortex. Yet how SARS-CoV-2, either directly (from the virus itself) or indirectly (from the immune response to the virus), affects neuron function in the medial prefrontal cortex (mPFC) of people, especially those suffering from neurocognitive deficits caused by HIV-associated neurocognitive disorder (HAND) and substance use disorders, remains unknown. To address this gap, we used a rat model of neuroHIV controlled by combined antiretroviral therapy (HIV-1 Tg rats, with no live HIV but expression of viral proteins) with cocaine self-administration (Coc-SA). After a 2-week Coc-SA followed by a 3-week withdrawal, drug-seeking behaviors were assessed at days 3 and 21. Immediately following their last drug-seeking assessment, rats were transcardially perfused with an artificial cerebral spinal fluid (aCSF), brains collected and slices containing the mPFC were dissected and used for whole-cell patch-clamping. We then assessed the effects of SARS-CoV-2 spike protein (in nM: 1, 2.5, 5) or human CSF antibodies (5μg/mL, which may injure neurons) isolated from COVID-19 patients on firing activity of rat pyramidal neurons, with or without Coc-SA. We found that both HIV-1 Tg and non-Tg rats displayed similar drug-taking behaviors, including the Coc volume, suggesting that drug-taking behavior is not altered by neuroHIV. During the long-term withdrawal, drug-seeking activity was reduced, but not fully eliminated, in Coc-SA rats, regardless of genotype, indicating a persistence of drug-induced neuroplasticity. Meanwhile, Coc-SA rats exhibited abnormally increased firing frequency and altered membrane properties, revealing mPFC neuron hyperactivity. SARS-CoV-2 S protein applied in bath increased hyperactivity of mPFC pyramidal neurons in non-Tg/SAL-yoked control rats, and worsened overactivation in Coc-SA rats, regardless of genotype. Moreover, our preliminary data also suggest that the SARS-CoV-2 antibodies isolated from CSF of COVID-19 patients also induce mPFC neuron hyperactivity in SAL-yoked rats, while worsening neuronal overactivation in Coc-SA rats. These findings suggest that SARS-CoV-2, either directly or indirectly, alters mPFC neuronal activity by causing overactivation and possible injury, which could contribute to the neurocognitive deficits seen in COVID-19 patients.

Disclosures: S.L. Cassoday: None. **L. Chen:** None. **A. Donner:** None. **D. Imeri:** None. **T.L. Shull:** None. **J. Schneider:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.12/W2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21DA056309
R37DA015014
R01DA032444

Title: Adult human brain tissue cultures to study neuroHIV

Authors: *E. IROLLO[1], R. VAN DUYNE[1], E. V. O'BRIEN[1], J. JOHNSON[1], J. G. JACKSON[1], A. SARKAR[1,2,5], Z. KLASE[1,3], O. MEUCCI[1,3,4];
[1]Pharmacol. & Physiol., [2]Neurosurg., [3]Ctr. of Neuroimmunology and CNS Therapeut., [4]Microbiology and Immunol., Drexel Univ. Col. of Med., Philadelphia, PA; [5]Global Neurosciences Inst., Philadelphia, PA

Abstract: People with HIV often present with HIV-associated neurocognitive disorders (HAND) that range from mild impairment to severe dementia. HAND can persist even when HIV is fully suppressed by antiretrovirals (ARV), suggesting a complex pathogenesis that is hard to reproduce in animal models. This study seeks to develop a tractable human tissue-based experimental model of neuroHIV that preserves the tissue environment, architecture, and cellular networks of the human brain and recapitulates critical aspects of HIV infection in the CNS. To model this ex vivo, we have generated organotypic human cortical cultures from surgical resections of adults. These cultures are subsequently exposed to patient-matched cells infected with HIV-1 ex vivo. In validating this model, we require the following: 1) that brain slices remain viable, stable, and functionally active for the entire duration of the experiments; 2) that appropriate CNS resident cells are infected with HIV; 3) that infection is controlled by ARV. After optimization of culture conditions, we assessed brain slice cells viability using vital staining and flow cytometry. We consistently observed >90% viability in the first week of culture and no less than 80% in 2-week-old cultures, with both CNS cells and immune cells viable. Confocal microscopy studies of the neuronal dendritic arbor reveal that overall dendritic spine density is stable until at least 14 days in vitro (DIV), decreasing at the end of the third week in culture along with overall decreases in slice viability. Importantly, mature spine phenotypes are predominant in the first 2 weeks. We are currently testing microelectrode array technologies to monitor neuronal network activity. To test the utility of these cultures in modeling HIV infection, we generated monocyte-derived macrophages and CD4+ T-cells from patient-matched peripheral blood, infected these cells with a GFP-expressing R5 HIV-1, and co-cultured these cells with the slices. Up to nine days post-infection, we detected viral RNA (PCR), GFP+ resident microglia (flow cytometry) and HIV replication (p24 AlphaLISA). Cell-free virus also successfully infected brain slices. Preliminary studies in the presence of ARV regimen Biktarvy effectively controls viral RNA in the infected slices, suggesting we can adequately model a suppressed HIV infection in the brain. In summary, though still at an early stage of development, this model offers an impactful tool to study the mechanisms of neuronal injury in

HIV infected brain, including the contribution of ARV and/or HIV comorbidities (such as substance use disorders), as well as a novel means for testing potential neuroprotective agents.

Disclosures: E. Irollo: None. **R. Van Duyne:** None. **E.V. O'Brien:** None. **J. Johnson:** None. **J.G. Jackson:** None. **A. Sarkar:** None. **Z. Klase:** None. **O. Meucci:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.13/W3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NIAID R01-AI145435
NIH NIAID 1K08AI139371

Title: Dendrimer-conjugated N-acetyl cysteine in pediatric rabbit model of tuberculous meningitis affects white matter density and amyloid deposition

Authors: *N. N. L. DAMIBA[1,5,6], N. SAH[2], J. KIM[2,6,5], W. G. H. M. LIYANAGE[3], M. SHARMA[3], C. ERICE[2,6,5], R. M. KANNAN[3], S. JAIN[4,5], S. KANNAN[2,7], E. W. TUCKER[2,6,5]; [2]Anesthesiol. and Critical Care Med., [3]Ctr. of nanomedicine, [4]Pediatrics, [1]Johns Hopkins Univ. - Main Campus, Baltimore, MD; [5]Ctr. for Infection and Inflammation Imaging Res., [6]Ctr. of Tuberculosis Res., [7]Ctr. of nanomedicine, Johns Hopkins Univ., Baltimore, MD

Abstract: Background and Aim: Tuberculous meningitis (TBM) is the most devastating form ofextrapulmonary tuberculosis (TB), especially in children and immunocompromised hosts.Although pathogenesis is incompletely understood, neuroinflammation plays a key role andcorticosteroids (i.e., dexamethasone) are the standard of care host-directed therapy (HDT).However, they do not significantly improve neurobehavioral outcomes and novel therapeuticsare urgently needed. N-acetyl cysteine (NAC) is an anti-inflammatory and antioxidant with poorbioavailability. Through conjugation with dendrimer nanoparticles, dendrimer-NAC (D-NAC)permeates through the blood-brain barrier, targets activated microglia, reduces white matterinjury and improve outcomes in several animal models of neuroinflammation. We previouslydeveloped a pediatric rabbit model of TBM with neurobehavioral deficits and postmortemhistopathological features of human disease, including neuroinflammation with microgliaactivation, decreased myelination (i.e., white matter density) and amyloid deposition associatedwith neurogenerative. Here, we tested the ability of D-NAC to improve white matter injury anddecrease amyloid deposition in our TBM model.**Method:** Young New Zealand White rabbits (post-natal day 5) received an intraparenchymalinjection of PBS (uninfected, control) or live *Mycobacterium tuberculosis* H37Rv (TB-infected).After 3 weeks, TB-infected rabbits received treatment with saline (treatment control), D-NAC ordexamethasone for 2 weeks before brain tissue was harvested. Brains were fixed with 4% PFA,paraffin embedded, sectioned to 10 µm and stained with Luxol Fast Blue to identify whitematter, or

Congo Red to identify amyloid deposition. White matter and amyloid depositiondensity (mm^2) were quantified with ImageJ.**Results and Conclusions:** TB-infected rabbits treated with saline had decreased white matterdensity and increased amyloid deposition compared to uninfected rabbits (P<0.01). D-NACtreatment increased white matter density in TB-infected rabbits compared to those treated withdexamethasone (P<0.01) but did not restore it to the density of uninfected rabbits, except whenmales were examined separately. Additionally, D-NAC decreased amyloid deposition comparedto those treated with saline or dexamethasone only when males were examined separately(P<0.01). These sex differences were studied in a small sample and will need to be reproducedin a larger study. These preliminary data demonstrate that D-NAC is a promising HDT but itsefficacy may be impacted by sex differences that need to be studied further.

Disclosures: N.N.L. Damiba: None. **N. Sah:** None. **J. Kim:** None. **W.G.H.M. Liyanage:** None. **M. Sharma:** None. **C. Erice:** None. **R.M. Kannan:** None. **S. Jain:** None. **S. Kannan:** None. **E.W. Tucker:** 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 NIAID, Hartwell Foundation.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.14/W4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5T32GM081740
NIH Grant DA013137
NIH Grant NS100624
NIH Grant DA059310

Title: Hiv associated gut dysbiosis: correlations with dendritic spine morphology in the medium spiny neurons of the nucleus accumbens

Authors: *M. T. RODRIGUEZ, C. F. MACTUTUS, R. M. BOOZE;
Psychology, Univ. of South Carolina, Columbia, SC

Abstract: HIV-1 infection has been found to be associated with microbial translocation and dysbiosis. These alterations are associated with the progression and severity of HIV-1-associated neurocognitive disorder (HAND) symptoms in humans. HIV-1-associated dysbiosis is characterized by an increase in the genera Prevotella and a decrease in Bacteroides and Akkermansia Muciniphila, a combination that may alter neurocognitive function by reducing the production of γ-aminobutyric acid (GABA) and increasing the translocation of LPS generated via the gastrointestinal microbiome. The present study utilized HIV-1 Transgenic (Tg) rats and

F344 control animals to investigate the effects of S-Equol (SE) on the gastrointestinal microbiome and subsequent dendritic spine morphology. Alpha diversity analysis between HIV-1 Tg vs. control rats at baseline was approaching significance ($p \leq 0.051$) with beta diversity analysis indicating a clear difference in phylogenetic composition ($p \leq 0.001$), but not abundance. At the genus level, baseline differences were found between HIV-1 Tg rats vs. controls, with Alloprevotella being increased in the HIV-1 Tg rats. Weighted UniFrac analysis of phylogenetic differences and taxonomic abundance provided evidence of a significant effect of S-equol in HIV-1 Tg rats ($p \leq 0.007$) and controls ($p \leq 0.047$). Preliminary results further suggest a correlation between alterations in dendritic spine morphology in medium spiny neurons of the nucleus accumbens (i.e., head diameter) and the overall gastrointestinal microbiome composition (Operational Taxonomic Units; OTUs). These results suggest a link between HIV-1-associated dysbiosis and synaptic function, as indicated by alterations in dendritic spine morphology, and targeting HIV-1 gut dysbiosis as a therapeutic approach for HAND.

Disclosures: **M.T. Rodriguez:** None. **C.F. Mactutus:** None. **R.M. Booze:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.15/W5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH P30 MH 097488
NIH P30 AI 045008
NIH NINDS 1R01NS122570-01A1
NIH NIDDK R01DK115762
NIH U01-MH121260
NIH R01-MH096875
NIH R01-AG060931
NIH R00-AG051764
P40OD012217
C06OD026690

Title: snRNA-seq revealed progressive loss of inhibitory neurons in the frontal cortex of SIV-infected Rhesus Macaques during the early post-infection window

Authors: *S. NASSER CHEHIMI[1], R. C. CRIST[1], M. J. MONTAGUE[2], N. SNYDER-MACKLER[5], M. O. BOHLEN[7], K. L. CHIOU[5], T. ZINTEL[6], M. L. PLATT[3], M. R. HAYES[4], D. L. KOLSON[1], B. C. REINER[1];
[2]Neurosci., [3]Neuroscience, Perelman Sch. of Med., [4]Psychiatry, [1]Univ. of Pennsylvania, Philadelphia, PA; [6]Ctr. for Evolution and Med., [5]Arizona State Univ., Tempe, AZ; [7]Biomed. Engin., Duke Univ. Neurobio. Grad. Program, Durham, NC

Abstract: People living with acquired immunodeficiency syndrome (AIDS) are subject to opportunistic infections and the development of long-term cognitive impairments, termed HIV-associated neurocognitive disorders (HAND). Postmortem studies have identified cortical transcriptome alterations, with limited data available at the cell type-specific level. Moreover, the inaccessibility of brain tissue prevents the investigation of the acute stage of HIV infection and disease progression. To address this knowledge gap, we used the rhesus macaque non-human primate (NHP) model of simian immunodeficiency virus (SIV) infection. We employed single-nuclei RNA sequencing (snRNA-seq) approach to examine NHP frontal cortex (FC) brain tissue at 10- and 20-days post-infection (DPI) post-SIV infection, and non-infected controls, to evaluate the molecular alterations underlying the disease progression during the early SIV post-infection window. We identified 15 distinct cell types, encompassing expected neuronal and non-neuronal cells in the FC. Examining the frequency of specific cell type clusters, an increase in microglia was detected at both post-infection time points, indicative of the acute phase of viral infection. Additionally, we identified a progressive decrease of inhibitory interneurons, suggesting the frontal cortex is a susceptible region prone to neuronal damage during the acute stage of SIV infection. Examination of differentially expressed genes (DEGs) determined that the majority of DEGs were limited to the 10-DPI time point and that DEGs shared between 10- and 20-DPI groups were predominantly downregulated. This suggests a transient gene expression state during the early infection stage, followed by increased transcriptome stability. Cell type-specific pathway analysis after SIV infection was associated with immune system signaling and antiviral mechanisms, especially at 10-DPI. Cell-cell interaction networks identified altered intercellular communication networks within the FC, with the majority of interaction vectors defined by reduced ligand expression, but a mixture of increased or decreased receptor expression. In summary, this project used snRNA-seq to provide valuable insights into the molecular mechanisms associated with the acute phase of SIV infection in the rhesus macaque FC, which potentially contribute to the progression and severity of the disease and HAND. These findings represent thrilling opportunities to explore additional time points and gain a comprehensive understanding of the causes of the long-term neurocognitive decline associated with pharmacotherapy-induced extended survival after HIV infection.

Disclosures: S. Nasser Chehimi: None. **R.C. Crist:** None. **M.J. Montague:** None. **N. Snyder-Mackler:** None. **M.O. Bohlen:** None. **K.L. Chiou:** None. **T. Zintel:** None. **M.L. Platt:** None. **M.R. Hayes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cantius Therapeutics, LLC. Other; Novo Nordisk, Boehringer Ingelheim, Eli Lilly & Co.. **D.L. Kolson:** None. **B.C. Reiner:** Other; Novo Nordisk, Boehringer Ingelheim.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.16/W6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5 T32 NS121780-02

Title: Dysregulated Amyloid in HIV-Associated Neurocognitive Disorder: The Role of Gp120 induced Endoplasmic Reticulum Stress

Authors: *C. AGBEY, L. CAMPBELL, I. MOCCHETTI;
Georgetown Univ. Med. Ctr., Washington DC, DC

Abstract: People living with Human Immunodeficiency Virus (HIV) (PLWH) may develop HIV-Associated Neurocognitive Disorder (HAND), a disease characterized by cognitive, motor, and behavioral impairments. HAND remains prevalent despite combination antiretroviral therapy (cART). Studies have linked HIV infection of the CNS to increased accumulation of the amyloid beta (Aβ) peptide, and Aβ aggregation may promote neurotoxicity and neuroinflammation. However, the mechanisms underlying Aβ accumulation in HAND are unclear. The HIV envelope protein gp120 causes neuronal degeneration after its internalization into neurons. Internalized gp120 is transported retrogradely towards the cell body in close proximity to the endoplasmic reticulum (ER). Notably, Aβ may be generated from amyloid precursor protein (APP) in the ER and excessive ER stress has been linked to increased buildup of Aβ. Thus, **we propose that gp120-mediated neurotoxicity relies on the ability of gp120 to disrupt ER function and increase Aβ accumulation.** Here, we present preliminary findings that exposure to the HIV-1 protein gp120 promotes an increase of β-Amyloid, which correlates with gp120 induced ER calcium depletion.

Disclosures: C. Agbey: None. **L. Campbell:** None. **I. Mocchetti:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.17/W7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R21NS106640 (SV)
Silvateam/ Indunor SA (MMP)
Houston Methodist NeuralCODR Fellowship program (SS)

Title: Gut microbiota dysbiosis and cytokines alterations in COVID patients correlate with long-COVID symptoms three years after discharge.

Authors: *S. SORIANO[1], M. M. PISKORZ[2], K. CURRY[3], T. J. TREANGEN[3], S. VILLAPOL[1];
[1]Houston Methodist Res. Inst., Houston, TX; [2]Hosp. de Clínicas José de San Martín, Univ. de Buenos Aires, Buenos Aires, Argentina; [3]Dept. of Computer Sci., Rice Univ., Houston, TX

Abstract: Post-acute COVID-19 symptoms or Long COVID, a debilitating illness that affects at least 10% of individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is characterized by a wide range of symptoms, including fatigue, cognitive impairment, and gastrointestinal (GI) issues. These symptoms can persist for more than three months after the initial infection. Gut dysbiosis, an imbalance in the gut microbiome, may play a role in immune dysfunction and chronic inflammation associated with SARS-CoV-2 infection. Our study aimed to explore the potential connections between gut dysbiosis, inflammation, and the development of Long COVID. To investigate this, we recruited 124 hospitalized COVID-19 patients (50% male and female, aged 18-90) and collected longitudinal blood and stool samples. We compared their results with those of 53 matched healthy controls. Gut microbiome profiling was performed through full-length 16S rRNA gene sequencing using the Oxford Nanopore platform. We found changes in gut microbiota community composition and a significantly decreased alpha diversity in COVID patients compared to healthy volunteers. Intriguingly, this decrease in bacterial diversity was more pronounced in female and obese COVID patients. Furthermore, we observed a significant decrease in the abundance of *Blautia sp.* and *Faecalibacterium prausnitzii* at the species level. To explore the link between gut dysbiosis and inflammation, we measured 27 serum cytokine biomarkers that we correlated with the gut microbiome alterations observed in COVID patients. Among the cytokines analyzed, Fibroblast Growth Factor (FGF) and Macrophage Inflammatory Protein-1b (MIP-1b) showed a positive correlation with the abundance of *Blautia sp.* in the gut. Three years after their COVID-19 infection, 57% of the enrolled patients reported Long COVID symptoms, predominantly neurological. Our study concludes that COVID-19 leads to dysbiosis in the gut microbiome, as evidenced by a decreased bacterial diversity and the abundance of specific bacterial species. These changes correlate with acute inflammatory markers and subsequent Neuro Long COVID symptoms.

Disclosures: S. Soriano: None. **M.M. Piskorz:** None. **K. Curry:** None. **T.J. Treangen:** None. **S. Villapol:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.18/W8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R01DA035714
NIH grant R01DA041932

Title: SRI-32743 allosterically modulates norepinephrine transporter and attenuates HIV-1 Transactivator of transcription (Tat) protein-induced inhibition of the transporter-mediated dopamine uptake

Authors: *A. C. JIMENEZ-TORRES[1], A. HASTIE[1], S. T. NESTOR[2], O. MOUKHA-CHAFIQ[2], T. H. NGUYEN[2], S. ANANTHAN[2], C. E. AUGELLI-SZAFRAN[2], J. ZHU[1];

[1]Drug Discovery and Biomed. Sci., Univ. of South Carolina, Columbia, SC; [2]Chem., Southern Res. Inst., Birmingham, AL

Abstract: Dysregulation of dopamine neurotransmission induced by the HIV-1 Tat protein has been implicated as a major pathogenic factor in the development of HIV-1 associated neurocognitive disorders. HIV-1 Tat transgenic mice displayed a decrease in dopamine transport in the prefrontal cortex via both dopamine (DAT) and norepinephrine (NET) transporters. We have demonstrated that SRI-32743, a novel allosteric modulator, attenuates HIV-1 Tat protein-induced inhibition of DAT and alleviates the potentiation of cocaine reward in HIV-1 Tat transgenic mice. This study determined whether SRI-32743 allosterically modulates NET and attenuates Tat-induced inhibition of NET-mediated dopamine uptake. SRI-32743 was found to partially inhibits [^{3}H]dopamine uptake in CHO-K1 cells expressing hNET (IC_{50} value, 12.03 ± 3.22 µM, E_{max} value 61.42 ± 10.71 %). SRI-32743 decreased the maximal velocity (V_{max}) and K_m values of [^{3}H]dopamine uptake in a concentration dependent manner. SRI-32743 inhibited [^{3}H]Nisoxetine binding with an affinity of 26.4 ± 5.17 µM, whereas the affinity for desipramine, a NET inhibitor, is 6.0 ± 4.0 nM. SRI-32743 (50 nM) decreased the cocaine-mediated dissociation of [^{3}H]Nisoxetine binding in hNET (K_{-1} = 0.087 ± 0.028 min^{-1}) relative to cocaine alone (K_{-1} = 0.233 ± 0.021 min^{-1}). While 50 nM SRI-32743 alone did not alter NET-mediated dopamine uptake, the addition of SRI-32743 attenuated Tat (8.7 or 17.5 nM)-induced decrease in [^{3}H]DA uptake via hNET. These findings demonstrate that Tat and cocaine interactions with both DAT and NET may be regulated by compounds interactions at the allosteric modulatory sites on these transporters, suggesting a potential therapeutic intervention for HIV-infected patients with concurrent cocaine abuse.

Disclosures: **A.C. Jimenez-Torres:** None. **A. Hastie:** None. **S.T. Nestor:** None. **O. Moukha-Chafiq:** None. **T.H. Nguyen:** None. **S. Ananthan:** None. **C.E. Augelli-Szafran:** None. **J. Zhu:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.19/W9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant U01DA054170
NIH Grant GM60655
NIH Grant GM007717
NIH R21AG066496
SAPPT SCI-12776

Title: Human iPSC-derived brain organoids to model the effect of APOE-isoforms on SARS-CoV-2 neurotropism

Authors: *A. MUNIZ PEREZ, C. MCMAHON, K. K. MEYER-ACOSTA, J. HSIEH; Neuroscience, Developmental, and Regenerative Biol., Univ. of Texas at San Antonio, San Antonio, TX

Abstract: Up to 30% of COVID-19 patients report the manifestation of neurological symptoms, highlighting the need to better understand the neurotropism of SARS-CoV-2. It has been reported that patients carrying two copies of *APOE4,* the leading genetic risk factor for Alzheimer's disease, may also have an elevated risk for contracting SARS-CoV-2. We have previously found that SARS-CoV-2 can infect astrocytes in human brain organoids grown from embryonic stem cells (ESCs) that have the *APOE3/4* genotype. It is unclear how the *APOE4/4* genotype may lead to more severe SARS-CoV-2 infection. In order to understand why *APOE4/4* carriers are at higher risk for more severe COVID-19 infection, we have established human cortical spheroids (hCS) and human subpallium spheroids (hSS) from induced pluripotent stem cells (iPSCs) derived from *APOE3/3* and *APOE4/4* patients to see if *APOE4/4* alters the susceptibility and severity of SARS-CoV-2 infection in astrocytes and neurons. Preliminary data shows that *APOE4/4* hCS and hSS are more susceptible to SARS-CoV-2 infection compared to *APOE3/3*. This study suggests that *APOE*-isoform could affect severity of SARS-CoV-2 neurotropism, highlighting the need for genetic screening of vulnerable populations.

Disclosures: A. Muniz Perez: None. **C. McMahon:** None. **K.K. Meyer-Acosta:** None. **J. Hsieh:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.20/W10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P30 MH075673
NIH Grant R01 AG068130

Title: Brain N-acetylaspartyl-glutamate (NAAG) is associated with cognitive function in virally-suppressed people living with HIV

Authors: *R. WISEMAN[1,2,3], R. DASTGHEYB[4], I. SANTIUSTE[5], C. RIGGS[1,5], R. RAIS[3,4,2], J. ALT[3], K. BIGOS[2,6], P. BARKER[7], L. RUBIN[4,6,8,5], B. SLUSHER[4,3,6,9,10];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Pharmacol. and Mol. Sci., [3]Johns Hopkins Drug Discovery, [4]Neurol., [5]Brain Hlth. Program, [6]Psychiatry and Behavioral Sci., [7]Radiology and Radiological Sci., [8]Epidemiology, [9]Neurosci., [10]Oncology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Suppressive antiretroviral therapy has improved and extended the lives of people living with HIV (PLWH); despite these advances, cognitive impairment persists especially in the domains of working memory (WM) and executive function (EF). One brain circuit linked to

these domains is regulated by the neurotransmitter N-acetyl-aspartyl glutamate (NAAG), the endogenous agonist of metabotropic glutamate receptor 3. NAAG levels are modulated by its catabolic enzyme glutamate carboxypeptidase II (GCPII), which is robustly upregulated in neuroinflammation. Inhibition of GCPII increases brain NAAG and has improved learning and memory in multiple rodent and primate models. As neuroinflammation is present in virally suppressed (VS)-PLWH, we investigated if brain NAAG levels measured by magnetic resonance spectroscopy (MRS) were associated with cognitive function. We utilized 7-Tesla MRS data to examine relationships between regional NAAG levels in frontal white matter (FWM), posterior cingulate, mesial precuneus, left basal ganglia (BG), and left hippocampus (Hp) and domain-specific cognitive performance in VS-PLWH (n=40) and seronegative individuals (n=20) after adjusting for depressive symptoms and premorbid function. All participants were $\geq$50 years of age, had no prior 3-month illicit drug use, and were negative for affective and neurologic disorders. In the total study population, higher NAAG levels in FWM were associated with better attention/WM (r=0.33, P=0.01) and processing speed (r=0.24, P=0.06). Higher BG NAAG levels correlated with better verbal fluency (r=0.35, P=0.01) and higher NAAG in the Hp correlated with better EF (r=0.40, P=0.006). Conversely, higher NAAG levels in the mesial precuneus were associated with poorer attention/WM (r=-0.27, P=0.06), which may indicate that NAAG plays a different role in regions less-activated by the cognitive tasks employed. The same relationships were also observed when data from VS-PLWH and HIV-uninfected individuals were analyzed separately, suggesting a global role for NAAG in cognition outside of disease. In a separate pilot study, we analyzed plasma NAAG levels in PLWH to investigate whether plasma NAAG also related to cognition. Plasma from 20 VS-PLWH (n=10 cognitively impairment and n=10 unimpaired) were analyzed via LC/MS. We observed a significant difference between the two groups (Cohen's d=0.72), with VS-PLWH demonstrating cognitive impairment having higher plasma NAAG compared to cognitively intact VS-PLWH. Collectively, these data suggest brain and plasma NAAG may serve as biomarkers of cognition in VS-PLWH, and modulation of brain NAAG could represent a novel therapeutic avenue.

Disclosures: **R. Wiseman:** None. **R. Dastgheyb:** None. **I. Santiuste:** None. **C. Riggs:** None. **R. Rais:** None. **J. Alt:** None. **K. Bigos:** None. **P. Barker:** None. **L. Rubin:** None. **B. Slusher:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.21/W11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPQ, 303051/2023-1
FAPERJ, E26/202.667/2019

Title: Phenolic glycolipid-1 of Mycobacterium leprae is involved in human Schwann cell line ST8814 neurotoxic phenotype

Authors: *B. MIETTO[1], K. GIRARDI[2], K. LIMA[3], G. C. ATELLA[3], D. S. DA SILVA[2], A. M. PEREIRA[2], P. S. ROSA[4], F. A. LARA[2];
[1]Federal Univ. of Juiz de Fora, Juiz de Fora, Brazil; [2]Oswaldo Cruz Fndn., Rio de Janeiro, Brazil; [3]Federal Univ. of Rio de Janeiro, Rio de Janeiro, Brazil; [4]Lauro de Souza Inst., Bauru, Brazil

Abstract: Leprosy is a chronic infectious disease caused by Mycobacterium leprae infection in Schwann cells. Axonopathy is considered a hallmark of leprosy neuropathy and is associated with the irreversible motor and sensory loss seen in infected patients. Although M. leprae is recognized to provoke Schwann cell dedifferentiation, the mechanisms involved in the contribution of this phenomenon to neural damage remain unclear. In the present work, we used live M. leprae to infect the immortalized human Schwann cell line ST8814. The neurotoxicity of infected Schwann cell-conditioned medium (SCCM) was then evaluated in a human neuroblastoma cell lineage and mouse neurons. ST8814 Schwann cells exposed to M. leprae affected neuronal viability by deviating glial 14C-labeled lactate, important fuel of neuronal central metabolism, to de novo lipid synthesis. The phenolic glycolipid-1 (PGL-1) is a specific M. leprae cell wall antigen proposed to mediate bacterial-Schwann cell interaction. Therefore, we assessed the role of the PGL-1 on Schwann cell phenotype by using transgenic M. bovis (BCG)-expressing the M. leprae PGL-1. We observed that BCG-PGL-1 was able to induce a phenotype similar to M. leprae, unlike the wild-type BCG strain. We next demonstrated that this Schwann cell neurotoxic phenotype, induced by M. leprae PGL-1, occurs through the protein kinase B (Akt) pathway. Interestingly, the pharmacological inhibition of Akt by triciribine significantly reduced free fatty acid content in the SCCM from M. leprae- and BCG-PGL-1-infected Schwann cells and, hence, preventing neuronal death. Overall, these findings provide novel evidence that both M. leprae and PGL-1, induce a toxic Schwann cell phenotype, by modifying the host lipid metabolism, resulting in profound implications for neuronal loss. We consider this metabolic rewiring a new molecular mechanism to be the basis of leprosy neuropathy.

Disclosures: B. Mietto: None. **K. Girardi:** None. **K. Lima:** None. **G.C. Atella:** None. **D.S. da Silva:** None. **A.M. Pereira:** None. **P.S. Rosa:** None. **F.A. Lara:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.22/W12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Texas Christian University Invest in Scholarship Grant (2022-2023)
Texas Christian University Science and Engineering Research Center (SERC) Grant

Title: Long-term Mediterranean diet consumption provides neuroprotection against soluble amyloid-beta production in the cortex and hippocampus, in comparison to a typical American diet

Authors: *M. BERTRAND[1], P. BRADEN-KUHLE[2], V. LACY[1], K. N. BRICE[3], G. W. BOEHM[2], M. J. CHUMLEY[1];
[1]Biol., [2]Psychology, Texas Christian Univ., Fort Worth, TX; [3]Psychology, Rice Univ., Houston, TX

Abstract: Approximately 1 in 9 Americans over the age of 65 has Alzheimer's disease (AD). As the size of this age group is expected to more than double by 2040, the prevalence of AD is likewise predicted to rapidly increase. Two key risk factors for late-onset AD include poor diet and obesity. Therefore, long-term nutritional strategies could potentially reduce the development of hallmark AD biomarkers, such as amyloid beta (Aβ), later in adulthood. High saturated fat Western diets (WD)have been associated with an increased risk of AD. A WD is comprised of refined carbohydrates, sugars, and saturated fat. The relationship between a WD and AD biomarkers has been explored in prior animal research. However, the majority ofprior studies examined the effects of extremely high fat diets (typically providing over 40-60% kcal from fat) that do not mimic that of a typical American diet (TAD). Researchers have found that diets extremely rich in saturated fat increase Aβproduction in both the cortex and hippocampus of rodents. Conversely, the Mediterranean diet (MD) is a plant-based diet that provides complex carbohydrates and plentiful unsaturated fatty acids, which were shown to mitigate Aβ in rodents. However, prior research has primarily explored the effects of a standard rodent diet supplemented with a single component of the MD, rather than a cohesive MD. To address this limitation, we designed two, comprehensive MD and TADs that mimic human diets in Mediterranean regions and the U.S., respectively. Further, we matched the macronutrient density ranges betweenthe two experimental diets to control for energy availability. The current study examined the effects of the MD versus the TADdiet on Aβ1-42 production in the cortex and hippocampus of male and female C57BL/6J mice. Mice were weaned onto one of the two diets at postnatal day 21. Following 6 months of diet consumption, we collected cortical and hippocampal tissue to quantify the levels of soluble Aβ1-42 via ELISA. Our results showed that long-term consumption of TAD significantly increased the production of soluble Aβ1-42 in both the cortex and hippocampus of male C57BL/6J mice in comparison to the MD. Additionally, we found that TAD increased the production of soluble Aβ1-42 in the cortex, but not the hippocampus, of female C57BL/6J mice. Overall, these results suggest that the MD plays a neuroprotective role against Aβ1-42 production in comparison to the TAD in male C57BL/6J mice. In future studies, we aim to further examine sex differences observed in the current research.

Disclosures: M. Bertrand: None. **P. Braden-Kuhle:** None. **V. Lacy:** None. **K.N. Brice:** None. **G.W. Boehm:** None. **M.J. Chumley:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.23/W13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Texas Christian University Invest in Scholarship Grant
Texas Christian University Science and Engineering Research Center Grant (SERC)

Title: Exploring the effects of a comprehensive Mediterranean diet verses a typical American diet on peripheral inflammation and the expression of inflammation-related genes in the dorsal hippocampus

Authors: *V. LACY[1], P. BRADEN-KUHLE[2], M. BERTRAND[1], K. N. BRICE[2], G. W. BOEHM[3], M. J. CHUMLEY[1];
[1]Biol., [2]Psychology, [3]Dept Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Approximately 72% of Americans are overweight or obese, partially due to the consumption of a Western diet (WD). The WD is a pro-inflammatory diet due to a high intake of simple carbohydrates, sugars, and saturated fat. Recent research has demonstrated that saturated fat functions as a Toll-like receptor 4 (TLR4) agonist in rodents, ultimately inducing an inflammatory state. Previous research has demonstrated that the WD and obesity increase pro-inflammatory cytokine levels in serum and mRNA levels in the hippocampus of rodents. While pro-inflammatory cytokines are a vital portion of the immune response, chronic inflammation associated with excess pro-inflammatory cytokines is affiliated with several diseases. For example, middle-aged adults with high levels of pro-inflammatory cytokines such as TNF-α and IL-1β, have an increased risk of Alzheimer's disease (AD). In contrast to the WD, the Mediterranean diet (MD) is a plant-based, mostly unsaturated fat diet. Research has shown that it is crucial to consume a balanced omega-6 to omega-3 ratio of 1:1 or 2:1, like that in the MD, as elevated ratios found in the WD lead to increased inflammation. However, previous studies generally administered an extremely high-fat Western rodent diet that does not resemble that of the typical American. Thus, our study aims to address this limitation by designing two experimental diets to mimic the typical American or Mediterranean diet and control for available energy via matching the macronutrient density of both diets. Therefore, in the current study, we examined the effects of the typical American diet (TAD) versus the MD in relation to pro-inflammatory cytokine production in serum and gene expression in the dorsal hippocampus of C57BL/6J mice. C57BL/6J mice were weaned onto one of the two diets, and following 6 months of diet administration, mice were treated with one intraperitoneal injection of lipopolysaccharide (LPS) or saline 4 hours prior to euthanasia. LPS administration leads to non-pathogenic induction of peripheral and central inflammation. We collected serum to conduct multiplex electrochemiluminescence assays to measure TNF-α and IL-1β in the periphery and collected dorsal hippocampus to measure TNF-α and IL-1β gene expression via qRT-PCR. We found that males on the TAD were significantly heavier than those on the MD, even with energy controlled. Further, the TAD significantly increased TNF-α production in the serum, which was largely correlated with increased body weight. We are currently conducting qRT-PCR, and trending patterns of ongoing data collection suggest that diet and LPS treatment may increase expression of TNF-α in the dorsal hippocampus.

Disclosures: **V. Lacy:** None. **P. Braden-Kuhle:** None. **M. Bertrand:** None. **K.N. Brice:** None. **G.W. Boehm:** None. **M.J. Chumley:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.24/W14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Texas Christian University Invests in Scholarship Grant 2022–2023
Texas Christian University Science and Engineering Research Center Grant

Title: Exploring the effects of a comprehensive Mediterranean diet verses a typical American diet on spatial memory and behavior in C57BL/6J mice

Authors: *P. N. BRADEN-KUHLE, V. LACY, K. N. BRICE, M. E. BERTRAND, C. SHOFFNER, M. J. CHUMLEY, G. W. BOEHM;
Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Over 6 million Americans have Alzheimer's disease (AD), and this is estimated to triple by 2060. A hallmark pathology of AD is amyloid beta (Aβ), a protein that disrupts neuronal function, and is associated with cognitive impairment. Although about 94% of all AD cases are a subtype of AD known as late-onset AD (LOAD), the etiology of LOAD is largely unknown. A risk factor for LOAD is poor diet, such as a Western diet (WD). In rodents, a WD has been shown to increase cognitive impairment and Aβ in the cerebral cortex and hippocampus. The brain region hardest hit by AD pathologies is the hippocampus, which is critical in neural mechanisms of learning and memory. Conversely, plant-based diets like the Mediterranean diet (MD), have been shown to protect against cognitive impairment. For example, standard rodent diets supplemented with one or two dietary factors found in the MD have been shown to support cognitive function. Although there is evidence for the benefits of Mediterranean dietary factors in rodents, the combined effects of a comprehensive, macronutrient-matched MD on cognitive function remain uncertain. A key limitation in the scientific literature is that the majority of prior animal studies have only examined the effects of extremely high-fat WDs (providing over 40-60% kcal from fat), or a MD with only one or two key factors. We aimed to fill a gap in the literature by designing a rodent diet that mimicked the typical American diet (TAD), rather than an exaggerated WD, and a macronutrient-matched rodent diet that mimicked a typical Mediterranean diet. C57BL/6J mice were weaned onto one of the two diets at postnatal day 21. Following six months of diet, we conducted behavioral tests, including open field, elevated zero, and object-location memory task (OLMT). We found that the TAD decreased locomotor activity and exploratory behavior in open field, such that mice on the TAD traveled less distance and exhibited fewer vertical counts in comparison to mice on the MD. Additionally, we found that the TAD increased anxiety-like behavior, as mice on the TAD

spent less time in the open quadrant of the elevated zero maze, in comparison to mice on the MD. Further, the results of OLMT demonstrated that mice on the MD had stronger spatial memory in comparison to mice on the TAD. In an additional study, we analyzed the amount of soluble, cortical and hippocampal $A\beta_{1-42}$ and found that mice on the TAD had significantly more $A\beta_{1-42}$ than mice on the MD. Further, analyses showed that higher levels of $A\beta_{1-42}$ were negatively correlated with spatial memory. These data suggest that increased $A\beta_{1-42}$ production induced by the TAD may impact spatial memory abilities and other behavioral outcomes in C57BL/6J mice

Disclosures: P.N. Braden-Kuhle: None. **V. Lacy:** None. **K.N. Brice:** None. **M.E. Bertrand:** None. **C. Shoffner:** None. **M.J. Chumley:** None. **G.W. Boehm:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.25/W15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R44MH108481
NIH Grant R44DA044050

Title: Clathrin-nanoparticles efficiently deliver BDNF to the hippocampus, reverse depression and neuroinflammation and increase neurogenesis, dendritic length, and spine density in HIV/Tat mouse model

Authors: *G. VITALIANO[1], C. W. ADAM[1], C. DEPALISTE[1], Y. RAZAVI[1], F. VITALIANO[2];
[1]McLean Hospital/ Harvard Med. Sch., Belmont, MA; [2]EXQOR, Boston, MA

Abstract: Rationale: Advances in treatment of neurodegenerative/ neuroinflammatory disorders and depression have been made by administering BDNF directly to the brain, or by using drugs or brain stimulation (e.g., TMS, ECT) that increase BDNF indirectly. BDNF reverses depression, promotes neuroregeneration and restores brain functions but cannot easily cross a blood-brain barrier (BBB). We delivered up to 400-fold higher BDNF concentrations to the hippocampus than other nasal delivery methods (Vitaliano at al. 2022). Our goal was to decrease neurotoxic/neuroinflammatory effects of HIV/transactivator of transcription (Tat) protein and reverse depressive symptoms in Tat+ mice. **Methods:** iTat mice (n=40) were treated daily for 7 days with doxycycline (100 mg/kg/d i.p.) that induces Tat expression (Tat+). Concurrently, animals received intranasally either BCNPs (0.3 mg/kg of BDNF with 2.4 mg/kg of clathrin) or saline (40 µl). Subsequently, Tat+ mice were tested with Tail Suspension (TS), Grip strength and Rotarod tests. Hippocampal sections were stained with Golgi, and dendritic length, arborization and spine densities were assessed using Neurolucida software (MBF). Doublecortin (DCX) positive cells were counted using Stereo Investigator (MBF). Cytokine concentrations were

measured using V-PLEX Proinflammatory Panel 1 kit (MSD). **Results:** In BCNP vs. saline treated Tat+ mice, hippocampal granule neurons exhibited significantly higher dendritic length ($F_{(1,9.3)}$=11.131, P=0.0083) and arborization ($F_{(1,7.9)}$=18.024, P=0.0029). BCNPs compared to saline significantly increased thin (P=0.0004), mushroom (P=0.0042) and total (P=0.0001) spine densities but decreased stubby spine densities (P=0.0361). DCX+ cell densities also significantly increased (p=0.0494) in the granule cell layer of BCNP vs. saline treated Tat+ mice. BCNPs reversed Tat-induced neuroinflammation by significantly decreasing hippocampal concentrations of proinflammatory cytokines (TNFα P=0.0247, IL1β P=0.0185, and IL6 P=0.0472) and by increasing levels of anti-inflammatory IL10 cytokine (P=0.0277). Time spent immobile was significantly decreased (P=0.0023) in the TST, and grip strength (P=0.0189) and rotarod performance were increased (P=0.0089) in BCNP vs. saline treated Tat+ mice. **Conclusions:** BCNPs bypassed the BBB, increased hippocampal neurogenesis, spine density, dendritic length and arborization, and reversed depressive symptoms and neuroinflammation in Tat+ HIV/neuroAIDS mouse model. Clathrin-nanotechnology may be able to enhance neuronal regeneration and plasticity and restore mood quickly and more efficiently than existing treatment methods.

Disclosures: **G. Vitaliano:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EXQOR Technologies Inc.. **C.W. Adam:** None. **C. Depaliste:** None. **Y. Razavi:** None. **F. Vitaliano:** A. Employment/Salary (full or part-time):; EXQOR Technologies Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EXQOR Technologies Inc..

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.26/W16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P30AI050409
NIH Grant K01 NS129895-02
Burroughs Wellcome

Title: Non-classical estrogen receptor, GPER, may reduce HIV-induced neuroinflammation

Authors: J. DE VASTY, K. DAVIS, K. PIERRE, T. FLETCHER, ***K. S. WILLIAMS**; Envrn. and Hlth. Sci., Spelman Col., Atlanta, GA

Abstract: Currently, there are 1.2 million patients living with HIV in the US with a median age of 50 or older. Despite the success of combined antiretroviral treatments, 30-50% of persons living with HIV are affected by HIV-associated neurocognitive disorders (HAND). As persons living with HIV age, it is predicted that the prevalence of HAND will increase. Therefore,

understanding how aging with HIV affects the brain is important for therapeutic discovery. Macrophages and microglia (M/M) play pivotal roles in the pathogenesis of HIV-associated neurocognitive disorders. The ensuing inflammatory M/M activation causes neuronal damage. Studies utilizing exogenous anti-inflammatory and antioxidants to mitigate disease progression have been unsuccessful; however, targeting endogenous pathways, such as estrogen signaling may be advantageous. Loss of estrogen due to the onset of menopause leads to reduced cognitive functions in women while classical estrogen receptors have been shown to be neuroprotective. It has been reported that 17β-estradiol can inhibit HIV infection in primary macrophages and peripheral blood mononuclear cells and protect neurons against HIV proteins, gp120, and tat. However, recent studies have elucidated the non-genomic protective roles of non-classical estrogen receptor GPER1, however, how it contributes to HIV-induced neuroinflammation is unknown. We hypothesize that activation of GPER1 via natural estrogen, 17β- estradiol, and specific GPER1 inducer G-1 will reduce HIV-induced neuroinflammation. To test this, we utilized a dual cell *in vitro* model consisting of human monocyte-derived macrophages (MDMs) and rat cortical neurons. GPER1 was blocked with a specific antagonist (G15) in MDMs prior to HIV stimulation with inactivated HIV_{ada} in the presence and absence of 17β-estradiol or GPER1 agonist, G-1 treatment. Whole-cell and cytoplasmic lysates, mRNA, and conditioned medium were collected at various time points. We found that 17β-estradiol and G-1suppressed neurotoxin production and inflammatory phenotypes in HIV-infected macrophages in a GPER1-dependent manner. Given these studies, estrogen signaling, via GPER1, may reduce neuroinflammation seen during neurodegenerative disorders, such as HIV-associated neurocognitive disorders.

Disclosures: **J. De Vasty:** None. **K. Davis:** None. **K. Pierre:** None. **T. Fletcher:** None. **K.S. Williams:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.27/W17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant F31NS116924
NIH Grant R01NS103212

Title: Vegf-a upregulation by neurons during experimental cerebral malaria contributes to blood-brain barrier disruption

Authors: *C. E. FAIN, S. RANA, F. JIN, M. T. LILLEY, M. A. MAYNES, G. C. SIECK, A. J. JOHNSON;
Mayo Clin., Rochester, MN

Abstract: Cerebral malaria (CM) is a severe complication of *Plasmodium falciparum* infection. Biomarkers of disease severity include blood-brain barrier (BBB) permeability, severe edema,

and vascular endothelial growth factor (VEGF) upregulation in post-mortem brain tissue. We previously showed via T1 and T2-weighted small-animal MRI, the recapitulation of vascular permeability using the *Plasmodium berghei ANKA* murine model of experimental cerebral malaria (ECM). Vascular permeability was noted to be regional, occurring in olfactory bulb, hippocampus and brainstem regions on day 6-8 of PbA infection. In this work we determined through RNAscope *in situ* hybridization assay that at day 6 post infection, VEGF-A mRNA transcripts were upregulated, specifically in these regions of permeability. We next sought to identify the main cell-types responsible for this upregulation, with focus on brainstem pathology. Here we report that mature NeuN+ neurons in brainstem display significantly increased production of VEGF-A mRNA transcripts during ECM. VEGF-A upregulation is observed in neurons near regions of tight junction protein disruption, and engagement of VEGF-A protein with brain microvessels is observed via immunofluorescence in these regions as well. Neurons positive for VEGF-A protein were often seen being engulfed by microglia and/or astrocytes in late ECM; indicating a possible role for VEGF-A in neuronal pathology. In mice lacking perforin, the cytotoxic molecule employed by CD8 T cells, VEGF-A mRNA did not increase in neurons day 6 of infection. These findings indicate an integral role for cytotoxic CD8 T cells in the upregulation of VEGF-A in neurons during ECM. Using a tamoxifen inducible mouse strategy, we induced neuron-specific ablation of VEGF-A during PbA infection, which resulted in decreased proportion of CD8 T cell brain infiltration. This in turn, prevented disruption of tight junction proteins. These changes resulted in overall extended survival of the animals. In conclusion, these findings highlight a potential role for CD8 T cells in promoting BBB disruption through neuronal-VEGF upregulation, identifying this cytokine as one that could be targeted therapeutically to treat human CM.

Disclosures: C.E. Fain: None. **S. Rana:** None. **F. Jin:** None. **M.T. Lilley:** None. **M.A. Maynes:** None. **G.C. Sieck:** None. **A.J. Johnson:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.28/W18

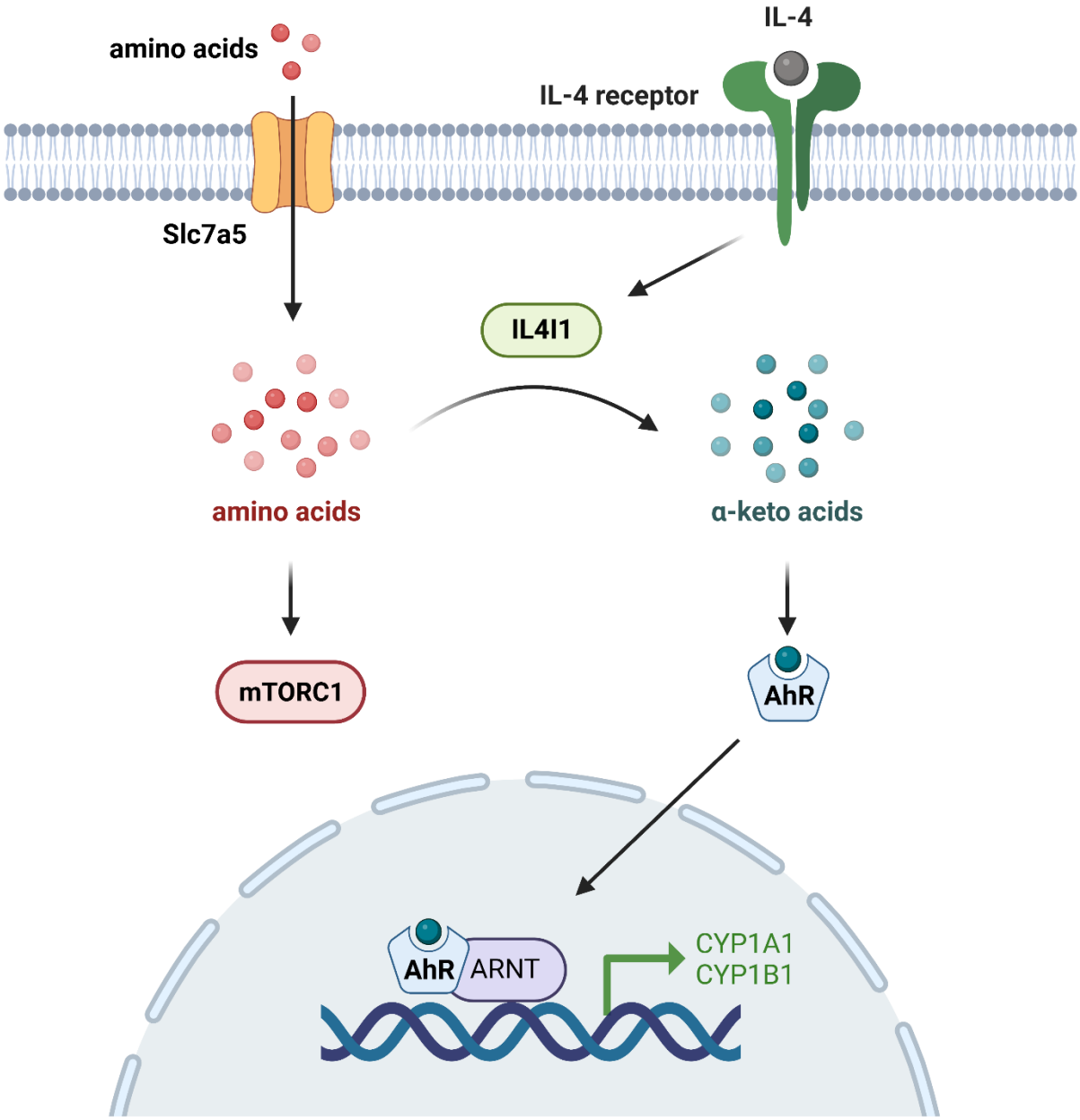
Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R01 NS107523-01

Title: Myeloid cell-associated aromatic amino acid metabolism facilitates myelin regeneration in thecentral nervous system

Authors: *J. HU[1], H. KIM[1], D. LADAKIS[2], J. REGER[1,3], K. A. CHAMBERLAIN[1], N. V. SHULTS[1], H. C. OFT[1], V. N. SMITH[1], L. M. ROSKO[1], G. S. MELCHOR[1], E. LI[1], M. BAYDYUK[1], M.-M. FU[3], P. BHARGAVA[2], J. K. HUANG[1];
[1]Georgetown Univ., Washington DC, DC; [2]Johns Hopkins Univ., Baltimore, MD; [3]Natl. Inst. of Neurolog. Disorders and Stroke (NINDS), Bethesda, MD

Abstract: Multiple sclerosis (MS) is a neuroinflammatory disease where myelin sheaths from multiple regions of the central nervous system (CNS) are destroyed. Regulation of myeloid cell activity is critical for successful myelin regeneration (remyelination). We found that myeloid cells upregulate an amino acid oxidase downstream of IL-4 signaling, interleukin-4 induced 1 (IL4I1), during remyelination, and its deletion in myeloid cells impaired remyelination efficiency. Mice lacking IL4I1 expression exhibited a reduction in the alpha-keto acids (AKAs) generated from the enzymatic activity of IL4I1 in demyelinated lesions. Decreased AKA levels were also observed in people with multiple sclerosis (MS), particularly in the progressive phase when remyelination is impaired. AKA treatment modulated inflammation in microglia culture, and promoted oligodendrocyte differentiation in the presence of microglia. However, AKA treatment did not influence the differentiation of pure oligodendrocyte culture, indicating the effect of AKAs on oligodendrocytes was indirect, likely through the regulation of microglia. In mice, the administration of AKAs after demyelination decreased microglia-associated inflammation, and increased oligodendrocyte maturation and remyelination. Transcriptomic analysis revealed AKAs induced a metabolic shift in myeloid cells in lesions and upregulated aryl hydrocarbon receptor (AhR) activity. As an amino acid oxidase, IL4I1 treatment also inhibited the amino acid-induced mammalian target of the rapamycin complex 1 (mTORC1) pathway. Blocking amino acids transportation into the cells during autoimmune responses attenuated experimental autoimmune encephalomyelitis (EAE) clinical disabilities and reduced mTORC1 activity of myeloid cells in the spinal cords. Our results suggest myeloid cells-derived amino acid oxidase IL4I1 facilitates remyelination, and that therapeutic approaches targeting amino acid metabolism in inflammatory demyelinating diseases, such as MS, may improve remyelination.



Disclosures: **J. Hu:** None. **H. Kim:** None. **D. Ladakis:** None. **J. Reger:** None. **K.A. Chamberlain:** None. **N.V. Shults:** None. **H.C. Oft:** None. **V.N. Smith:** None. **L.M. Rosko:** None. **G.S. Melchor:** None. **E. Li:** None. **M. Baydyuk:** None. **M. Fu:** None. **P. Bhargava:** None. **J.K. Huang:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.29/W19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ZIA NS 003119
APND-MD

Title: A 4D transcriptomic map for the evolution of multiple sclerosis-like lesions in the marmoset brain

Authors: J.-P. LIN[1], A. BRAKE[1], M. DONADIEU[1], A. LEE[1], R. KAWAGUCHI[3], P. SATI[4,1], D. H. GESCHWIND[3,5], S. JACOBSON[2], D. P. SCHAFER[6], D. S. REICH[1];
[1]Translational Neuroradiology Section, [2]Viral Immunol. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Natl. Inst. of Hlth., Bethesda, MD; [3]Departments of Neurol. and Human Genet., UCLA, Los Angeles, CA; [4]Dept. of Neurol., Cedars Sinai Med. Ctr., Los Angeles, CA; [5]Psychiatry, Semel Inst. for Neurosci. and Human Behavior, David Geffen Sch. of Medicine, UCLA, Los Angeles, CA; [6]Univ. of Massachusetts Med. Sch., Univ. of Massachusetts Chan Med. Sch. Grad. Program in Neurosci., Upton, MA

Abstract: The current understanding of the pathophysiology of multiple sclerosis (MS) lesions is primarily derived from studies conducted on human biopsy and postmortem tissue. However, relying on a single time point, especially at the end of life, fails to capture the dynamics of lesion evolution, making it challenging to develop therapies for earlier lesion stages. To close this gap, we employed a clinically relevant model to study the development and repair of MS-like lesions. Common marmosets (*Callithrix jacchus*) have high genetic, physiological, and immunological similarities to humans, and induction of experimental autoimmune encephalitis (EAE) recapitulates the evolution of white matter (WM) lesions in MS more accurately than rodent models. Using magnetic resonance imaging (MRI)-guided RNA profiling, we analyzed ~600,000 transcriptomes with single-nucleus resolution together with ~55,000 spatial transcriptomes. These were analyzed as a function of EAE inoculation status, longitudinal quantitative MRI signal, and histopathological features. We observed substantial diversity and expansion of immune and glial cells in the developing lesion, with cycling dendritic cells (DC), monocytes, microglia, and oligodendrocyte progenitor cells (OPC) being dominant in the early stages of the lesion when the blood-brain barrier is open. While the overall proportion of astrocytes remains relatively constant over time, their transcriptomes underwent significant changes associated with EAE (AST_{EAE}), altering their intercellular communications. The AST_{EAE} cluster accumulates at the lesion edge and is more strongly influenced by extracellular matrix signals compared to homeostatic astrocytes. By computing the centrality of signaling networks with CellChat program, we further identified that AST_{EAE} is the main influencer and receiver of ANGPTL (Angiopoietin-like protein) signal sent from differentiating OPC, suggesting its roles in regulating lipid and glucose metabolism. AST_{EAE} is also predicted to be a player in EAE-specific networks, such as IL16 (Interleukin 16) and CD30 (TNF-receptor superfamily) signaling that encompass ependyma, DC, and microglia, suggesting its role in mediating the initiation of neuroinflammation. Furthermore, by mapping the distribution of cells and transcripts with

demyelination as a function of distance from the lesion center, informed by MRI and lipid-staining, we identified microenvironment niches that mark the initiation/expansion and settlement/containment of WM lesions. Overall, these findings provide a detailed accounting of the cells and molecules involved in the growth and resolution of MS-like lesions.

Disclosures: **J. Lin:** None. **A. Brake:** None. **M. Donadieu:** None. **A. Lee:** None. **R. Kawaguchi:** None. **P. Sati:** None. **D.H. Geschwind:** None. **S. Jacobson:** None. **D.P. Schafer:** None. **D.S. Reich:** None.

Poster

PSTR469. Neuroinflammation: HIV, HSV, COVID19, and Other Infections II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR469.30/W20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DoD CDMRP GW130053

Title: Stress and sarin surrogate prime the epigenetic response to LPS in brain and blood in a mouse model of Gulf War Illness

Authors: A. SASAKI[1], D. ASHBROOK[2], S. WIJENAYAKE[3,1], ***P. O. MCGOWAN**[1]; [1]Biol. Sci., Univ. of Toronto, Toronto, ON, Canada; [2]Dept. of Genetics, Genomics and Informatics, The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]Dept. of Biol., Univ. of Winnipeg, Winnipeg, MB, Canada

Abstract: Gulf War Illness (GWI) is a disorder characterized by multiple symptoms and neuroimmune dysfunction, exhibiting resemblances to sickness behavior. Unfortunately, existing approaches for treating GWI primarily concentrate on symptom management rather than targeting the fundamental etiology of the illness. A leading hypothesis for the cause of GWI is that the high physiological stress of the combat theatre interacted with nerve agent exposure in theatre. Using a preclinical mouse model, we have found that acute exposure to the glucocorticoid corticosterone (CORT) and the sarin surrogate diisopropyl fluorophosphate (DFP) associate with DNA methylation modifications and genome-wide changes in transcript abundance. Long term models have found that these exposures exacerbate the neuroinflammatory response to a subsequent immune challenge with lipopolysaccharide (LPS). In this long-term exposure model, mice are initially exposed to CORT (200 mg/L) in the drinking water for 7 days followed by a single, acute injection of DFP (4 mg/kg, i.p.). This is then followed by periodic administration of CORT for 7 days every other week to a total of 5 weeks with LPS (0.5 mg/kg, s.c.) on the final day. In the present study, mice exposed to CORT and DFP were sacrificed 6, 12, and 24 hours after LPS challenge. Hippocampus, frontal cortex and whole blood gene expression and DNA methylation were evaluated by RNA-seq and Reduced Representation Bisulfite sequencing. Similar classes of genes increased in expression in the hippocampus and frontal cortex across all three timepoint, however differential methylation

was largely confined to the hippocampus. Compared to no LPS control, differential methylation and expression were observed in 37 genes at 6hrs post-LPS challenge and 24 genes and at all three timepoints. Of these genes, 29 and 16 genes at 6hrs post-LPS and at all three timepoints, respectively, were also differentially methylated in blood. Interestingly, FKBP5, a gene involved in glucocorticoid binding to its receptor and in stress-related disorders, was conserved across brain and blood. These findings suggest potential mechanisms by which GWI exposures alter the neuroinflammatory response to immune challenge, and potential blood biomarkers that could be pursued in future studies.

Disclosures: A. Sasaki: None. **D. Ashbrook:** None. **S. Wijenayake:** None. **P.O. McGowan:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.01/W21

Topic: C.08. Ischemia

Title: Microglia Initiated Astrocyte-Mediated Ischemic Tolerance

Authors: *T. TATEOKA, H. YOSHIOKA, T. WAKAI, K. HASHIMOTO, N. FUKUDA, H. KINOUCHI;
Univ. of Yamanashi Fac. of Med. Grad. Sch. of Med., Chuo city, Japan

Abstract: Background: Astrocyte-mediated ischemic tolerance has attracted much attention recently as a neuroprotective mechanism induced by ischemic preconditioning (IPC). The interaction between microglia and astrocytes is expected to play an important initiator; however, it has not been elucidated. **Aim:** We investigated the effects of pharmacological microglial depletion on the neuroprotective efficacy and the phenotype of reactive astrocytes after IPC. **Method:** Transient focal cerebral ischemia using mice was induced by intraluminal filament occlusion. Using a colony stimulating factor 1 receptor antagonist PLX5622, we created a situation of microglial depletion at the induction of IPC, and recovered to the normal levels at the loading of lethal ischemia, 7 days after IPC. **Results:** This controlled microglial depletion diminished protective effects of IPC against lethal ischemia, with larger infarction volume (control 37.9 ± 19.5 vs PLX 64.0 ± 16.5 mm^3, $P<0.05$) and worse neurological Longa's scores (control 1.2 ± 0.78 vs PLX 2.4 ± 0.88, $P<0.05$). Reactive astrocytes were observed after IPC, which was significantly more enhanced with microglial absence during IPC (control 11.4 ± 3.0 vs PLX 21.8 ± 4.4 $/3*10^{-2}$ mm^2, $P<0.01$). Furthermore, IPC without microglia enhanced the proliferation of neurotoxic C3d-positive astrocytes (control 3.0 ± 3.1 vs PLX 12.1 ± 4.5 $/3*10^{-2}$ mm^2, $P<0.05$), and suppressed the induction of neuroprotective P2X7 receptor-positive astrocytes (control 8.8 ± 4.1 mm^2 vs PLX 4.4 ± 2.9 $/3*10^{-2}$ mm^2, $P<0.01$). **Conclusions:** The present study demonstrates that Microglia are essential for the induction of ischemic tolerance by cross-talk with astrocytes in a sophisticated manner.

Disclosures: **T. Tateoka:** None. **H. Yoshioka:** None. **T. Wakai:** None. **K. Hashimoto:** None. **N. Fukuda:** None. **H. Kinouchi:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.02/W22

Topic: C.08. Ischemia

Title: Single-cell RNA sequencing identifies the activation of neurodegeneration-associated microglial cells in an animal model of ischemic white matter degeneration

Authors: *Y. OH;
Ajou university, Gyeonggi-do, Korea, Republic of

Abstract: Chronic impairment of cerebral blood flow due to hypertension may underlie white matter degeneration in the elderly. The potential mechanisms of how ischemic stress leads to demyelination and axonal degeneration in the white matter are largely unknown. We developed a novel subcortical white matter degeneration model in renovascular hypertensive (RVH) rats. RVH markedly induced demyelination and axonal degeneration within the corpus callosum and cingulum. White matter hyperintensities on T2 MRI accompanied these white matter pathologies, and the fractional anisotropy in diffusion tensor imaging was also significantly reduced. Single-cell RNA-sequencing (sc-Seq) was performed to profile cell-type specific gene expression in this model. Sc-Seq revealed the activation of microglial cells that express a set of proinflammatory genes. Pathway analysis indicated that the gene profile in microglial cells in this condition is highly enriched with the gene sets that are associated with various neurodegenerative diseases. In contrast, oligodendrocytes increased expression of the genes linked to Toll-like receptor signaling, suggesting the activation of the innate immune pathway. Furthermore, macrophage subclusters substantially increased in animals with white matter lesions and were found to obtain the genotype of the neurodegenerative-associated microglial cells. These results suggest that microglial cells and monocyte-derived macrophages take the neurodegenerative-associated microglial phenotype and may together contribute to ischemic white matter degeneration.

Disclosures: **Y. Oh:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.03/W23

Topic: C.08. Ischemia

Support: NIH R01 Grant

Title: Characterization of a 3D Printed Microfluidic Chip for Localized and Sustained Stimulation in Ex Vivo Hippocampal Slices

Authors: *C. E. WITT[1], A. ROSS[2];
[1]Chem., [2]Univ. of Cincinnati, Cincinnati, OH

Abstract: Strokes (ischemic and hemorrhagic) are the fifth leading cause of death in the United States thus leading to a national health burden. Over the years, work has gone into understanding the neurochemical tone during global ischemic events; however, there is a large shift within the neuroscience community to these events at the site of injury (focal ischemia). Focal ischemia is of particular interest because of its confounding effects in localized regions. By coupling microfluidics with fast-voltammetry, our lab has been able to make great strides toward making measurements at the localized sight of ischemia. Our previous work has shown that are able to fabricate a 100 um port on a microfluidic platform to deliver injury with a 583.5 +/- 65.9 um spatial resolution over a minute period. Though this initial work shows promise, a more user-friendly fabrication approach is necessary to improve translatability of the device. Likewise, improvements in the spatial resolution of delivery would enable more precise localization of the stimulus. Therefore, in this talk, we will discuss a 3D printing approach and a new geometric design for stimulus delivery to achieve our overarching goals. Ultimately, this new device enables sustained, spatially resolved delivery of injury in ex vivo tissue slices and shows significant improvement form our previously published device. Overall, our device bridges a critical gap in the literature and will directly impact our ability to understand local physiology during injury when coupled to fast-voltammetry.

Disclosures: C.E. Witt: None. **A. Ross:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.04/W24

Topic: C.08. Ischemia

Support: AHA grant 20TPA35490411
NIH grant NS122808

Title: Recurrent hypoglycemia exacerbates cerebral ischemic damage in ITD rats by promoting mitochondrial dysfunction

Authors: *S. MALLEPALLI[1,2], L. DALCO[1,2], A. K. REHNI[1,2], S. CHO[1,2], K. DAVE[1,2,3]; [1]Peritz Scheinberg Cerebral Vascular Dis. Res. Labs., Miami, FL; [2]Dept. of Neurol., [3]Neurosci. Program, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Diabetes is a risk factor for stroke. Recurrent hypoglycemia (RH) exposure is common in treated diabetes patients. Repeated RH exposure in diabetic rats worsens outcomes post-cerebral ischemia. However, the underlying mechanism is not well understood. Mitochondrial autophagy and dynamics play an important role in cerebral ischemic damage. In the present study, we examined markers of autophagy (HSP60, Beclin1, and LC3-II) and mitochondrial dynamics (OPA1, mitofusin1, and Fis1) in hippocampus (the most vulnerable area to ischemic damage). We used non-synaptic (NSM) and synaptic mitochondria (SM) of RH-exposed diabetic rats subjected to global cerebral ischemia (GCI) or sham surgery overnight after the last hypoglycemia exposure. Male insulin-treated streptozotocin diabetic rats (insulin-treated diabetes, ITD) were exposed to RH (hyperinsulinemic hypoglycemia) or ITD+RH+glucose (hyperinsulinemic euglycemia) (controls). After ~24 h post-surgery, mitochondria were harvested, and protein levels were assayed using western blots. Data from sham groups were pooled as no intergroup difference was observed, and results were expressed as % of the pooled sham group. The levels of HSP60 in NSM were higher by 255% (355±30, n=6, $p<0.001$) and 146% (246±15, n=5, $p<0.01$) in the RH+glucose+GCI and RH+GCI groups, respectively, when compared to the sham group (100±22, n=9). HSP60 levels in the RH+glucose+GCI group were higher by 44 % ($p<0.05$) as compared to the RH+GCI group in NSM. We observed a 173% increase in HSP60 levels in SM in the RH+glucose+GCI (273±18, n=6, $p<0.01$) group as compared to the sham group (100±32, n=11). In NSM, levels of LC3-II were higher by 145% in the RH+GCI (245±28, n=5, $p<0.01$) group when compared to the sham group (100±22, n=10). LC3-II in the RH+GCI group was significantly higher by 98% ($p<0.05$) as compared to RH+glucose+GCI (123±19, n=6) in NSM. Levels of OPA1 in NSM were higher by 144% (244±29, n=5, $p<0.01$) and 172% (272±43, n=6, $p<0.001$) in the RH+glucose+GCI and RH+GCI groups, respectively, when compared to the sham group (100±18, n=10). In NSM, the levels of mitofusin1 were higher by 87% (187±12, n=5 $p<0.01$) in the RH+GCI group as compared to the sham group (100±11, n=8). However, the RH+GCI group showed a significantly increased expression by 45% as compared to the RH+glucose+GCI group (129±19, n=6 $p<0.05$) in NSM. We observed no significant difference between groups in levels of Fis1 and Beclin1 in NSM and Beclin1, LC3-II, OPA1, mitofusin1, and Fis1 from SM. Our results demonstrate that RH exposure to ITD rats impacts mitochondrial dynamics and mitophagy markers more in NSM than SM. Acknowledgement: AHA grant 20TPA35490411 and NIH grant NS122808.

Disclosures: S. Mallepalli: A. Employment/Salary (full or part-time):; full, Univ of Miami Sch. of Med. **L. Dalco:** A. Employment/Salary (full or part-time):; full, Univ of Miami Sch. of Med. **A.K. Rehni:** A. Employment/Salary (full or part-time):; full, Univ of Miami Sch. of Med. **S. Cho:** A. Employment/Salary (full or part-time):; full, Univ of Miami sch. of med. **K. Dave:** A. Employment/Salary (full or part-time):; full, Univ of Miami sch. of med..

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.05/W25

Topic: C.08. Ischemia

Support: CIHR (MOP-4227791)
CIHR (MOP-198081)
CIHR PDF award (JR)

Title: Consequences of brain-wide microinfarction on microglial activation and vascular pathophysiology

Authors: *J. ROYEA[1,2,3,4], D. ALOMAR[2], M. VANNI[3], G. SILASI[2];
[2]Cell. and Mol. Mechanisms, [1]Univ. of Ottawa, Ottawa, ON, Canada; [3]Lab. de Neurophotonique, Univ. of Montreal, Montreal, QC, Canada; [4]Lab. de Neurophotonique, Univ. de Montréal, Montréal, QC, Canada

Abstract: Background: Cerebral microinfarction is a form of ischemic injury caused by impairments in microvascular blood flow. Their presence is a common occurrence within the aging population and represents an important biomarker for many brain pathologies, including vascular cognitive decline, dementia and Alzheimer's disease[1,2,3]. Despite the presence of hundreds to thousands of microinfarcts within the ageing brain[4], microinfarction is vastly understudied relative to its prevalence within the general population and clinical studies on microinfarcts are limited. Indeed, the presence of a large number of microinfarcts within individuals may be sufficient to alter vascular pathophysiology, disrupt the function of neural networks, and activate gliosis. For these reasons, this study examined the impact of microinfarction on the brain using histological markers of damage, including microglial activation, blood brain barrier (BBB) leakage, and vascular pathophysiology.**Methods/Results:** We utilized a mouse model of microinfarction triggered through the intracarotid injection of fluorescent microspheres that broadly distributed microinfarcts throughout the targeted hemisphere[5]. Transgenic mice expressing green fluorescent protein (GFP) in microglial cells (Tmem119-eGFP) were used to perform brain-wide evaluation of microgliosis following microinfarction. The BBB was assessed by Evans blue dye extravasation histologically. Wholebrain imaging was performed and validated widespread distribution of microvascular occlusions, with the majority of beads present within the cortices (~33% of the total distribution). Induction of microinfarction resulted in an acute form of damage to the brain whereby microgliosis was significantly correlated with BBB disruption.**Conclusions:** Understanding the impact of microinfarction within the brain is central towards the development of preventative and therapeutic interventions that can mitigate the damaging effects of microinfarcts in humans.**References:**1. Westover et al, Neurology, 2013, 1365-1369; 2. Smith et al, Lancet Neurology, 2012, 272-282 ; 3. van Veluw et al, Lancet Neurology, 2017, 730-740 ; 4. Shih et al, Stroke, 2018, 803-810 ; 5. Silasi et al, JCBFM, 2015, 734-738.**Acknowledgements:** Supported by CIHR (MOP-427791 (GS); MOP-198081 (MV)), CIHR PDF award (JR).

Disclosures: J. Royea: None. **D. Alomar:** None. **M. Vanni:** None. **G. Silasi:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.06/W26

Topic: C.08. Ischemia

Support: NIH Grant Guanosine

Title: Transient Guanosine Signaling Changes as a Function of Ischemic Severity

Authors: *K. CALDWELL;
Univ. of Cincinnati, Cincinnati, OH

Abstract: Disruption of the ability to exchange glucose and oxygen in the brain leads to one of the most common forms of brain damage, ischemic stroke. The increases in inflammation, glutamatergic excitotoxicity, and oxidative stress enable detrimental outcomes of this disease. Work on understanding various biomarkers important during stroke is useful for the development of therapeutics; however, there remains a lack of understanding of the neuropathological impact of ischemic events. Guanosine is a purine signaling molecule and an emerging biomarker of interest for neuroprotection. Many have shown the ameliorating effects of exogenous guanosine treatment in ischemic stroke yet the molecular mechanism of how endogenous guanosine in the brain recovers stroke has not been uncovered. Previously, our lab has shown an increase in rapid, endogenous guanosine signaling during severe ischemic events using fast-scan cyclic voltammetry (FSCV) at a carbon fiber microelectrode (CFME). Despite this finding, an understanding of how this subsecond signaling changes as a function of ischemic severity is not well understood yet could advance fundamental knowledge on the brain's immediate neuroprotective response during varying severities of injury. In this work, we use an optical oxygen sensor to characterize an *ex-vivo* oxygen glucose deprivation (OGD) model with controllable severity (normoxia, mild, and severe). In doing so, we have cultivated a standard for *ex vivo* slice ischemic studies which better correlate to the varying ischemic severity models which exist for in vivo analysis. Immunohistochemistry and 2,3,5-Triphenyltetrazolium chloride (TTC) were used to help further correlate the changes observed as a function of OGD model to the changes measured in guanosine signaling as a function of ischemic severity. Overall, this work provides the first method to specifically control ischemic severity ex vivo with correlations to how these changes influence neural signaling.

Disclosures: K. Caldwell: None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.07/W27

Topic: C.08. Ischemia

Title: Supplementation with a symbiotic induced neuroprotection and improved memory in rats with ischemic stroke

Authors: *Y. CRUZ[1], A. ROMO[1], V. GÁLVEZ-SUSANO, Jr.[2], E. GARCÍA[1], A. IBARRA[1]; [1]Ctr. de Investigación en Ciencias de la Salud (CICSA), Facultad de Ciencias de la Salud., Univ. Anáhuac México, México, Mexico; [2]Inst. Politécnico Nacional, México, Mexico

Abstract: After ischemic stroke, there are several deleterious mechanisms involved in tissue damage, including the inflammatory response. The increase in pro-inflammatory cytokines has been related to greater damage to neural tissue and promotion of neurological alterations, including cognitive impairment. Recent research has shown that the use of prebiotics and/or probiotics counteracts inflammation and improves cognitive function through the production of growth factors, such as brain-derived neurotrophic factor (BDNF), by reducing inflammatory molecules. Therefore, in this proof-of-concept study, the effect of the symbiotic inulin and *Enterococcus faecium* was evaluated on memory improvement and neuroprotection in a murine model of transient middle cerebral artery occlusion (tMCAO). In order to accomplish this, the animals were subjected to ischemia; the experimental group was supplemented with the symbiotic and the control with the vehicle. The neurological deficit, spatial and working memory were evaluated using the Zea Longa scale, Morri's water maze and the 8-arm maze tests, respectively. Infarct size, expression of BDNF and tumor necrosis factor-alpha (TNF-α) were also assessed. The results show that supplementation with the symbiotic significantly diminished neurological deficit, improved memory and learning, increased BDNF expression, reduced infarct size and TNF-α production. These findings provide new evidence about the therapeutic use of symbiotics for ischemic stroke and open a possibility for the design of further studies.

Disclosures: Y. Cruz: None. **A. Romo:** None. **V. Gálvez-Susano:** None. **E. García:** None. **A. Ibarra:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.08/W28

Topic: C.08. Ischemia

Title: Longitudinal Tracking of Neurovascular Response Following Microinfarcts Using Multi-Modal Neural Platform

Authors: *Y. JIN[1], F. HE[1], H. RATHORE[2], Y. SUN[1], R. YIN[3], J. ZHANG[1], C. XIE[1], L. LUAN[1];

[1]Electrical Computer Engin., [2]Applied Physics, Rice Univ., HOUSTON, TX; [3]Rice Neuroengineering Initiative, HOUSTON, TX

Abstract: Microinfarcts, often referred to as “silent stroke”, are common in aged and injured brain, particularly in individuals with cognitive impairment or underlying small vessel diseases. However, the neurophysiological impact of individual cerebral microinfarcts remains largely unknown owing to their small size and lack of behavioral impairments. In this study, we induced microinfarcts in aged mice model by targeted photo-thrombosis that selectively blocked the cerebral blood flow in a single penetrating arteriole and investigated how neural and vascular activities responded longitudinally to such a micro-occlusion. We employed ultra-flexible electrodes nanoelectrode threads (NETs) to track intracortical neural activities, while used two-photon imaging, along with laser speckle contrast imaging to track microvasculature, microcirculation, and regional cerebral blood flow longitudinally from pre-infarct baseline to two weeks post-microinfarct. To assess the neurophysiological impact of microinfarcts in the nearby tissue, we examined neural activity in region 0.3-1.2mm from the microinfarct core with little observable damage on the microvasculature. Our findings revealed that neural activities were suppressed by the microinfarct, with time course of recovery tracking that regeneration and reperfusion of microvasculature at the microinfarct core. In addition, the decrease of neural activities was cortical-depth related, with shallower layers being more severely affected than deeper layers. This differed from microvascular damage where the deeper cortical layer was more severe. Furthermore, putative cell-type-specific analysis of single neurons revealed that the excitability of fast spiking narrow interneurons was severely dampened while pyramidal neurons were minimally affected. Consistently, spike phase locking at the low gamma band, driven by the fast-spiking interneurons, was compromised by microinfarcts, indicating an interruption in large range neuronal assembly communication. These findings suggest that microinfarcts induce profound neural impairment that extend much further than the microscopic vascular damages.

Disclosures: **Y. Jin:** None. **F. He:** None. **H. Rathore:** None. **Y. Sun:** None. **R. Yin:** None. **J. Zhang:** None. **C. Xie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patent filed by The University of Texas on ultraflexible neural electrode technology used in the study and hold equity ownership in Neuralthread, Inc. **L. Luan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor on patent filed by The University of Texas on ultraflexible neural electrode technology used in the study and hold equity ownership in Neuralthread, Inc..

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.09/X1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH-R01HL139492
W81XWH1810166 & W81XWH1810167
W81XWH2210461 & W81XWH2210462
NIH T32 Training Grant for Clinician Scientists in Pediatric Critical Cardiopulmonary Disease

Title: A Novel Neuroimmunomodulatory Cocktail Treats Existing Posthemorrhagic Hydrocephalus of Prematurity and Associated Neurological Sequelae in Rats

Authors: *T. HECK[1], Y. KITASE[1], A. ODUKOYA[3], X. JIA[4], L. L. JANTZIE[2], S. ROBINSON[4];
[2]Pediatrics, [1]Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Neurosci., Johns Hopkins Univ., Bowie, MD; [4]Johns Hopkins Univ., Baltimore, MD

Abstract: Along with the effects of altered cerebrospinal fluid (CSF) dynamics, children with PHHP are prone to epilepsy and cerebral palsy (CP). While some infants recover from severe intraventricular hemorrhage (IVH) without sequelae, we posit that the failed recovery causing PHHP also leads to neurological comorbidities from chronic inflammation and neural-immune dysfunction. We hypothesize that neuro-immunomodulation can aid in recovery, normalize CSF dynamics, and mitigate increased intracranial pressure (ICP). Melatonin (MLT) may potentiate Roxadustat (ROX), a member of a new drug class of oral prolyl hydroxylase domain inhibitors. We tested a regimen of ROX+MLT in an established rat model of existing PHHP. We induced PHHP with bilateral intracerebroventricular injection of littermate lysed red blood cells on postnatal day 1 (P1) in rats of both sexes with in utero exposure to chorioamnionitis. P21 (toddler equivalent) PHHP rats were randomly allocated to 10 days of intraperitoneal ROX+MLT or vehicle with blinded observers. Intra-aural distance (proxy head circumference) was measured. Opening pressure was measured at P30. A second cohort received 10 days of ROX+MLT induction at P21 then maintenance dosing thrice weekly. Computerized, digital gait analyses and touchscreen chamber testing of visual discrimination and reversal learning were performed, followed by seizure threshold testing with escalating doses of pentylenetetrazol. Differences between groups were compared with parametric or nonparametric tests and post-hoc corrections, $p<0.05$ as significant. At P30, vehicle-treated PHHP rats (n=13) had larger heads than shams (n=22) and higher OP ($p<0.0001$). ROX+MLT-treated PHHP rats (n=11) had smaller heads than vehicle-treated PHHP rats ($p=0.02$) and lower ICP ($p=0.04$). Head size and ICP for ROX+MLT-treated PHHP rats did not differ from shams. Vehicle-treated PHHP rats exhibited an abnormal gait reminiscent of CP, compared to shams and ROX+MLT-treated PHHP rats ($p<0.05$). In visual discrimination, vehicle-treated PHHP rats (71% pass) performed worse than shams (91% pass) and ROX+MLT PHHP rats (92% pass). Reversal learning and cognitive flexibility also improved with ROX+MLT therapy. Vehicle-treated PHHP rats had a lower seizure threshold than shams, but this normalized with ROX+MLT therapy ($p<0.05$). Our results suggest that ROX+MLT begun at toddler-equivalent age in a preclinical model of PHHP improves CSF dynamics, gait, and seizure threshold. Persistent inflammation from IVH early in life may deter endogenous neural cell repair, and immunomodulation offers a pharmacologic strategy to reduce shunt burden and sequelae like CP and epilepsy.

Disclosures: T. Heck: None. **Y. Kitase:** None. **A. Odukoya:** None. **X. Jia:** None. **L.L. Jantzie:** None. **S. Robinson:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.10/X2

Topic: C.08. Ischemia

Support: NIH NINDS R01NS105899

Title: Asymmetric gait in rodents following photothrombotic stroke

Authors: *H. KHANNA[1], *H. KHANNA[2], H. H. CHAN[1], K. B. BAKER[1];
[1]Neurosciences, Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Cleveland Clin., Cleveland, OH

Abstract: Stroke is a devastating condition that can result in chronic motor impairments that severely impact activities of daily living and quality of life. When these effects extend to lower limb function, they can impact weight bearing and result in gait abnormalities. Healthy gait is symmetrical, with both legs spending a similar proportion of time in the swing and stance phases of the gait cycle. Following a stroke, however, individuals often adopt compensatory methods to reduce loading on the affected leg. This imbalanced distribution of body weight results in asymmetrical gait where the patient increases its reliance on the unaffected limb to ambulate. To better characterize the natural history of gait abnormalities over time following a cortical infarct, we evaluated baseline and post-lesion motor function using a photothrombotic stroke (PTS) model in female Long-Evans rats aged approximately four months. A total of 19 animals underwent baseline evaluation using the Noldus CatWalk system and the horizontal ladder task followed by PTS via open craniotomy targeting primary motor cortex. Beginning one-week post-infarct, animals underwent repeat testing weekly for a period of four weeks. The two parameters we used to characterize gait on the CatWalk - overall temporal symmetry and stance time symmetry - showed statistically significant gait asymmetry following stroke. This was followed by improvement two weeks after stroke and plateau from week 2 to week 4 of the post-stroke period. This was mirrored by other CatWalk parameters which also showed similar trends. Motor deficits also were observed in the ladder rung walking task, where a significant increase in the number of slips was seen the first week after stroke. This was followed by improved performance after week 2 post-stroke with performance stabilizing from that point to the end of the study. Based on trends in intensity, duty cycle, and base of support amongst other metrics, we propose that this stabilization in performance is due to a combination of spontaneous recovery and compensatory mechanisms adopted by the rats to ambulate while reducing the extent to which weight is loaded on the afflicted hind paw. The results of this study provide insight as to how compensatory mechanisms and improvement in the degree of gait asymmetry are reflected by changes in the gait cycle over time. This analysis of natural history supports the use of gait asymmetry as a measure of deficit and functional improvement over time for in preclinical stroke models, particularly in relation to novel therapeutic approaches.

Disclosures: **H. Khanna:** None. **H. Khanna:** None. **H.H. Chan:** None. **K.B. Baker:** None.

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.11/X3

Topic: C.08. Ischemia

Support: NIH NINDS R01NS105899

Title: Evaluating Fine Motor Deficits in Photothrombotic Stroke-Treated Rats

Authors: *M. D. PORTER[1,4], H. H. CHAN[1], C. SONNEBORN[2], H. KHANNA[1], A. MACHADO[3], K. B. BAKER[1];
[2]Quantitative Hlth. Sci., [3]Neurosurg., [1]Cleveland Clin., Cleveland, OH; [4]Neurosciences, Kent State Univ., Kent, OH

Abstract: The preclinical photothrombotic stroke (PTS) model results in an ischemic injury that emulates human stroke. The pasta matrix retrieval task is used to evaluate skilled forepaw use in rats and corresponds to measures of human upper limb function, but it has never been used to evaluate motor deficits in rats with PTS lesions. We sought to characterize affected paw use across the natural history of the PTS model and to evaluate the relationship between pasta task performance and cylinder and ladder rung walking test results. Our goal was to determine whether the pasta matrix provides unique insight into the nature and time course of deficits. 19 adult female Long-Evans rats were trained to perform a pasta matrix retrieval task. Following training and baseline characterization, all animals underwent PTS of the dominant motor cortex. After one week of recovery, pasta matrix, cylinder, and ladder tasks were administered to characterize the severity and time course of motor deficits over a period of four weeks. Rats were evaluated by the ladder and cylinder tasks once per week and by the pasta matrix 3 times weekly. After sacrifice, lesion volume was calculated and compared to performance on each metric. Pasta matrix task results show evidence of a deficit persisting 4 weeks after stroke, with rats breaking an average of 1.9 fewer pieces each week than at pre-stroke baseline when all time points are included ($p = 0.0002$, random intercept mixed effects model). The cylinder task supports evidence of a continued deficit after stroke, with rats using their affected forepaw an average of 2.1 fewer percentage points each week than at pre-stroke baseline when all time points are included ($p = 0.0013$, random intercept mixed effects model). The ladder task also showed a continued deficit after stroke, with rats slipping an average of 0.86 more times each week than at pre-stroke baseline when all time points are included ($p < 0.0001$, random intercept mixed effects model). Pasta matrix performance was correlated to cylinder task performance (0.63, Pearson correlation), but not as strongly to ladder performance (-0.47, Pearson correlation). No relationship was identified between lesion volume and behavior. For the first time, the pasta matrix retrieval task shows that PTS impairs fine motor ability of the rat forepaw for up to one month following stroke induction. Pasta task performance is positively correlated with cylinder

task performance, but not with ladder task performance, possibly due to compensation in the ladder task by the other 3 limbs.

Disclosures: **M.D. Porter:** None. **H.H. Chan:** None. **C. Sonneborn:** None. **H. Khanna:** None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc., US Patent 7640063. **K.B. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS Therapy, Inc. F. Consulting Fees (e.g., advisory boards); Enspire DBS Therapy, Inc..

Poster

PSTR470. Ischemia and Hemorrhage: Animal Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR470.12/X4

Topic: C.09.Stroke

Title: Early and long-lasting white matter damage in the perforant pathway following subarachnoid hemorrhage in mice

Authors: *A. REGNIER-GOLANOV, C. KARMONIK, R. LE, H. GOODWIN, N. KVIRKVELIA, E. GOLANOV, G. BRITZ;
Neurosurg., Houston Methodist Hosp., Houston, TX

Abstract: Subarachnoid hemorrhage (SAH), when the blood extravasates into the subarachnoid space, is the least frequent stroke but has a high fatality rate (40%) and often debilitates people of working age (<55-65 years) and impedes them from going back to work. In our mouse model of SAH, we observed the development of chronic behavioral abnormalities consistent with those observed in humans. Our RNA next-generation sequencing study of the hippocampus (HPC), structure of learning and memory, at 4-days, showed a significant downregulation in the myelin/oligodendrocytes-related genes. The aim of the present study is to show the myelin/oligodendrocytes and white matter damages occurring in the perforant pathway, connecting the entorhinal cortex (EC) and the HPC that may play a major role in the following cognitive deficits observed. SAH was induced by unilateral endovascular perforation of the circle of Willis, in the left side. In Sham mice, the filament was inserted without perforation. At 96h, brains were processed for immunohistochemistry (IHC) of MBP (Myelin Basic Protein). At 10-12months following SAH, we conducted diffusion tensor magnetic resonance imaging in Magnetic Resonance Imaging (DTI-MRI) in ex vivo brain to assess white matter alterations in the long-term consequences. Significant increase in fractional anisotropy (FA), measure of the directionality of the white fibers, was observed in the left EC in SAH vs Sham and Naïve ($P=0.0023$ and $P<0.001$, respectively n=3/4) and in the right EC in SAH vs Naïve ($P=0.0074$, n=3). No significant difference was observed in FA in the right or left HPC. At 96h, IHC for MBP tended to increase in all layers of the hippocampus and significantly in the stratum

radiatum (P=0.002) and the pyramidal layers (P=0.03; n=3 SAH, 3 Sham) confirming myelin disturbances 4-days following SAH. FA measurements showed alterations in the EC's white fibers and MBP expression showed changes in the oligodendrocytes/myelin in the hippocampus after SAH, in line with the decrease in hippocampal volume that we previously reported. Taken together, these results confirmed damages in the white matter, and most probably in the perforant pathway, shedding the light on possible mechanisms underlying cognitive impairments following SAH.

Disclosures: A. Regnier-Golanov: None. **C. Karmonik:** None. **R. Le:** None. **H. Goodwin:** None. **N. Kvirkvelia:** None. **E. Golanov:** None. **G. Britz:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.01/X5

Topic: C.10. Brain Injury and Trauma

Title: Determining strain thresholds of calcium dysfunction following biaxial stretch in vitro

Authors: I. M. BERKE, L. V. FORTUNO, *A. S. CARRIZALES, A. DILEONARDI; United States Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Traumatic brain injury (TBI) is a leading cause of disability and mortality for the modern-day warfighter. While progress has been made to diagnose and treat TBI, fundamental questions regarding disease initiation and progression remain unanswered. Specifically, *in vitro* strain and strain-rate injury thresholds, and their relationship to those observed *in vivo*, are of interest given current and emerging threats to the warfighter. To this end, Sprague Dawley E18 hippocampal cultures were grown upon custom-manufactured stretchable micro-electrode arrays (sMEAs, [BMSEED]) and on day *in vitro* (DIV) 10 were transduced (AAV1-hSyn1-GCaMP6s-P2A-nls-dTomato). Cultured were used for experiments on DIV21. To induce relevant *in vitro* TBI-like pathology the MEASSuRE-X system (BMSEED) was used to stretch sMEAs bi-axially at the following strain and strain-rate combinations: Sham, 5% $25s^{-1}$, 10% $25s^{-1}$, 30% $25s^{-1}$, 30% $50s^{-1}$ ($n \geq 4$/group). Recordings of spontaneous calcium dynamics were collected prior to, immediately following, and 60 minutes following injury. Recordings were analyzed and normalized firing frequencies were calculated. Cultures were fixed and immunocytochemistry-based interrogation of microtubule-associated protein 2 (MAP2) was used to assay morphological features. To test for an effect of treatment a one-way ANOVA with Tukey's post-hoc was performed, statistical significance was set at $p < 0.05$. Following stretch, overt changes to spontaneous calcium signaling were observed, normalized firing frequency decreased in 10% $25s^{-1}$, 30% $25s^{-1}$, and 30% $50s^{-1}$ when compared to Sham but not 5% $25s^{-1}$ treated groups. Brightfield micrographs of sham, 5% $25s^{-1}$, and 10% $25s^{-1}$ groups appeared similar following treatment whereas cultures undergoing 30% strain exhibited mild changes to cell morphology with sparse somal granularity in neuronal cells. Fluorescent micrographs followed a similar

trend, cultures undergoing 30% strain showed MAP2 blebbing in neurites as well as somal staining without overt nuclear exclusion. Taken together, these *in vitro* results suggest that genetically encoded calcium indicator-based interrogation of Ca^{2+} signaling following high-speed biaxial stretch serves as a sensitive measure of cellular dysfunction that may precede overt changes to cellular structure or protein localization. Further, these results highlight the potential biological impact of low strain, high strain-rate loading paradigms with relevance to the modern-day warfighter.

Disclosures: I.M. Berke: None. **L.V. Fortuno:** None. **A.S. Carrizales:** None. **A. DiLeonardi:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.02/X6

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1R01NS104368

Title: Network effects of traumatic brain injury: from infra slow to high frequency oscillations

Authors: *B. MARSH[1], M. BAZHENOV[2];
[1]The Univ. of California San Diego, La Jolla, CA; [2]Med., Univ. of California San Diego, La Jolla, CA

Abstract: Traumatic brain injury (TBI) can have a multitude of effects on neural functioning. In extreme cases, TBI can lead to seizures both immediately following the injury as well as persistent epilepsy over years to a lifetime. Mechanisms of neural dysfunctioning and epileptogenesis after TBI remain poorly understood, and aftercare for TBI remains minimal. To address these questions, we developed a biophysical network model implementing effects of ion concentration dynamics and homeostatic plasticity to test immediate network effects of TBI. TBI was modeled as a loss of baseline activity from severed long range connections; this was applied to the neurons in the middle of the network, surrounded by uninjured healthy zones. We focus on two primary phenomena that have both been reported in vivo after TBI and occur natively in the model: an increase in infra slow oscillations, and the emergence of high frequency oscillations. The infra slow oscillations are seen as < 0.1 Hz oscillations in the network local field potential, while the high frequency oscillations are seen as bursting events in individual neurons in the high gamma range (40 - 60 Hz). These bursts are highly synchronized across locally connected neurons, but do not propagate outside of the injured zone. We show that the infra slow oscillations can be directly attributed to extracellular potassium fluctuations, while the existence and mean frequency of the high frequency oscillations is related to the increase in strength of synaptic weights from homeostatic synaptic plasticity after TBI. We then show that buildup of high frequency oscillations can directly precede biophysically realistic seizure-like ictal events

that span all neurons in the network. Once one of these events has occurred, additional seizures can then be initiated in previously healthy uninjured regions. This study brings greater understanding to the underlying network effects of TBI, and how they can give rise to epileptic activity. We further lay the foundation to begin to investigate how injured networks can be healed and how seizures following traumatic brain injury may be prevented.

Disclosures: B. Marsh: None. **M. Bazhenov:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.03/X7

Topic: C.10. Brain Injury and Trauma

Support: NIH NS 111378
NIH NS117148
NIH NS 116838

Title: TBI disrupts circadian rhythmicity and thermoregulatory capacity

Authors: *B. DA CRUZ WEBER FULCO[1], Z. YING[1], P. B. VANDER[2], C. J. VILLANUEVA[1], S. M. CORREA[1], F. GOMEZ-PINILLA[1];
[1]Dept. Integrative Biol. and Physiol., [2]Molecular, Cell. & Integrative Physiol. Program, UCLA, Los Angeles, CA

Abstract: Traumatic brain injury (TBI) is a serious public health concern, affecting thousands of people around the world and resulting in death and disability. Clinical evidence indicates that TBI disrupts autonomic function, increasing the pathological burden of TBI. The autonomic nervous system (ANS) innervates peripheral organs and autonomic dysregulation can have crucial consequences for death and survival. Regulation of body temperature is primordial to maintain brain and body physiology and here we aim to study how TBI influences metabolic thermogenesis. We also aimed to determine whether there is a relationship between the core body temperature rhythm and the response to cold challenge and metabolic thermogenesis. We used implantable temperature transponder transmitter recordings to show that animals exposed to moderate lateral fluid percussion (FPI) experience acute hypothermia, slowly returning to baseline temperature after 12 hours. FPI animals also reduced amplitude of circadian rhythmicity until post-injury day 4. Mice were exposed to a cold chamber (4 ºC) 7 days after FPI to assess their thermoregulatory capacity. In contrast to the Sham group, which showed a slow decrease in temperature across the challenge duration, the TBI animals showed an acute decline between 3 and 4 hours with an abrupt increase after 5 hours. Liver and brown adipose tissue play a crucial action on the regulation of body temperature, and we have recently shown that TBI affects liver function (Khandelwal et al., PMID 37137432; BBA 2023). We performed transcriptomic analysis to determine possible genes associated with control of temperature regulation in the

liver and adipose tissue. We found a decrement in RNA levels of thermoregulatory genes in liver (*Apoa4, Fgf21* and *PPARGC1A*) and brown adipose tissue (*Elovl3*) in TBI animals exposed to cold. Results showed that TBI affects temperature regulation and that these changes are associated with circadian rhythmicity. These results emphasize the action of body physiology on the TBI pathogenesis.

Disclosures: B. Da Cruz Weber Fulco: None. **Z. Ying:** None. **P.B. Vander:** None. **C.J. Villanueva:** None. **S.M. Correa:** None. **F. Gomez-Pinilla:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.04/X8

Topic: C.10. Brain Injury and Trauma

Support: NJCBIR20IRG003

Title: Changes to neural circuit dynamics in neurons after stretch injury as a model of TBI

Authors: *D. SULLIVAN[1], B. VAGLIO[2], R. PONCE WONG[3], O. GRAUDEJUS[4], B. L. FIRESTEIN[2];
[2]Cell Biol. and Neurosci., [1]Rutgers Univ., Piscataway, NJ; [3]BMSEED Llc, Tempe, AZ; [4]Sch. of Mol. Sci., BMSEED Llc/Arizona State Univ., Tempe, AZ

Abstract: Mild traumatic brain injury (TBI) is the leading cause of accident-related death and disability in the world and can lead to long-term neuropsychiatric symptoms, such as a decline in cognitive function and neurodegeneration. TBI includes primary and secondary injury, with head trauma and deformation of the brain caused by the physical force of the impact as primary injury, and cellular and molecular cascades that lead to cell death as secondary injury. Currently, there is no treatment for TBI-induced cell damage and neural circuit dysfunction in the brain, and thus, it is important to understand the underlying cellular mechanisms that lead to this cell damage. Previous work has identified the toxic effects of physical and pharmacological injury on cells, but as of yet, there are no studies that explore changes to electrical communication of brain cells after physical injury. Our laboratory uses a stretch injury model to study the effects of TBI in cultures of cortical cells. In the current study, we use stretchable multi-electrode arrays (sMEAs) to model the primary injury of mild TBI and study the electrophysiological effects of physically injuring cortical cells. We recorded before injury and then stretched the flexible membrane of the sMEAs to injure the cells to varying degrees. At 1, 24, and 72 hours post-stretch, we recorded activity to analyze differences in spike rate, spike variability, spike bursting, and spike synchronization, which represent the activity of and connections between neurons. We found significant changes in spike rate, fano factor, individual bursting properties, synchronization of firing, and local and global efficiency over time within and between stretch strength groups. Our

results suggest that changes to electrophysiological properties after stretch are dependent on the strength of synchronization between neurons prior to injury.

Disclosures: D. Sullivan: None. **B. Vaglio:** None. **R. Ponce Wong:** None. **O. Graudejus:** None. **B.L. Firestein:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.05/X9

Topic: C.10. Brain Injury and Trauma

Support: DoD CDMRP W81XWH-21-1-0884
CRIM Research Scholar Award EDUC4-12822

Title: Loss of inhibition following mild traumatic brain injury to primary visual cortex

Authors: *B. HOU, J. EOM, D. LYON, R. HUNT;
Univ. of California, Irvine, Irvine, CA

Abstract: Visual impairments are among the most common health related challenges for individuals with traumatic brain injury (TBI). In addition to cognitive, motor and neuropsychiatric changes, as many as 75% of military veterans' self-report visual symptoms as a result of TBI. Here we performed a series of slice electrophysiology studies in a mouse model of central visual system TBI. Approximately 2 months after TBI, whole-cell voltage-clamp recordings of layer II/III neurons revealed ~50% reduction in the frequency of spontaneous (s) and miniature (m) inhibitory post-synaptic currents (IPSCs) in brain-injured V1 neurons versus uninjured controls. No concurrent change in event amplitude was found, but preliminary results indicate a potential change in event kinetics. These slice electrophysiology results suggest mild TBI to V1 produces a long-lasting reduction of GABA-mediated inhibition to principal neurons and are consistent with our prior studies describing interneuron loss throughout brain-injured V1. Ongoing experiments are testing the effect of transplanting interneuron progenitors, derived from mouse embryonic medial ganglionic eminence (MGE), on inhibition and *in vivo* electrophysiological responses to visual stimuli in V1 neurons. To date, our results suggest MGE cells survive, migrate and integrate into adult brain-injured V1 circuits.

Disclosures: B. Hou: None. **J. Eom:** None. **D. Lyon:** None. **R. Hunt:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.06/X10

Topic: C.10. Brain Injury and Trauma

Title: Assessment of seizure-like activity in neuronal networks post-traumatic brain injury utilizing novel TBI-on-a-chip model

Authors: *S. J. MUFTI[1,2,3], E. A. ROGERS[1,4], J. MARTINEZ[1,2,3], T. B. BEAUCLAIR[1,2,3], N. KRISHNAN[1,2], J. CRODIAN[1], M. GONZALEZ[1], D. KIM[1], R. SHI[1,2,3];
[1]Ctr. for Paralysis Res., [2]Weldon Sch. of Biomed. Engin., [3]Dept. of Basic Med. Sciences, Sch. of Vet. Med., Purdue Univ., West Lafayette, IN; [4]Indiana Univ. Sch. of Med., West Lafayette, IN

Abstract: Traumatic brain injury (TBI) is a leading cause of death and long-term disability worldwide. Further, seizure development is one of the most serious sequalae post-TBI. Unfortunately, the underlying mechanisms linking TBI and seizures are not well understood. Identifying cell-scale pathophysiological changes in the brain post-injury will be paramount for identifying therapeutic targets. While *in vivo* models offer crucial insight, it is often difficult to precisely control the extent of internal brain injury in animals (e.g., degree of deformation or pressure exerted on tissue) even with the ability to command injury application (e.g., rate of rapid acceleration injury or weight-drop), which could account for the variation produced between approaches. Further, it is often not possible to accurately isolate distinct injury types by using these methods, which can adversely affect subsequent modeling of the resulting symptoms, such as seizures. Taken together, these limitations likely contribute to the continuous failure of clinical trials investigating TBI therapies that showed promising results in preclinical studies. In response, we utilized our unique TBI-on-a-chip system to investigate seizure-like activity (SLA) in neuronal networks post-TBI, with the ability to manipulate multiple factors in a highly controlled environment, minimizing systemic confounding variables. This novel system simulates the pathophysiology of concussive TBI by applying clinically relevant, rapid acceleration injuries to murine cortical networks on custom microelectrode arrays, while providing real-time, cell-scale monitoring of electrophysiological and morphological changes. Utilizing extracellular recordings of network spike activity, we reveal the spontaneous appearance of SLA in networks exposed to 10 rapidly (4-6 sec) administered 30 g impacts. Furthermore, we attempt to better characterize SLA and burst features to gain critical information about post-injury electrophysiological changes. While we observed significant increases in the synchronization of neuronal networks post-injury, a generally accepted measure of SLA, we also have identified additional metrics for SLA characterization in networks using bicuculline (a GABA antagonist) for SLA induction, such as the presence of a “transition period” and a “spike-free interval” immediately preceding synchronized activity initiation. In summary, our TBI-on-a-chip model could provide vital insights into functional and morphological changes post-TBI, while enabling the investigation of underlying SLA mechanisms, which could lead to the identification of potential therapeutic targets.

Disclosures: **S.J. Mufti:** None. **E.A. Rogers:** None. **J. Martinez:** None. **T.B. Beauclair:** None. **N. Krishnan:** None. **J. Crodian:** None. **M. Gonzalez:** None. **D. Kim:** None. **R. Shi:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.07/X11

Topic: C.10. Brain Injury and Trauma

Support: ONR Grant 34

Title: Network-wide analysis of hyper- and hypo-excitation responses to an in vitro traumatic brain injury model for assessing critical mechanical thresholds

Authors: *J. SERGAY[1], A. HAI[1], C. FRANCK[2];
[1]Biomed. Engin., [2]Mechanical Engin., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Traumatic brain injury (TBI) is a mechanically induced trauma to the head known to cause both short-term cognitive deficits and long-term neurodegeneration. Even mild TBIs (mTBIs) present these effects and account for over 80% of TBI cases worldwide. Despite the prevalence of mTBIs among athletes, soldiers, and civilians alike, so much is still unknown about the cellular consequences of the injury and targets for universally effective treatments. One well-established molecular response is an increase in calcium (Ca^{2+}) ion concentration in neural cells post-injury. Many molecular pathways involved in neuronal network activity and Ca^{2+} dynamics have been found to alter cellular morphology, cell viability, and the extracellular environment. However, TBI literature assessing the effect of mechanical loading on neuronal signaling is scarce and often fails to look at both hyperacute and acute reactions. This study uses large-scale data at a single-cell scale to conduct parametric studies to characterize network disruption under variable and realistic mechanical loading. Neuronal co-cultures are established on stretchable, dogbone-shaped polydimethylsiloxane (PDMS) substrates. Primary cortical neural cells from P0 rats are seeded on poly-d-lysine (0.1 mg/ml) and laminin (0.4 mg/ml) treated PDMS substrate at about 1,800 cells/mm^2. To simulate the deformation in the range of mTBI, a custom-built tension device applies a uniaxial stretch at a prescribed strain magnitude (0.1, 0.3, or 0.5) and strain rate (1 s^{-1} or 50 s^{-1}). The fluorescent Ca^{2+}-probe Fluo-4 AM optically captures spontaneous network Ca^{2+} fluxes immediately before (0^-), after (0^+), and 24 hours post stretch in two-minute timelapses. Preliminary data of samples stretched at 50 s^{-1} shows several noteworthy hyperacute post-injury events. Immediately after stretch, there is a global network increase in mean Ca^{2+} intensity and a hyperexcitation that lasts between 5 to 10 seconds in all strain experimental groups. After this initial period, Ca^{2+} activity decreases compared to the 0^- data. This effect is not seen in sham samples. The initial data is consistent with effects discussed in literature from glutamate-induced excitotoxicity. This study presents a novel protocol to quantitatively characterize the strain and strain rate threshold for significant neuronal cell signaling perturbation enabling first-of-its-kind recordings at the single-neuron level of network-wide dynamics immediately after applying controlled injury mechanics. Quantitative, acute injury characterization will allow for accurately informed predictive models to mitigate TBI and develop protective measures.

Disclosures: **J. Sergay:** None. **A. Hai:** None. **C. Franck:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.08/X12

Topic: C.10. Brain Injury and Trauma

Title: Btbi-on-a-chip: recapitulating blast trauma in vitro with simultaneous morphological and electrophysiological monitoring

Authors: *T. B. BEAUCLAIR[1,2,3], E. A. ROGERS[6,4], J. MARTINEZ[1,2,3], S. MUFTI[1,2,3], Z. ZHANG[1,2,3], N. KRISHNAN[1,2,3], R. SHI[1,2,5,3];
[2]Ctr. For Paralysis Res., [3]Weldon Sch. of Biomed. Engin., [4]Ctr. for Paralysis Res. Consultant, [5]Basic Med. Sci., [1]Purdue Univ., West Lafayette, IN; [6]Indiana Univ. Med. Sch., West Lafayette, IN

Abstract: Traumatic brain injuries (TBI) induced via exposure to explosive blast waves (bTBI) are an increasingly common wartime injury sustained by combatants and civilians alike. Unfortunately, current treatment options are limited and the underlying mechanisms remain unknown. While multiple animal models of trauma have provided key insights towards uncovering the underlying pathogenesis (alterations in oxidative stress, neuroinflammation, and neuronal signaling), they generally lack the type of spatial and temporal resolution necessary to conduct investigations on the cellular level. In response, we developed “bTBI-on-a-chip”, a cellular model of blast trauma capable of simultaneously monitoring both electrophysiological and morphological parameters of neuronal networks exposed to overpressure (OP) injuries in real-time.The bTBI-on-a-chip system consists of a shock tube for OP wave generation, and a stainless-steel chamber for cellular maintenance. This proprietary “mini-incubator” system is capable of preserving cellular integrity during high pressure blast exposures (160 kPa) while sustaining physiological parameters via a complimentary life-support system. This novel approach, combined with optically transparent microelectrode arrays (MEAs) fabricated in-house, provides visual and electrical access to murine cortical networks during OP injury. Here, we demonstrate the system and incorporate standard ICC and electrophysiological techniques to investigate the effect of mild (120 kPa, 92% viability) blast wave exposure in networks immediately following and 24 hr post-injury. Further, by introducing a slightly modified protocol we are able to separate secondary, media-tied biochemical injuries from primary, mechanical injuries, by exposing non-injured networks to the media from injured cells. Preliminary results reveal average, significant increases of 24% in levels of intracellular acrolein, a product and initiator of lipid peroxidation, in both neuronal and glia cells at 24-hours post-injury. These increases are coupled with a 35% elevation in the inflammatory cytokine TNF-α. Further, a significant, asymmetrical loss in pre- and post-synaptic terminals was observed during this time-period. Furthermore, we describe an OP-injury response profile using average spike rates for networks. Finally, we mitigate several of these OP-induced alterations utilizing the acrolein scavenger Hydralazine, suggesting that acrolein may play a key role in bTBI. It is our hope that

this novel apparatus will be utilized to investigate the underlying mechanisms of trauma while providing a tool for investigating intervention strategies.

Disclosures: T.B. Beauclair: None. **E.A. Rogers:** None. **J. Martinez:** None. **S. Mufti:** None. **Z. Zhang:** None. **N. Krishnan:** None. **R. Shi:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.09/X13

Topic: C.10. Brain Injury and Trauma

Support: Canadian Institute for Health Research FDN-148397
Fondation Leducq 15CVD02

Title: Brain pericytes and perivascular fibroblasts are stromal progenitors with dual functions in cerebrovascular regeneration after stroke

Authors: *L.-P. BERNIER[1], J. K. HEFENDEHL[1], W. SCOTT[1], L. TUNG[1], C.-A. LEWIS[1], H. SOLIMAN[1], S. SIMM[2], L. DISSING-OLESEN[1], J. HOFMANN[3], D. GUO[1], M. DEMEGLIO[3], F. ROSSI[1], M. UNDERHILL[1], B. A. MACVICAR[1];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. Med. Greifswald, Greifswald, Germany; [3]Goethe Univ. Frankfurt, Frankfurt, Germany

Abstract: Functional revascularization is key to stroke recovery and requires remodelling of blood vessels, around which is located the brain's only stromal compartment. Stromal progenitor cells (SPC) form a functional grouping of cells critical for tissue regeneration following injury in many organs, yet their identity in the brain remains elusive despite implications in neovascularization and scar formation. Here we show that the perivascular niche of brain SPCs includes pericytes, venular smooth muscle cells and a distinct population of perivascular fibroblasts, that together help regenerate the cerebral microvasculature following stroke. The ischemic injury triggers amplification of pericytes and perivascular fibroblasts in the infarct region where they associate with endothelial cells inside a reactive astrocyte border. Fate-tracking of *Hic1*$^{+}$ SPCs uncovers a transient functional and transcriptional phenotype of stroke-activated pericytes and perivascular fibroblasts, where both populations remain segregated, displaying dichotomous angiogenic and fibrogenic profiles. In the adult brain, pericytes and perivascular fibroblasts are therefore distinct subpopulations of stromal progenitors that coordinate revascularization and scar formation after injury.

Disclosures: L. Bernier: None. **J.K. Hefendehl:** None. **W. Scott:** None. **L. Tung:** None. **C. Lewis:** None. **H. Soliman:** None. **S. Simm:** None. **L. Dissing-Olesen:** None. **J. Hofmann:** None. **D. Guo:** None. **M. DeMeglio:** None. **F. Rossi:** None. **M. Underhill:** None. **B.A. MacVicar:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.10/X14

Topic: C.10. Brain Injury and Trauma

Title: Cortical neurons are differentially vulnerable to degeneration following mild traumatic brain injury

Authors: *M. ALKASLASI[1], E. LLOYD[1], A. GABLE[3], H. YARUR[1], V. TSAI[1], H. TEJEDA[1], C. E. LE PICHON[2];
[2]NIH, [1]NIH, Bethesda, MD; [3]NIH, NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, Bethesda, MD

Abstract: Mild traumatic brain injury (mTBI) occurs when there is sudden and rapid movement of the brain within the skull. This mild and indirect brain injury leads to a pathological cascade involving many cell types and structures in the brain, and induces injury responses including excitotoxicity, impaired axonal transport, and neuroinflammation. While the overall pathology of mTBI has been widely studied, the neuron-intrinsic responses and subtype-specific outcomes have not been elucidated. Here, we evaluate whether and which injured cortical neurons activate neuron-intrinsic stress responses in a mouse model of mTBI. Anesthetized mice were administered a unilateral closed-skull controlled cortical impact injury over the motor cortex and followed up to 10 weeks post injury to evaluate degeneration, stress response activation, neuroinflammation, and cell survival. We find that layer V projection neurons (PNs) are particularly vulnerable to damage in the acute phase following mTBI, exhibiting axon swellings characteristic of diffuse axonal injury as well as dendrite degeneration. A subset of PNs upregulate Activated Transcription Factor 3 (*Atf3*), a transcription factor that is activated in response to axon injury. By permanently labeling Atf3-expressing neurons, we show that layer V PNs express markers of axon injury, upregulate pre-apoptotic genes, undergo cell death, and are ultimately phagocytosed following mTBI. Conversely, layer II/III Atf3-expressing neurons lack degenerative morphology and survive long term, indicating differential vulnerability of cortical neurons to the same insult. Understanding the differential stress responses initiated in cortical neurons following mTBI may elucidate the neuron-intrinsic barriers to survival following CNS injury.

Disclosures: **M. Alkaslasi:** None. **E. Lloyd:** None. **A. Gable:** None. **C.E. Le Pichon:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.11/X15

Topic: C.10. Brain Injury and Trauma

Support: NIH/NINDS R01 NS112212
NIH/NINDS R01 NS122777

Title: Decoding the mechanisms of mitochondrial electron transport chain components loss upon pro-inflammatory microglial activation

Authors: *N. ZHANG[1], S. VONGDEUANE[2], N. YADAVA[2], R. P. MAYERS[3], H. HWANG[2], B. M. POLSTER[2];
[1]Neuroscience, Sch. of Med., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Anesthesiol., [3]Program in Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Aberrant or excessive pro-inflammatory microglial activation contributes to many neurodegenerative diseases. This activation is modeled *in vitro* by combined exposure to the Toll-like receptor 4 (TLR4)-activating molecule lipopolysaccharide (LPS) and the cytokine interferon-gamma (IFN-γ), which cause microglia to adopt a neurotoxic state. Inducible nitric oxide synthase (iNOS)-mediated nitric oxide (NO) production, mitochondrial electron transport chain (ETC) dysfunction, and NLRP3 inflammasome-dependent caspase-1 activation are implicated in the pro-inflammatory activation. The mitochondrial ETC dysfunction, which includes large decreases in multiple ETC complex subunits, is thought to depend on NO production. However, the precise mechanisms of subunit loss are unclear. Here, we tested the hypothesis that both TLR4-dependent caspase-1 protease activation and NO production are required for mitochondrial ETC subunits loss in pro-inflammatory microglia. Using an OXPHOS antibody cocktail, we examined the level of ETC complex proteins by western blot at 18 hours post LPS+IFN-γ stimulation in wild type (WT) and *Nos2* knockout HAPI mouse microglial cells ± caspase-1 inhibitor and/or TLR4 inhibitor. Complex I subunit NDUFB8, Complex II subunit SDHB, and Complex IV subunit COX1 were all reduced relative to α-tubulin or total protein in LPS+IFN-γ-activated WT HAPI cells whereas Complex III UQCRC2 subunit and Complex V ATP5A subunit were unchanged. CRISPR knockout of *Nos2* and inhibition of NO production in WT cells each rescued the decrease in Complex II and IV subunits but did not preserve the Complex I subunit NDUFB8. Addition of caspase-1 inhibitor VX-765 or TLR4 antagonist TAK-242 failed to rescue any of the ETC complex proteins. However, each inhibitor moderately reduced iNOS protein expression and their combined addition led to a partial rescue of COX1. Unexpectedly, iNOS expression was partially suppressed by caspase-1 inhibitor and, also, a decreased level of the caspase-1 p20 active fragment was detected when NO production was prevented by the iNOS inhibitor 1400W or by *Nos2* ablation. These results suggest that there is a positive feedback loop between caspase-1 activation and nitric oxide production. In addition, findings indicate that mitochondrial ETC dysfunction is mediated by multiple mechanisms, as SDHB and COX1 but not Complex I subunit NDUFB8 were rescued by nitric oxide elimination and only COX1 loss was sensitive to the caspase-1-TLR4 inhibitor combination. Additional work is needed to elucidate the TLR4-, caspase-1-, and nitric oxide-independent mechanisms recruited by LPS+ IFN-γ that impair the mitochondrial ETC in microglia.

Disclosures: **N. Zhang:** None. **S. Vongdeuane:** None. **N. Yadava:** None. **R.P. Mayers:** None. **H. Hwang:** None. **B.M. Polster:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.12/X16

Topic: C.10. Brain Injury and Trauma

Support: P41EB027062
S10OD021624
HU00012020015

Title: Injury-induced metabolic dysfunction is consistent between different types of traumas.

Authors: *V. LIAUDANSKAYA[1], C. J. O'CONNELL[2], A. SYMES[4], M. J. ROBSON[3], D. L. KAPLAN[5];
[1]Biomed. Engin., [2]Div. of Pharmaceut. Sciences, James L. Winkle Col. of Pharm., [3]Div. of Pharmaceut. Sciences, James L. Winkle Col. of Pharmacy; Neurosci. Grad., Univ. of Cincinnati, Cincinnati, OH; [4]Pharmacol., Uniformed Services Univ., Bethesda, MD; [5]Biomed. Engin., Tufts Univ., Medford, MA

Abstract: Traumatic Brain Injury is initially a mechanical injury, which launches a complex cascade of molecular and metabolic events amplified by cell interactions and the severity of the initial impact. Patients suffer from mental and physical disabilities after either type of injury, as currently, no effective therapy is available. Thus, there is a critical need to understand trauma-specific mechanisms that drive injury progression to develop diagnostic biomarkers and treatments effectively. Metabolic dysfunction is one potential driver of injury-induced neurodegeneration, as alterations in tricarboxylic acid (TCA) cycle-related enzymes, lipid peroxidation, mitochondrial and fructose metabolism have all been noted following injuries in human athletes, rodent *in vivo*, and human *in vitro* studies. However, the trauma-specific changes in metabolism that control secondary damage progression after the injury remain to be elucidated. To address this urgent need, we used a human in vitro triculture (3D) model of blunt injury to discover potential metabolic drivers of damage progression and compared our findings with a mouse model of head acceleration injury. 3D *in vitro* model was composed of human iPSC-derived neurons, microglia, and primary astrocytes seeded in silk protein porous scaffolds (d=6mm) with a collagen gel central window (d=2mm) at 2:0.5:0.1 million cells, respectively. By 5 weeks, the 3D *in vitro* model forms mature neural networks and is used at that time for blunt injuries with a controlled cortical impactor. RNAseq analysis detected decreased expression of glycolysis-associated genes and increased rate of fatty acid oxidation; intriguingly, despite glycolytic depression, the expression of genes associated with downstream OXPHOS was increased 24h after the injury, potentially using acetyl-CoA from fatty acids oxidation to fuel TCA cycle and OXPHOS. Additionally, our data demonstrated a significant shift in the expression of epigenetic regulators of metabolic function. At last, when we compared the results of gene expression between blunt *in vitro* and head acceleration *in vivo* models, we observed altered expression (with the same directionality) of over 250 convergent gene transcripts. Of

these, 126 were directly linked to metabolic processes, indicating acute progression of metabolic dysfunction. We are currently focused on determining the contribution of the metabolic pathways isolated from RNA sequencing analysis to neurodegeneration progression post-TBI; future efforts will establish a cell-specific profile of the metabolic dysfunction in short- and long-term (6-12 months) cultures.

Disclosures: V. Liaudanskaya: None. **C.J. O'Connell:** None. **A. Symes:** None. **M.J. Robson:** None. **D.L. Kaplan:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.13/X17

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX005015))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (I01RX001520))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (IK2RX003253))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX004561))
The Veterans Bio-Medical Research Institute

Title: Strain specific influences on gene dysregulations induced by mild blast TBI

Authors: *B. A. CITRON[1,3,4], K. E. MURRAY[1,4], V. A. STIRITZ[2,4], T. P. COMINSKI[2], A. RAVULA[5], V. DELIC[1,3,4], K. D. BECK[2,3,4], B. J. PFISTER[6,7];
[1]Lab. of Mol. Biol., [2]Neurobehavioral Res. Lab., VA New Jersey Hlth. Care Syst., East Orange, NJ; [3]Pharmacology, Physiology, and Neurosci., Rutgers New Jersey Med. Sch., Newark, NJ; [4]Sch. of Grad. Studies, Rutgers Univ., Newark, NJ; [5]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [6]Biomed. Engin., [7]Ctr. for Injury Biomechanics, Materials and Med., New Jersey Inst. of Technol., Newark, NJ

Abstract: Approximately 25% of the two million service personnel deployed since 2000 have reported receiving a traumatic brain injury (TBI). The vast majority of these are mild, and, in the military, due to exposure to blast waves from a variety of sources. Unfortunately, an effective treatment is still needed; meanwhile, Veterans cope with long-term effects of impaired brain

processing and neurodegeneration. A better understanding is needed to address the problems caused by TBI. Genetic predispositions to neuronal damage susceptibility versus predispositions to neuroprotective resilience and repair represent an understudied gap in our understanding of how to best treat the brain following a TBI. Moreover, as therapeutic approaches become more personalized, a robust understanding of the contributions of genetic background to TBI resilience and recovery is essential in order to develop solutions for our affected Veterans. We studied a set of eight model strains of mice that provide a high degree of genetic variation and we have been examining the effects of blast-induced injury and the subsequent recovery. The injury model utilizes a well-established blast tube system mimicking pressure waves experienced during a field exposure. We compared gene expression levels within the entire transcriptome by RNA-seq of 96 hippocampal samples (n=3/group; 32 different groups comprising the full matrix of 8 different strains x 2 sexes x sham vs. TBI). We uncovered sex and strain-specific injury-induced alterations in gene expression at the pathway, gene ontology, and individual gene level. For example, synaptic plasticity factors, e.g., Camk2a, and stress response factors, e.g., Hsp90aa1, responded differently to blast exposures dependent on the strain and sex. Acoustic startle testing provided a quantitative measure of injury induced deficits and recovery where we could detect reductions in sensitivity to the stimulus recovering at a 90 day post-injury chronic time point. There were genes implicated in startle response circuitry that were downregulated or upregulated >1.5 fold by the TBI ($P<0.05$). Our results provide essential starting points for future investigations to understand how these genes play roles in post-injury outcomes, to develop optimal models for blast studies, and to lead towards the definition of therapeutic targets that could be modulated to eventually improve the health of Veterans and others with histories of blast exposures.

Disclosures: **B.A. Citron:** None. **K.E. Murray:** None. **V.A. Stiritz:** None. **T.P. Cominski:** None. **A. Ravula:** None. **V. Delic:** None. **K.D. Beck:** None. **B.J. Pfister:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.14/X18

Topic: C.10. Brain Injury and Trauma

Support: VA BLR&D Grant I01BX005015
VA RR&D Grant I01RX001520
VA BLR&D Grant I01BX004561
VA RR&D Grant IK2RX003253
VA Research Career Scientist Award IK6BX006188
Veterans Bio-Medical Research Institute

Title: Identification of cell-specific differential gene expression profiles by snRNA-seq following repetitive weaponry-type blast-induced traumatic brain injury

Authors: *K. E. MURRAY[1,3], F. J. VELLOSO[4,5], A. RAVULA[6], V. DELIC[1,3,5], K. D. BECK[2,3,5], B. J. PFISTER[7,8], S. W. LEVISON[4,3,5], B. A. CITRON[1,3,5];
[1]Lab. of Mol. Biol., [2]Neurobehavioral Res. Lab., VA New Jersey Hlth. Care Syst., East Orange, NJ; [3]Sch. of Grad. Studies, Rutgers Univ., Newark, NJ; [4]Lab. for Regenerative Neurobio., [5]Dept. of Pharmacology, Physiol. & Neurosience, Rutgers-New Jersey Med. Sch., Newark, NJ; [6]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [7]Dept. of Biomed. Engin., [8]Ctr. for Injury Biomechanics, Materials and Med., New Jersey Inst. of Technol., Newark, NJ

Abstract: Mild traumatic brain injuries (mTBIs) among military and law enforcement personnel are frequently caused by low-level blast exposures from use of heavy weaponry, including 0.50-caliber rounds, grenades, and breaching devices, during training and active service. Service personnel who sustain repetitive weaponry-type blast-induced TBI (rwbTBI) do not display overt pathological symptoms immediately but rather develop mild symptoms including cognitive impairments, attention deficits, mood changes, irritability, and sleep disturbances over time. We are interested in neuronal health and understanding mechanisms to improve cognitive outcomes for Veterans and others. We hypothesize that rwbTBI results in upregulation of neuroinflammatory and other detrimental genes and downregulation of neuroprotective genes. Furthermore, we investigated whether modulation of neuroprotective transcription factors can help combat neuroinflammatory responses and neuronal loss following rwbTBI by influencing gene expression profiles of cell populations within the hippocampus, a critical region involved in memory and cognition. We used a well-established shock tube system to reproduce the pressure waves experienced during occupational use of heavy weaponry. Male C57Bl/6J mice received five 70 kPa blast exposures at 1-min intervals to model occupational use of heavy weaponry followed by intraperitoneal (i.p.) administration of tert-butylhydroquinone (tBHQ), a Nrf2 activator, and pioglitazone, a PPARγ agonist, at 30 minutes post-injury. Single nucleus RNA sequencing (snRNA-seq) was performed using nuclei isolated from mouse hippocampal tissue at 24 hours post-injury to assess gene expression changes by cell type in response to injury and/or treatment. Preliminary analysis identified differential expression of genes of interest including a 3-fold upregulation of *Nfe2l2*, the gene that encodes Nrf2, in dentate granule cells due to injury. We also conducted gene ontology and pathway analysis to identify functional profiles in various cell types after injury and/or treatment. This study provides a deeper understanding of the mechanisms that underlie neuropathological changes following repetitive weaponry-type blast exposure and provides a foundation for the identification of therapeutic targets that could be modulated to improve the health of Veterans and others with histories of occupational blast exposures.

Disclosures: K.E. Murray: None. **F.J. Velloso:** None. **A. Ravula:** None. **V. Delic:** None. **K.D. Beck:** None. **B.J. Pfister:** None. **S.W. Levison:** None. **B.A. Citron:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

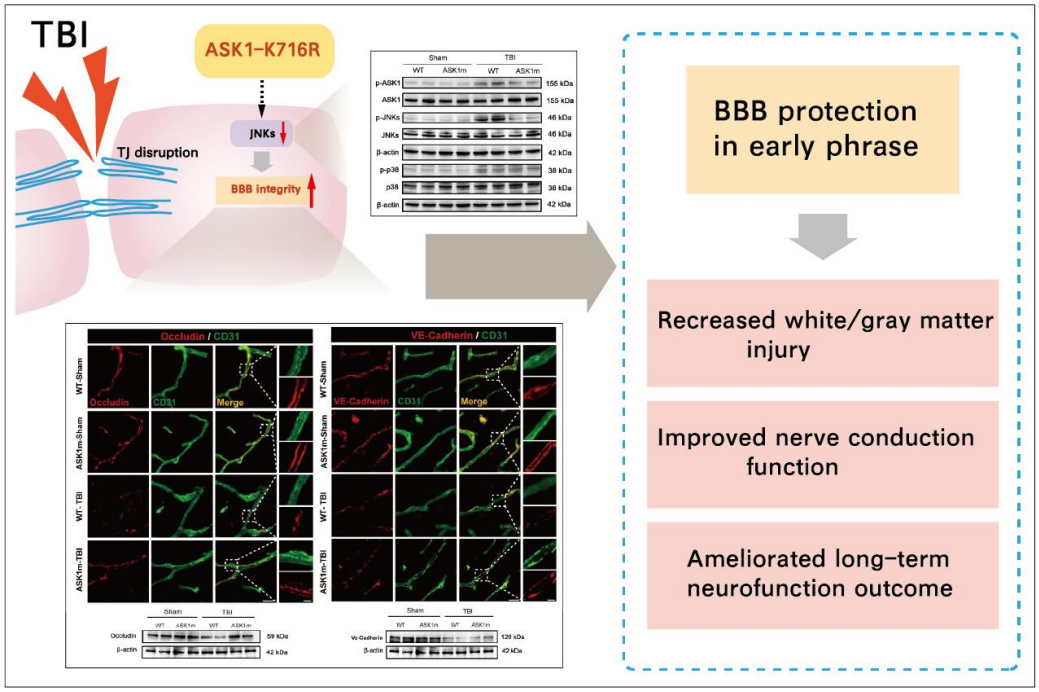
Program #/Poster #: PSTR471.15/X19

Topic: C.10. Brain Injury and Trauma

Title: Ask1-k716r reduces neuroinflammation and white matter injury by preserving blood-brain barrier integrity after traumatic brain injury

Authors: *S. CHEN, Y. GAO, S. MENG, Y. HUANG, Z. SHI, J. LI;
Inst. of Brain Sicence, Fudan Univ., Shanghai, China

Abstract: Background: Apoptosis signal-regulating kinase 1 (ASK1), an evolutionarily highly conserved protein, is involved in neuronal death and neuroinflammation in several central nervous system disorders. Specifically, the deficiency of ASK1 attenuates blood-brain barrier (BBB) permeability after injury, suggesting its contribution to BBB integrity. Despite this knowledge, it remains unclear how ASK1 affects traumatic brain injury (TBI) outcomes and how it regulates BBB function following TBI.**Methods:** Map3k5-e site-specific mutation(ASK1-K716R) transgenic mouse model were generated by using the CRISPR/Cas9 system. TBI model were established via the controlled cortical impact method. ASK1 expression and distribution were detected using Western blotting and IF, respectively. ASK1 kinase activity after TBI was detected using a kit of the ASK1 kinase activity. A battery of behavioral tests was used to assess neurofunction. TEM, IF and electrophysiology were employed to evaluate white matter injury and BBB integrity after TBI. WB was performed for tight junctions proteins and ASK1 downstream signaling pathway proteins. IF, qPCR, and flow cytometry were used to monitor neuroinflammation after TBI.**Results:** The activity of ASK1-K716R was significantly reduced after TBI. By inhibiting ASK1/JNK activity in endothelial cells, ASK1-K716R improved blood-brain barrier (BBB) integrity and ameliorated long-term outcome after TBI. ASK1-K716R decreased infiltration of peripheral immune cells into the brain parenchyma, restored TBI-induced proinflammatory phenotype transition of microglia, and downregulated the expression of several proinflammatory cytokines. Additionally, ASK1-K716R decreased white/gray matter injury and improved nerve conduction function in both myelinated and unmyelinated fibers after TBI. Conclusions: Inhibiting ASK1/JNK pathway, ASK1-K716R reduces cerebral vascular endothelial apoptosis and preserves blood-brain barrier integrity post-TBI. These effects lead to improved TBI-induced histological and functional outcome.



Disclosures: **S. Chen:** None. **Y. Gao:** None. **S. Meng:** None. **Y. Huang:** None. **Z. Shi:** None. **J. Li:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.16/X20

Topic: C.10. Brain Injury and Trauma

Support: NIH-NINDS R01 NS113921

Title: Calretinin and Parvalbumin Aberrant Trapping of TDP-43 and XRCC1 May Drive Neocortical Interneuron Death and Seizures in Neonatal Piglet and Human Hypoxic-Ischemic Encephalopathy

Authors: *L. J. MARTIN[1,2], D. PARK[1,2], C. PRIMIANI[2], C. O'BRIEN[2], M. CHEN[2], R. C. KOEHLER[2], P. KRATIMENOS[3], J. K. LEE[2];
[1]Neuropathology, [2]Johns Hopkins Univ., Baltimore, MD; [3]Children's Natl. Hospital, George Washington Univ. Sch. of Med. and Hlth. Sci., Washington, DC

Abstract: Interneuron degeneration could be a mechanism for seizure development in neonatal hypoxia-ischemic encephalopathy (HIE), but possible mechanisms are unknown. Neonatal piglets received hypoxia-ischemia (HI)+normothermia, HI+overnight hypothermia, sham+normothermia, or sham+overnight hypothermia. Some piglets had continuous electroencephalography (EEG) and videotaping during recovery. Piglets with intractable seizures were euthanized immediately. Piglets survived 1-7 days. Shams were time-matched euthanized. Naïve piglets were additional controls. Piglet brains were assessed using immunohistochemistry (single- and double-label) for interneuron markers calretinin, parvalbumin, and vasoactive intestinal peptide (VIP); TAR DNA binding protein-43 (TDP-43), a nuclear protein that binds DNA/RNA and suppresses cryptic exon inclusion into RNA transcripts; and the DNA repair scaffold protein X-ray repair cross complementing-1 (XRCC1). Other assessments were DNA fragmentation, co-immunoprecipitation (co-IP), and immunoblotting. Postmortem human neonatal HIE brains and non-HIE cases of spinal muscular atrophy and sudden-infant-death were comparators. Counts of immunopositive cells were done in piglet somatosensory cortex (n=4-6 piglets/group) and human parietal and temporal cortex. Calretinin interneurons in HI+normothermia layers II/III were severely depleted relative to sham. Parvalbumin interneurons were similarly vulnerable, but VIP neurons appeared unaffected. Hypothermia partially rescued the loss of interneurons in HI. Anesthesia also affected interneurons. Calretinin and parvalbumin formed nuclear and cytoplasmic inclusions that colocalized with TDP-43 and XRCC1; co-IP identified interactions among these proteins. Calretinin and parvalbumin interneurons accumulated DNA single- and double-strand breaks and died along the cell death continuum in a morphological form called aggrethanatosis. Cryptic exon-containing proteins were detected by immunoblotting in HIE brains. Calretinin and parvalbumin had tyrosine nitration signatures. EEG-detected seizures associated with interneuron degeneration. Calretinin and parvalbumin interneurons were similarly depleted in human HIE neocortex compared to non-HIE neocortex. We conclude that HI causes deletion of neocortical inhibitory interneuron subtypes with inherent vulnerabilities instructed by their intrinsic calcium-binding protein content and by mechanisms consistent with protein nitration-related toxic trapping and aggregation of XRCC1 and TDP-43 and loss of their function driving faulty DNA repair and accumulation of abnormal proteins.

Disclosures: **L.J. Martin:** None. **D. Park:** None. **C. Primiani:** None. **C. O'Brien:** None. **M. Chen:** None. **R.C. Koehler:** None. **P. Kratimenos:** None. **J.K. Lee:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.17/X21

Topic: C.10. Brain Injury and Trauma

Title: Analysis of cell proliferation, neovascularization, and myelination in adult zebrafish optic tectum redeveloping in organotypic culture

Authors: *I. T. MASSARO[1,2], A. BIMBO-SZUHAI[1,2], R. L. PEGUERO[2], S. GUARIGLIA[1,3], C. P. CORBO[1,4,2];
[1]New York State Inst. for Basic Res., New York City, NY; [2]Wagner Col., Staten Island, NY; [3]St. Joseph by the Sea High Sch., Staten Island, NY; [4]Jacksonville Univ., Jacksonville, FL

Abstract: Zebrafish (Danio rerio) have emerged as a prominent model for understanding neurogenesis in the central nervous system. While zebrafish have been widely used as a model organism to study early development, our group has demonstrated the adult zebrafish optic tectum as a useful vertebrate-model for studying neurogenesis post-traumatic brain injury. Using a model where optic tectal explants are cultured in organotypic media, our group has shown that explants of the adult zebrafish optic tectum can form structures resembling the neural tube seen in the embryonic stage of neuronal development. This is characterized by the formation of an organized cortical structure predominantly with radial glia, a forming ependymal surface with microvilli, new blood vessels, and a forming ventricular space. Interestingly, we have shown mast cell presence in the regenerating optic tectal explant. In this study, we set out to investigate cell migration and proliferation as well as formation of new myelin and neovascularization in the neural-tube-like structures formed in the optic tectum explants. Combining laser scanning confocal microscopy and transmission electron microscopy, we have demonstrated mast cell degranulation at the sites of the newly forming neural tube-like structures as well as evidence of neovascularization all within close proximity to these neural tube-like structures. These data strongly suggest a correlation between these newly forming structures and the neighboring mast cell degranulation and cell migration. As our previous research demonstrated presence of a neural tube-like structure post-traumatic brain injury, this study allows for a better understanding as to how the regenerative process is orchestrated on a cellular level and how this structure may be involved in adult neurogenesis. Furthermore, insights on how these underlying mechanisms can be influenced could yield novel treatments for related neurodegenerative diseases.

Disclosures: I.T. Massaro: None. **A. Bimbo-Szuhai:** None. **R.L. Peguero:** None. **S. Guariglia:** None. **C.P. Corbo:** None.

Poster

PSTR471. Brain Injury: Cellular Mechanisms

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR471.18/X22

Topic: C.10. Brain Injury and Trauma

Title: Analysis of cellular composition within redeveloping neural tube-like structures found in adult zebrafish optic tectum maintained in organotypic culture.

Authors: *A. BIMBO-SZUHAI[1,2], I. T. MASSARO[1,2], R. L. PEGUERO[2], M. SCOTTO[1], R. ARPAIO[1,2], S. GUARIGLIA[1,3], C. P. CORBO[1,4,2];
[1]New York State Inst. for Basic Res., Staten Island, NY; [2]Wagner Col., Staten Island, NY; [3]St. Joseph by the Sea High Sch., Staten Island, NY; [4]Jacksonville Univ., Jacksonville, FL

Abstract: Zebrafish (Danio rerio) has emerged as a promising model to study restorative neurogenesis after traumatic brain injury (TBI) due to the presence of neurogenic niches throughout the central nervous system (CNS). Our group has previously identified forming neural tube-like structures in regenerating explants of the optic tectum. We have morphologically characterized the main cellular components in these regenerative structures using scanning electron and brightfield microscopy. The architecture of these structures is largely comprised of undifferentiated cells, as well as glial and ependymal cells. Interestingly, in proximity to these structures we also frequently detect granulated cells resembling vertebrate mast cells (MC). Given the role that MCs play in early vertebrate brain development, here we investigate more robustly the identity of these granulated cells as well their relationship to restorative neurogenesis. To do this, our group employed laser scanning confocal microscopy to confirm the presence of CPA5, a mast cell-specific marker, within regenerating explants. Metachromasia staining using toluidine blue indicated abundant MC populations across our 7-day culturing period. Next, using SEM, we further investigated the surface morphology of the explants and found MCs in varying stages of degranulation, likely interacting with the surrounding tissue. Using our 3D rendering technique, we reconstructed virtual explants from mapped scanning electron micrographs to visualize the internal and external regenerative cytoarchitecture of the damaged tissue and the localization of MCs in 3D space. Finally, RT-PCR was employed and showed an increasing population of MCs across the culturing periods, complemented by β-hexosaminidase assay allowing the quantification of MC degranulation. Together, these findings suggest that MCs may play a critical role in the mechanism underlying zebrafish brain regeneration upon TBI.

Disclosures: A. Bimbo-Szuhai: None. **I.T. Massaro:** None. **R.L. Peguero:** None. **M. Scotto:** None. **R. Arpaio:** None. **S. Guariglia:** None. **C.P. Corbo:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.01/X23

Topic: C.10. Brain Injury and Trauma

Title: Uc-msc decreases inflammation in an vitro model of neonatal ivh

Authors: *C. BOLDEN[1,2], M. ZAMORANO[3], S. OLSON[4], B. A. MILLER[3];
[1]Pediatric Surgery, Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [2]Xavier Univ. of Louisiana, New Orleans, LA; [4]Pediatric Surgery 1, [3]Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Neonatal intraventricular hemorrhage IVH is a common complication in premature babies that can lead to long-term disability. IVH is an active process that includes the lysis of red blood cells and the release of hemoglobin (Hgb) over time, resulting in elevated pro-inflammatory cytokine production and oxidative phosphorylation. As the early postnatal period is a critical stage due to the vulnerability of neuronal-glial precursor cells in the developing brain, it is unclear how neuroinflammation plays a role in cell survival, migration, and differentiation in IVH pathology. Current therapies for the prevention or treatment of IVH are limited. The use of stem cell-based therapies offers a potential therapeutic approach to repair and/or restore critically injured brain tissue. To evaluate this approach, we implemented an in vitro model of mixed primary cell culture of oligodendrocytes, astrocytes, and microglia. This in vitro model of IVH provided an opportunity for mechanistic based screenings and therapeutic innovation. Mixed cultures were treated with Hgb (0.1%) and simultaneously administered umbilical cord-derived mesenchymal stem/stromal cells (UC-MSCs) for 24 h. Cell culture supernatant was collected and analyzed, and UC-MSCs treated wells demonstrated significantly decreased levels of hemoglobin-induced activation of IL-1β, TNF-α, and IGF-1. Cultures were examined for oligodendrocyte survival and microglial inflammation to qualitatively and quantitatively assess the effects of UC-MSC treatment after Hgb-exposure. Positive cells stained for Iba-1 showed a predominant amoeboid morphology in Hgb-treated wells, but cells treated simultaneously with UC-MSCs shaped the MG to a middle state of activation, showing a more ramified phenotype, which was consistent with the cytokine analysis described previously. Quantification of lactate dehydrogenase (LDH) was higher in Hgb-treated wells, but significantly reduced in UC-MSCs treated wells, supporting its therapeutic efficacy. Future studies will assess in vivo effect of UC-MSCs on Myelin integrity, synaptic connections and mitochondria function.

Disclosures: **C. Bolden:** None. **M. Zamorano:** None. **S. Olson:** None. **B.A. Miller:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.02/X24

Topic: C.10. Brain Injury and Trauma

Support: NINDS/NIH grant R21 NS114723-01A1
NINDS/NIH grant 5R01 NS111037

Title: Biodistribution of dye-loaded PgP nanoparticles after intranasal and intrathecal administration in a rat female controlled cortical impact TBI model

Authors: *B. ELLIOTT[1], C. E. JONES[1], E. DODS[1], K. HENRIE[1], T. A. MURRAY[2], J. LEE[1]; [1]Dept. of Bioengineering, Clemson Univ., Clemson, SC; [2]Biomed. Engin., Louisiana Tech. Univ., Ruston, LA

Abstract: Polymeric micelle nanoparticles (NPs) are attractive carriers for solubilization and delivery of hydrophobic drugs. In our lab, we developed an amphiphilic copolymer, poly(lactide-co-glycolide)-graft-polyethylenimine (PLGA-g-PEI: PgP) as a drug delivery carrier. Previously, we loaded a hydrophobic drug, rolipram (Rm) in PLGA core of PgP (Rm-PgP) and evaluated its therapeutic efficacy in a rat mild controlled cortical impact (CCI) TBI model. Rm-PgP nanoparticles administered by local, intraparenchymal injection reduced secondary injury and improved motor function recovery. Here, we investigate the biodistribution of Rm-PgP after administration by more clinically-relevant and minimally invasive intranasal (IN) and intrathecal (IT) routes. To visualize the NPs, the fluorescent dye, tetramethylindo tricarbocyanine iodide (DiR) was loaded in the PLGA core of PgP (DiR-PgP) instead of rolipram by solvent evaporation method. To generate moderate CCI TBI model, Sprague Dawley female rats were placed in a stereotaxic frame and a craniotomy (6 mm diameter) was made and injury was generated by impactor (4 m/sec, 2.5 mm depth, and dwell time 250 msec) using a 5 mm tip. Intrathecal catheters were inserted at lumbar (L4-5) level through a hole made in the dura. Rats were randomly assigned to three groups (n=3 /group): 1)) TBI+ IN inj. of DiR-PgP(50µL) , 2) TBI + IT inj. of DiR-PgP (40µL), and 3) Normal rats as a control. Rats were anesthetized and imaged at 1, 3, 7, and 14 days post-injury (DPI) by IVIS Luminar XR live animal imaging system (Caliper Life Sciences) using a normal rat to remove autofluorescence. At each time point, rats were sacrificed and organs (spinal cord, brain, kidneys, lungs, liver, heart, stomach, spleen) harvested and imaged. The fluorescence intensity of each organ was measured and expressed as percent radiant efficiency (% RE) and percent normalized radiant efficiency (% nRE) using the following equations: % Radiant Efficiency = RE_{organ}/RE_{total}, % Normalized Radiant Efficiency = Total RE @ nDPI/Total RE @ 1DPI. For IN administration, over 90% of DiR-PgP was distributed to the stomach and less than 0.1% of DiR-PgP was distributed to the brain at 1 DPI and over 90% of DiR-PgP was cleared from the body at 3 DPI. Interestingly, the % nRE in the brain increased 6 times at 7 DPI compared to 1 DPI. For IT administration, over 98% of DiR-PgP was distributed in the spinal cord and brain at 1 DPI and over 83% of DiR-PgP retained in the CNS at 14 DPI. In conclusion, IT administration of drug loaded PgP may offer an efficient route for sustained, localized drug delivery within the CNS to treat TBI.

Disclosures: B. Elliott: None. **C.E. Jones:** None. **E. Dods:** None. **K. Henrie:** None. **T.A. Murray:** None. **J. Lee:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.03/X25

Topic: C.10. Brain Injury and Trauma

Title: Conductive and bioactive supramolecular scaffolds promote CNS neurogenesis

Authors: *A. METLUSHKO, N. SATHER, N. TAKATA, D. SIMKIN, T. FRYER, E. KISKINIS, S. STUPP;
Northwestern Univ., Evanston, IL

Abstract: Traumatic injury in the central nervous system (CNS) causes immediate neuronal damage and death, with chronic symptoms such as damaged cognition, paralysis, and pain. While chemically engineered scaffolds can be delivered to provide a physical substrate and stimulate axon regrowth, many grafts lack long term efficacy largely due to inefficient electrical coupling of the material with the *in vivo* conductive environment. Here we show that incorporating a conductive component, such as poly[3,4-(ethelynedioxythiophene)] (PEDOT), into a biomaterial scaffold allows for electrical signal propagation, facilitating neuronal differentiation and maturation. Moreover, peptide amphiphiles (PAs) are designed to self-assemble into high-aspect ratio nanostructures that mimic the architecture of the extracellular matrix and can be functionalized with bioactive epitopes to provide specific signals to cells. To enhance neuronal maturation, we newly developed a conductive and bioactive scaffold consisting of functionalized PEDOT and a laminin mimetic (IKVAV) PA. Using transmission electron microscopy, Fourier transform microscopy and solution small/wide-angle X-ray scattering, we revealed that the functionalized PEDOT does not impact IKVAV PA self-assembly. This synthesized PEDOT was determined to be more biocompatible than previously described literature through cytotoxicity assays and live/dead imaging analysis in murine and human neurons *in vitro*. Interestingly, mouse E16 primary cortical neurons showed longer neurite length when cells were cultured for 5 days on PEDOT and PA coatings compared to controls of PA, PEDOT and PDL. Neurons stained with TUJ-1 and MAP2 showed that PEDOT:PA coatings significantly enhanced neurite outgrowth. Furthermore, Western blot analysis clearly showed the PEDOT:PA condition upregulated maturation and pre-/post-synaptic markers (MAP2, PSD 95, Synaptophysin). Further Western blot studies identified specific effector proteins, MAP-K, cAMP, and calcineurin as targets. To examine treatments in a more clinically relevant model, human induced pluripotent stem cell (iPSC) derived spinal cord neuron models were tested under PEDOT:PA treatments. We showed enhanced differentiation of iPSCs into human spinal cord progenitors through increased expression of TUJ-1/NESTIN. These results strongly suggest that PEDOT:PA promotes CNS neurogenesis in mouse and human systems. Our findings will open a new avenue to use this new biomaterial, PEDOT:PA, as a CNS therapeutic to enhance neuron regeneration and maturation.

Disclosures: A. Metlushko: None. **N. Sather:** None. **N. Takata:** None. **D. Simkin:** None. **T. Fryer:** None. **E. Kiskinis:** None. **S. Stupp:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.04/Y1

Topic: C.10. Brain Injury and Trauma

Support: Grant#: NHRI-EX110-10813NI
Grants# : MOST 108-2320-B-007 -005 -MY3; 111-2311-B-007-012

Title: Quantitative monitoring of neuronal regeneration by functional assay and wireless neural recording

Authors: *J.-W. YANG[1], M.-Z. LIANG[2], H. CHEN[3], L. CHEN[2,4];
[1]BioPro Scientific, Hsinchu, Taiwan; [2]Inst. of Mol. Med., Natl. Tsing Hua Univ., Hsinchu, Taiwan; [3]Electrical Engin., Natl. Tsing Hua Univ., HsinChu, Taiwan; [4]Dept. of Med. Sci., Natl. Tsing Hua Univ., Hsinchu, Taiwan

Abstract: Brain injury is heterozygous in nature and no single scheme is ideal for diagnosis and prognosis. Assessing proteins in cerebrospinal fluid is limited or not applicable after surgery whereas plasma biomarkers can only report the occurrence of injury. Thus far, there is no neuronal regeneration markers are available. This study aims to establish a potential reporter that correlates the regeneration progress of injured brain neurons. Local field potential (LFP) was measured at sites proximal to the injury region. The recorded neuronal activity reported stimulation of the right forelimb of mice. The experimental results indicate that the evoked LFP could potentially be used as a regeneration biomarker. This promising result further points towards the future application of a wireless, non-invasive recording system as a potential companion diagnosis device during drug development.

Disclosures: J. Yang: None. **M. Liang:** None. **H. Chen:** None. **L. Chen:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.05/Y2

Topic: C.10. Brain Injury and Trauma

Support: Korea Institute of Science and Technology Grant 2E32040-22-P016
Seoul national university Collage of Medicine Grant 800-20230198

Title: Innovations in Neurosurgery: Developing a Polymer Coated Dura to Prevent Post-Surgical Adhesion

Authors: *S. JEON[1], J. KIM[1], Y. LEE[1], M. LEE[1], K. KIM[1,2], J. LEE[1,2];
[1]Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Div. of Neurosurg., Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: (Background) Adhesion following brain or spinal surgery is a significant neurosurgical complication that often leads to pain and the need for additional surgeries.

Fibroblast cells are known to induce adhesion, causing tissues and organs to stick to surrounding tissues. In this study, we developed functional polymer coating-based substitutes (HEMA, EGDMA and V4D4) through the iCVD (initiated chemical vapor deposition) coating process to clinically utilize them as a replacement of a dura mater. (Materials and Methods) To compare the attachment of cells, mouse fibroblasts (NIH-3T3) and human fibroblasts (BJ-5TA, patient-derived fibroblasts) were cultured on HEMA, EGDMA, and V4D4-coated dura in vitro, and the cell attachment assay was performed. For in vivo studies, both bare dura and HEMA-coated dura were implanted on both sides of the brain in C57BL/6J mice (8-10 weeks old). After 2 weeks, the mice were humanely euthanized for evaluation of adhesion grading and histopathological changes using H&E staining, Masson's Trichrome staining, and immunohistochemistry. (Results) In vitro, the degree of adhesion was significantly reduced in the HEMA-coated group compared to the other groups, including the bare dura group. Gross adhesion grading was assessed in vivo, with the HEMA-coated group demonstrating lower adhesion grades than the bare dura group. Notably, the bare dura group exhibited a higher number of inflammatory cells and greater fibrosis compared to the HEMA-coated dura group. (Conclusion) This study suggests that HEMA-coated dura represents an effective polymer coating-based substitute for preventing adhesion, while also exhibiting reduced inflammatory responses in both in vitro and in vivo studies. Hence, our method could be clinically suitable to use as a dura mater in neurosurgery.

Disclosures: **S. Jeon:** None. **J. Kim:** None. **Y. Lee:** None. **M. Lee:** None. **K. Kim:** None. **J. Lee:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.06/Y3

Topic: C.10. Brain Injury and Trauma

Support: Ari and Regine Aprijaskis Fund, Grant number 347300-00
Dr. Miriam and Sheldon G. Adelson Center for the Biology of Addictive Diseases, Grant number 601133461
Sylvan Adams Sports Institute Grant number 0601133671

Title: Long-term behavioral alterations in mtbi: home-cage monitoring in mice

Authors: L. TSEITLIN[1], B. RICHMOND- HACHAM[3], L. BIKOVSKI[2], ***C. G. PICK**[3];
[1]Anat., [2]Myers Neuro-Behavioral Core Facility, Sackler school of Med., Tel Aviv - Yafo, Israel; [3]Anat., Tel Aviv Univ., Tel Aviv - Yafo, Israel

Abstract: Mild traumatic brain injury (mTBI) accounts for approximately 90% of all brain injury cases. However, diagnosing mTBI remains challenging, and patients often experience a wide range of short and long-term behavioral impairments. Animal models, particularly in rodents such as the weight drop model, have been instrumental in understanding the

consequences of mTBI. However, despite extensive research, our understanding of mTBI is still limited, and there is a need for innovative methods to improve our comprehension of this condition. One such innovative method is the use of home cage monitoring (HCM) systems. While traditional behavioral assessments used so far in mTBI studies suffer from limitations (e.g. lack of sensitivity, limited assessment time, stress from the human handler), HCM systems overcome these shortcomings and enhance longitudinal assessment. In fact, HCM provides a stress-free environment and allows comprehensive data collection encompassing various parameters throughout the circadian phases. In this study, we employed the Noldus PhenoTyper HCM system to assess long-term behavioral changes in a weight drop model of mTBI over a 72-hour period. By providing a stress-free -environment (e.g. human-free) for an extended duration, this study aimed to uncover behavioral alterations that could not be observed previously in this model using standard methods. The utilization of HCM systems in mTBI research allows for a comprehensive and detailed understanding of the behavioral consequences of mTBI, paving the way for improved treatments and interventions. mTBI mice displayed increased activity in the home cage, and in wheel use during their active phase, indicating a hyperactivity-related behavior. In addition, during both the active and rest phases, mTBI mice spent less time in the shelter, suggesting a disruption in their circadian rhythm. As for home cage cognitive assessment, when tested in discrimination learning, the mTBI mice showed no significant impairment in their ability to discriminate between different stimuli, as measured by the cognition wall method. However, they exhibited abnormal faster reversal learning, suggesting they quickly shifted their attention to the previously non-reinforced alternative, treating it as if it were a novel stimulus.

Disclosures: **L. Tseitlin:** None. **B. Richmond- Hacham:** None. **L. Bikovski:** None. **C.G. Pick:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.07/Web Only

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1R01NS122724-01A1

Title: Senolytic therapy improves long term functional outcome after traumatic brain injury

Authors: *D. BRANN, J. WANG, Y. LU, C. CARR, K. DHANDAPANI;
Neurosurg., Augusta Univ., Augusta, GA

Abstract: Chronic neuroinflammation can exist for months to years following traumatic brain injury (TBI), although the underlying mechanisms remain poorly understood. In the current study, we examined whether proinflammatory senescent cells are present in the brain long-term (months) after TBI and whether ablation of these cells via administration of senolytic drugs can

improve long-term functional outcome after TBI. The results revealed that astrocytes and microglia in the cerebral cortex, hippocampus, corpus callosum and lateral posterior thalamus colocalized the senescent cell markers, $p16^{Ink4a}$ or $p21^{Cip1/Waf1}$ at 5 weeks post injury (5wpi) and 4 months post injury (4mpi). Intermittent administration of the senolytic drugs, dasatinib and quercetin (D+Q) beginning 1-month after TBI for 13 weeks significantly ablated $p16^{Ink4a}$-positive- and $p21^{Cip1/Waf1}$-positive-cells in the brain of TBI animals, and significantly reduced expression of the major senescence-associated secretory phenotype (SASP) pro-inflammatory factors, interleukin 1b and interleukin 6. Senolytic treatment also significantly attenuated neurodegeneration and enhanced neuron number at 18 weeks after TBI in the ipsilateral cortex, hippocampus, and lateral posterior thalamus. Behavioral testing at 18 weeks after TBI further revealed that senolytic therapy significantly rescued defects in spatial reference memory and recognition memory, as well as depression-like behavior in TBI mice. Taken as a whole, these findings indicate there is robust and widespread induction of senescent cells in the brain long-term after TBI, and that senolytic drug treatment begun 1 month after TBI can efficiently ablate the senescent cells, reduce expression of proinflammatory SASP factors, reduce neurodegeneration, and rescue defects in reference memory, recognition memory, and depressive behavior.

Disclosures: **D. Brann:** None. **J. Wang:** None. **Y. Lu:** None. **C. Carr:** None. **K. Dhandapani:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.08/Y4

Topic: C.10. Brain Injury and Trauma

Support: Conacyt 252702

Title: Effect of hydrogen sulfide on the vascular dysfunction induced by severe traumatic brain injury in rats

Authors: *A. SÁNCHEZ-LÓPEZ[1], F. I. LÓPEZ-PREZA[2], S. HUERTA DE LA CRUZ[2], C. L. SANTIAGO-CASTAÑEDA[2], D. L. SILVA-VELASCO[2], J. H. BELTRAN-ORNELAS[2], J. A. TAPIA-MARTÍNEZ[2], L. ROCHA[2], D. CENTURIÓN[2];
[1]Pharmacobiology, Cinvestav-Coapa, Mexico, Mexico; [2]Pharmacobiology, Cinvestav-Coapa, CDMX, Mexico

Abstract: Traumatic brain injury (TBI) is a condition that affects the central nervous system and leads to systemic impairments. Particularly in the cardiovascular system, the blood pressure is altered after a TBI. Indeed, hypertension is one of the most frequent comorbidities in TBI survivors. Moreover, hypertension is related to a decrease in hydrogen sulfide (H_2S) synthesis. Therefore, this study aimed to assess the effects of i.p. subchronic administration with NaHS, an

exogenous H_2S donor, on TBI-induced vascular impairments. Animals underwent a lateral fluid percussion injury, and thoracic aortas were obtained seven days after TBI induction. The vascular function was measured using isolated organ bath chambers. The sensorimotor function was evaluated using the Neuroscore test. After seven days of the severe TBI induction, animals showed: (1) a decrease in body weight, (2) sensorimotor dysfunction, and (3) vascular dysfunction characterized by a decrease in the vasorelaxation induced by carbachol and an increase in contraction induced by norepinephrine. Interestingly, NaHS subchronic administration (3.1 mg/kg; i.p., every 24 h for seven days) reversed TBI-induced vascular dysfunction by restoring carbachol-dependent vasorelaxation and norepinephrine-induced vasoconstriction with no effect on the body weight or sensorimotor impairments. These results suggest the use of NaHS as a possible treatment to reverse post-TBI vascular dysfunction.

Disclosures: A. Sánchez-López: None. **F.I. López-Preza:** None. **S. Huerta De la Cruz:** None. **C.L. Santiago-Castañeda:** None. **D.L. Silva-Velasco:** None. **J.H. Beltran-Ornelas:** None. **J.A. Tapia-Martínez:** None. **L. Rocha:** None. **D. Centurión:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.09/Y5

Topic: C.10. Brain Injury and Trauma

Support: EU H2020 Marie Sklodowska Curie grant agreement No 740264
Academy of Finland
Sigrid Juselius Foundation
ANPCyT PICT 2019-01075

Title: Mitigating traumatic brain injury-induced status epilepticus and neuropathology with a systems biology selected compound

Authors: *N. P. T. KAJEVU[1], P. ANDRADE[1], I. BAÑUELOS[1], A. LIPPONEN[1,2], T. NATUNEN[3], N. PUHAKKA[1], L. GAVERNET[4], L. SABATIER[4], M. HILTUNEN[3], A. TALEVI[4], A. PITKÄNEN[1];
[1]Epilepsy Res. Group, A.I. Virtanen Inst. For Mol. Sciences, UEF, Kuopio, Finland; [2]Expert Microbiology Unit, Finnish Inst. for Hlth. and Welfare, Kuopio, Finland; [3]Sch. of Med., Inst. of Biomedicine, UEF, Kuopio, Finland; [4]Lab. of Bioactive Compound Res. and Develop. (LIDeB), Fac. of Exact Sci. – Argentinean Natl. Council of Scientific and Tech. Res. (CONICET), Univ. Nacional de la Plata, La Plata, Argentina

Abstract: BACKGROUND: Currently, no therapies can alleviate traumatic brain injury (TBI) induced status epilepticus or halt/reverse post-TBI neuropathology. **OBJECTIVE**: Our objective was to investigate whether a compound discovered through structure-based designing would demonstrate any disease-modifying acute effects after TBI. **METHODS AND RESULTS**:

Neuroprotective and anti-inflammatory effects of 11 compounds discovered by computer-assisted drug discovery were assessed in neuronal-BV2 microglial co-cultures. Amongst these, FBA (a sodium channel blocker) showed the best neuroprotective (MAP-2 as a tissue biomarker), antioxidative (nitrite production) and anti-inflammatory (TNF-α) effect *in vitro* [all $p < 0.01$]. Consequently, the *in vivo* neuroprotective and anti-seizure effects of FBA were assessed using lateral fluid-percussion injury in adult male Sprague-Dawley rats. FBA (30 mg/kg administered at 2 h and 24 h post-TBI, i.p.) reduced cortical lesion area (19 ± 4 mm^2, n=7) as compared to that of vehicle treatment (25 ± 6 mm^2, n=10, $p < 0.05$). In the FBA group the average frequency or cumulative duration of seizures during the first 72 h post-TBI was comparable to that in the vehicle group ($p > 0.05$). After a 5-minute continuous epileptiform activity, a single administration of FBA (100 mg/kg) increased the average of relative gamma power in the ipsilateral frontal (pre-treatment 5 % *vs.* post-treatment 9 %) and occipital (7 % *vs.* 12 %) regions as well as in the contralateral occipital (12 % *vs.* 19 %) region ($p < 0.05$). Diazepam (10 mg/kg) that was used as a reference drug, increased the average of relative delta power in the contralateral frontal area (39 % *vs.* 54 %, $p < 0.05$) accompanied with a decrease in average of relative theta power (27 % *vs.* 18 %, $p < 0.01$). **CONCLUSION AND FUTURE DIRECTIONS**: Structure-based designing is a promising approach to identify compounds to improve post-TBI structural and functional recovery.

Disclosures: **N.P.T. Kajevu:** None. **P. Andrade:** None. **I. Bañuelos:** None. **A. Lipponen:** None. **T. Natunen:** None. **N. Puhakka:** None. **L. Gavernet:** None. **L. Sabatier:** None. **M. Hiltunen:** None. **A. Talevi:** None. **A. Pitkänen:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.10/Y6

Topic: C.10. Brain Injury and Trauma

Support: U.S. DoD CDMRP grant# W81XWH2120040

Title: Acute 4-aminopyridine (4-AP) mitigates axon damage and node of Ranvier disruption after experimental traumatic brain injury, without increasing seizure behavior

Authors: *K. L. RADOMSKI, X. ZI, R. C. ARMSTRONG;
Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Axonal injury is a hallmark pathology of traumatic brain injury (TBI) that impairs the function of diverse neural circuits. No available treatment protects against acute axon damage, which is critical for preventing long term symptoms for patients with TBI. We previously showed significant reduction of axon damage after TBI in mice with acute administration of low dose 4-aminopyridine (4-AP; 0.5 mg/kg), a small molecule that inhibits voltage-gated potassium channels. Low dose 4-AP is used clinically to improve axonal conduction in several chronic

neurologic disorders. The current study evaluates repurposing of low dose 4-AP to inform risks and dosing as an acute therapy for TBI. We evaluated seizure threshold as the most likely adverse effect of 4-AP. We quantified 4-AP therapeutic benefit on axons with clinically applicable immunolabeling of β-amyloid precursor protein (β-APP) to detect impaired axonal transport and Nav1.6 sodium channel localization for node of Ranvier (NoR) disruption. Experiments in male and female mice were blinded and randomized with controls for sham injury and vehicle treatment. Adult mice received a closed skull concussive impact, with 4-AP initiated a day later (i.p. bolus, 2x/day for 6 days). Mice received 4-AP at either a high dose (5 mg/kg), low dose (0.5 mg/kg), or saline vehicle (0 mg/kg). 4-AP seizure threshold was assessed after a final injection on day 7 post-TBI/sham procedures. Seizure induction was analyzed using a Racine 8-point scale, as modified for mice, to non-invasively detect motor (tonic-clonic) and non-motor (arrest) seizure behavior. The high 4-AP dose induced clear seizure behavior (Racine score range 4-6) in all sham and TBI mice. In contrast, mice treated with the low 4-AP dose exhibited normal cage activities matching vehicle control mice. Neuropathological analysis of β-APP immunoreactivity in axonal swellings indicated diffuse axon damage in the corpus callosum after TBI. Both the high and low 4-AP doses had similar beneficial effects of approximately 30% lower levels of damaged axons as compared to vehicle-treated TBI mice. We also assessed the effect of acute low dose 4-AP on NoR disruption as a second measure and found a significant therapeutic benefit in this more subtle axonal damage. Taken together, these findings demonstrate that acute low dose 4-AP treatment did not induce seizures, even after TBI, and produced equivalent therapeutic benefit from axonal injury as the higher dose. These data provide further pre-clinical evidence for repurposing low dose 4-AP acute therapy as a new indication for patients after TBI.

Disclosures: K.L. Radomski: None. **X. Zi:** None. **R.C. Armstrong:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.11/Y7

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1RO1 NS126503-01A1

Title: The IRG1/itaconate pathway is a crucial modulator of microglial bioenergetics metabolism for ameliorating pro-inflammatory activation in traumatic brain injury

Authors: *N. LIU[1], Y. JIANG[1], Y. XIU[1], W. XIA[1], Y. WANG[1], A. NIAMNUD[1], S. A. GRAZIOSE[1], S. J. VODOVOZ[1], M. D. KILGORE[2], C. J. PEPER[1], P. S. SPENCER[1], X. WANG[1];
[2]Neurosurg., [1]Tulane Univ., New Orleans, LA

Abstract: Traumatic brain injury (TBI) rapidly triggers an inflammatory response in microglia, which contributes significantly to the pathophysiology of TBI. Emerging evidence indicates that microglial bioenergetics dysregulation is closely associated with pro-inflammatory activation, highlighting the potential of modulating microglial metabolism as a therapeutic strategy for TBI. However, the precise mechanisms underlying this relationship remain poorly understood. Itaconate, a newly identified metabolite in the tricarboxylic acid (TCA) cycle, is catalytically synthesized by immune responsive gene 1 (IRG1). IRG1/itaconate has emerged as a regulatory hub for immunity and metabolism in macrophages. In this study, we hypothesized that the IRG1/itaconate pathway might regulate the bioenergetics dysfunction-triggered pro-inflammatory activation of microglia after TBI. In this study, microglial-specific IRG1 deficiency and controlled mice were subjected to controlled cortical impact (CCI), and C57BL/6NJ mice received intraperitoneal itaconate derivative 4-octyl itaconate (OI) or vehicle at 1 h after CCI. The bioenergetics metabolism profile and pathways of brain isolated microglia were examined by single-cell RNA sequencing, LC-MS/MS-based metabolomics and metabolic flux analysis at 12 h after CCI. Neuroinflammation and neurodegeneration were determined with flow cytometry and immunostaining at 24 and 48 h after CCI. Neurobehavioral assessments were used to monitor motor and cognitive function up to 28 days after CCI in mice. We found that after CCI, IRG1 is specifically overexpressed while itaconate levels are significantly reduced in microglia. Furthermore, we demonstrated that microglial-specific IRG1 deficiency exacerbates the early bioenergetics decline in microglia after CCI, whereas supplementing with OI rescues this decline. OI administration restored the utilization and oxidative metabolism of fuels, including glucose, glutamine, and fatty acid metabolism in microglia following CCI. Moreover, microglial-specific IRG1 deficiency exacerbated microglial pro-inflammatory activation, neurodegeneration, and long-term neurological outcomes after CCI, whereas OI supplementation alleviated these effects. In summary, our study strongly suggests that TBI induces IRG1/itaconate axis dysregulation and bioenergetics dysfunction in microglia, targeting the dysregulation of the IRG1/itaconate pathway attenuates bioenergetics dysfunction, inhibits microglial pro-inflammatory activation and neurodegeneration, and ultimately ameliorates neurological deficits after TBI.

Disclosures: N. liu: None. **Y. Jiang:** None. **Y. Xiu:** None. **W. Xia:** None. **Y. Wang:** None. **A. Niamnud:** None. **S.A. Graziose:** None. **S.J. Vodovoz:** None. **M.D. Kilgore:** None. **C.J. Peper:** None. **P.S. Spencer:** None. **X. Wang:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.12/Y8

Topic: C.10. Brain Injury and Trauma

Support: NINDS/NIH grant R21 NS114723-01A1
NINDS/NIH grant 5R01 NS111037

Title: Rolipram delivered by PgP nanocarrier reduces secondary injury and improves motor and cognitive function in a rat moderate controlled cortical impact TBI model

Authors: C. E. JONES[1], B. ELLIOTT[1], Z. LIAO[1], K. HENRIE[1], T. A. MURRAY[2], ***J. LEE**[1]; [1]Clemson Univ., Clemson, SC; [2]180 Marina Pt, Louisiana Tech. Univ., Hot Springs, AR

Abstract: Traumatic brain injury (TBI) results in impaired motor and cognitive function due to primary mechanical insult and the progressive secondary injury including neuroinflammation. Rolipram (Rm), a phosphodiesterase IV inhibitor has been investigated to treat TBI by inhibiting the breakdown of the cyclic adenosine monophosphate (cAMP). In our lab, we developed poly(lactide-co-glycoside)-graft-polyethyleneimine (PgP) as a drug delivery carrier and Rm was loaded in the PgP nanocarrier by solvent evaporation method. In this work, we evaluated the effect of Rm-PgP on cAMP level restoration, secondary injury, and motor and cognitive function in a rat moderate controlled cortical impact (CCI) TBI model. To generate TBI model, Sprague Dawley male rats were placed in a stereotaxic frame and a craniotomy (6 mm diameter) was made and injury was generated by impactor (4 m/sec, 2.5 mm depth, and dwell time 250 msec) using a 5 mm tip. Rats were randomly assigned to three groups: 1) Normal rat, 2) untreated TBI (20µL, Saline) , and 3) Rm-PgP (20µL, 20µg Rm) treated group. Immediately after injury, Rm-PgP was administered by intraparenchymal injection using microinjection pump with Hamilton syringe (32 G) at 2 µl/min in the lesion site. At 3 days post -injury (DPI), brains were retrieved for cAMP level by ELISA assay. The effect of Rm-PgP on motor function was evaluated by rotarod test and on cognitive function was evaluated by Morris Water Maze starting on 8 DPI and continuing for 5 training days and a final probe test at 14 DPI. After functional studies, rats were sacrificed via cardiac perfusion by 4% PFA for the histological analysis. We observed that Rm-PgP treatment restores cAMP to a level not significantly different from normal level at 3DPI. Aditionally, we observed that Rm-PgP treatment improved motor function by rotarod compared to untreated TBI. For cognitive function, Rm-PgP treated group showed significant reduction in latency to target at 11 and 12 DPI for training period and increase in number of target crossing at 14 DPI (Probe test) compared to untreated TBI. Rm-PgP treatment showed a significant increase in phosphorylated CREB (pCREB). Rm-PgP treatment did not show any difference in lesion size compared to untreated TBI, but Rm-PgP treatment significantly decreases number of CD86 positive (pro-inflammatory M1 macrophages) cells and increases number of Arginase 1 positive (anti-inflammatory M2 macrophages) cells compared to untreated TBI. We also observed that Rm-PgP treatment significantly decreases in percent apoptotic cells compared to untreated TBI. In conclusion, Rm delivered by PgP improved motor and cognitive function and reduced secondary injury after TBI.

Disclosures: C.E. Jones: None. **B. Elliott:** None. **Z. Liao:** None. **K. Henrie:** None. **T.A. Murray:** None. **J. Lee:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.13/Y9

Topic: C.10. Brain Injury and Trauma

Support: Merit Review Grant I01BX004837
Merit Review Grant I01BX005750

Title: Loss-of-microrna-15a/16-1 function promotes neuropathological and functional recovery in experimental traumatic brain injury

Authors: C. ZHOU[1,2], **S. LI**[1,2], N. QIU[1,2], P. SUN[1,2], J. XUE[1,2], M. H. HAMBLIN[3], C. E. DIXON[1,2], J. CHEN[1,2], *K. YIN[1,2];
[1]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Geriatric Research, Educ. and Clin. Ctr., Veterans Affairs Pittsburgh Healthcare Syst., Pittsburgh, PA; [3]Tulane Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: MicroRNAs (miRs) have been implicated in a variety of human central nervous system (CNS) diseases, and the function and mechanisms of miRs in regulating long-term neurological outcomes are poorly understood in traumatic brain injury (TBI). Dysregulated plasma miR-15a/16-1 levels have been found in TBI individuals. However, the essential role and therapeutic potential of the miR-15a/16-1 cluster in TBI are poorly understood. In this study, to determine whether genetic deletion and pharmacological inhibition of the miR- 15a/16-1 cluster improve the functional recovery in TBI, experimental TBI was induced in miR-15a/16-1 knockout mice and wild-type controls by unilateral controlled cortical impact (CCI) for four weeks. A total of 8μg miR-15a/16-1 antagomir or scramble control was intranasally administered in each mouse at 2h, 5d, 10d, 15d, 20d, and 25d after TBI. The cognitive and sensorimotor neurobehavioral disorders, white matter injury, and neuronal loss were then extensively evaluated by multiple approaches and quantitatively analyzed. The parenchymal inflammatory cytokines were detected by inflammation antibody array. We found that mice underwent CCI for 28 days developed remarkable cognitive impairments, myelin loss and axonal damage in corpus callosum and external capsule, and neuronal death in cerebral cortex and hippocampal CA1 region. Genetic deletion of the miR-15a/16-1 cluster or intranasal delivery of miR-15a/16-1 antagomir significantly reduced CCI-induced neuronal loss, demyelination, and axonal injury, and improved long-term neurobehavioral outcomes in TBI mice. Inhibition of miR-15a/16-1 significantly decreased the expression of the proinflammatory cytokines interleukin-6, interleukin-9, monocyte chemoattractant protein-1, tumor necrosis factor alpha levels in the perilesional brain regions. These findings suggested that genetic deletion and pharmacological inhibition of the miR-15a/16-1 cluster improve histological and functional outcomes in an experimental TBI model. The miR-15a/16-1 cluster may be a novel therapeutic target for the treatment of TBI.

Disclosures: C. Zhou: None. **S. Li:** None. **N. Qiu:** None. **P. Sun:** None. **J. Xue:** None. **M.H. Hamblin:** None. **C.E. Dixon:** None. **J. Chen:** None. **K. Yin:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.14/Y10

Topic: C.10. Brain Injury and Trauma

Support: Research Foundation of SUNY (to DSFL)
Fellowship support from the NYS-IBR (to AMB)

Title: Anti-epileptogenic effects of early administration of brivaracetam in rats after severe traumatic brain injury

Authors: *A. MEJIA-BAUTISTA[1], H. B. MICHELSON[1], A. SANJANA[1], O. FAMUYIWA[1], J. H. GOODMAN[2], D. S. LING[1];
[1]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [2]Developmental Neurobio., NYS Inst. For Basic Res., Staten Island, NY

Abstract: Posttraumatic epilepsy (PTE) can occur in up to 40% of patients who sustain a severe traumatic brain injury (TBI). Despite decades of research, there are no therapeutic interventions to prevent PTE. Moreover, in many cases of PTE, seizures cannot be controlled with standard antiseizure medications (ASMs). Early intervention with ASMs may be one strategy to prevent PTE by intercepting the posttraumatic epileptogenic cascade. Using the controlled cortical impact (CCI) injury model of severe TBI, we have assessed the efficacy of brivaracetam (BRV) to prevent PTE. BRV is an FDA approved ASM that targets the synaptic vesicle protein 2A (SV2A) and appears to exert its anti-seizure actions by modulating synaptic glutamate release. We predict that BRV can prevent or mitigate the TBI-induced epileptogenic process and consequent cortical hyperexcitation. Sprague Dawley rats (P26-35) were subjected to a severe CCI injury (2 mm depth, 4 m/s). Randomly selected rats were given a single BRV dose (21 mg/kg, IP) immediately after injury (CCI-BRV). Sham-injured rats were not subjected to CCI. Due to the low incidence of spontaneous seizure activity in rodent TBI models, a challenge with a low dose of the voltage-gated potassium channel blocker 4-aminopyridine (4-AP) was used to assess seizure susceptibility as a metric for post-traumatic epileptogenesis. At 4-8 weeks after injury, rats were given a single dose of 4-AP (3.5 mg/kg, IP), and monitored for up to 70 min for the development of stage 4/5 behavioral seizures. CCI-injury led to a significant increase in seizure susceptibility, raising the proportion of CCI-injured rats that exhibited 4-AP-evoked seizures by two-fold relative to sham-injured controls. BRV treatment reduced injury-induced seizure susceptibility by 50%, bringing the proportion of CCI rats that exhibited seizures down to sham-control levels. These results demonstrate the utility of the 4-AP challenge as a test for epileptogenesis in this TBI model and suggest that early, post-injury administration of BRV may prevent the posttraumatic epileptogenic process.

Disclosures: A. Mejia-Bautista: None. **H.B. Michelson:** None. **A. Sanjana:** None. **O. Famuyiwa:** None. **J.H. Goodman:** None. **D.S. Ling:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.15/Y12

Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS 1R01NS112693-01A1
HHS PHS Ruth L. Kirschstein NRSA T32NS077889
Lexington VA Merit BX003405
KSCHIRT #20-7A

Title: The Direct Effect of 17β-Estradiol on Mitochondrial Dysfunction in a Mouse Model of Traumatic Brain Injury

Authors: *O. KALIMON[1], H. J. VEKARIA[2], P. PRAJAPATI[2], W. B. HUBBARD[3], P. G. SULLIVAN[4];
[1]Neurosci., [2]SCoBIRC, [3]Physiol., [4]Spinal Cord & Brain Injury research center, Dept. of Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Estrogen (E2) actions converge on mitochondria to influence their function. Mitochondria from the uninjured brain of female mice have higher respiration, lower peroxide (H_2O_2) production, and lower Ca^{2+} capacity compared to males; however, this is ablated by ovariectomy or E2 receptor β KO, indicating E2 is important for maintaining normal mitochondrial function. Further, direct administration of E2 to isolated mitochondria was shown to reduce H_2O_2 production, suggesting therapeutic potential. Mitochondrial dysfunction is a hallmark of traumatic brain injury (TBI) and is more severe in males than females. To understand the apparent sex-dependent outcomes after TBI, these studies explore the hypothesis that direct administration of E2 will rescue mitochondria from controlled cortical impact (CCI)-induced dysfunction. Age-matched male and female mice received a severe CCI (1mm depth) and were euthanized 24h later. Total mitochondria were isolated from a 4mm diameter region of the cortex containing the injury epicenter and penumbra (CCI), as well as a 4mm diameter region of the contralateral cortex to serve as the unimpacted control (uninjured). Upon isolation, mitochondria were treated with 0, 2, 20, or 2000nM E2 immediately prior to measuring bioenergetics or reactive oxygen species (ROS) production. E2 was present for the duration of the assay. The mitochondrial samples were reserved at -20ºC for assessment of electron transport chain (ETC) complex activities in the presence of E2. Male and female data were analyzed separately. The results found mitochondria from males had significant main injury effects in bioenergetics, which was abolished in State III respiration by 20nM E2 treatment. Mitochondria from females only showed a main injury effect in State V(CII) respiration. Consistent with the bioenergetic data, mitochondria from males had main injury effects in Complex I, II, & IV activities, but no treatment effect. Mitochondria from females had CCI-induced deficits in CI & II activities, but no treatment effect. At high and low membrane potential (ΔΨm), both sexes had a significant treatment effect in mitochondrial ROS production. Additionally, both sexes had a main injury effect at high ΔΨm; however, there was no interaction between injury & treatment. Overall, these exploratory studies found a distinct pattern of mitochondrial injury between males and females, which appears to be modulated by E2 only in males. This data suggests E2 can directly alter mitochondrial functions, though repetition of these assays is ongoing so that we may determine whether this modulation is beneficial or detrimental to mitochondrial health after TBI.

Disclosures: **O. Kalimon:** None. **H.J. Vekaria:** None. **P. Prajapati:** None. **W.B. Hubbard:** None. **P.G. Sullivan:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.16/Y13

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 5R01NS109477

Title: Therapeutic Effects of Vepoloxamer on Functional Outcomes After Traumatic Brain Injury in Male and Female Rats

Authors: *L. CHEN[1], Y. XIONG[1], M. CHOPP[1,2], H. PANG[1], Z. ZHANG[1], A. MAHMOOD[1], Y. ZHANG[1];
[1]Henry Ford Hlth. Syst., Detroit, MI; [2]Dept. of Physics, Oakland Univ., Rochester, MI

Abstract: Background: The effect of sex after traumatic brain injury (TBI) especially in potential therapeutic drugs is under-recognized. Better prognoses are found for young female patients and for female rodents after TBI, ischemic stroke, brain hypoxia, and subarachnoid hemorrhage. However, in other clinical studies of sex differences, sex disparity remains elusive in posttraumatic mortality or the incidence of acute complications after TBI and neuroprotective treatments. The aim of this study was to investigate whether there is a sex difference in functional outcomes and safety in TBI rats treated with Vepoloxamer. **Methods:** Male and female Wistar young rats, subjected to moderate TBI induced by controlled cortical impact injury, were treated randomly with 0 (saline as vehicle), 300 mg/kg of Vepoloxamer intravenously (IV) 2h after TBI. A battery of cognitive and neurological functional tests was performed after injury. Animals were killed 35 days after TBI and brain sections were stained for the analyses of lesion volume and hemorrhage area. Bleeding time assay was performed 3 h and 26 h post injury using a tail transection bleeding test. **Results:** Compared with saline treatment, IV administration of Vepoloxamer (300 mg/kg, 2 h post injury) significantly improved sensorimotor function, reduced lesion volume in both sexes. However, the therapeutic effect of Vepoloxamer on cognitive deficits was only found in male rats compared to saline. In both sexes, TBI significantly increased bleeding time at 3 and 26 h post injury. Although Vepoloxamer only normalized bleeding time in male rats at 3 h post injury, it normalized bleeding time compared to sham in both sexes at 26 h post injury later. Compared to saline, Vepoloxamer did not further increase hemorrhage but decreased hemorrhage area after TBI. One-way ANOVA followed by post hoc Tukey's tests was used to compare the differences in bleeding test, functional and histological outcomes. P value <0.05 was considered significant. **Conclusion:** We have demonstrated that Vepoloxamer (300 mg/kg, IV administered at 2 h post injury) is safe and has great potential therapeutic value for male and female patients with TBI. TBI increases bleeding time but Vepoloxamer normalizes bleeding time and decreases

hemorrhage area, suggesting that there is no increased risk for intracranial hemorrhage after TBI in both sexes treated with Vepoloxamer. However, compared to female TBI rats, Vepoloxamer appears more effective in improving cognitive function in male TBI rats, suggesting that there is a sex disparity. Additional experiments are required to investigate mechanisms underlying sex difference.

Disclosures: **L. Chen:** None. **Y. Xiong:** None. **M. Chopp:** None. **H. Pang:** None. **Z. Zhang:** None. **A. Mahmood:** None. **Y. Zhang:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.17/Y14

Topic: C.10. Brain Injury and Trauma

Support: R01 NS20099
R37 HD059288

Title: Investigating the Effects of Tetrahydrocannabinol on Contextual Fear Conditioning after Mild Traumatic Brain Injury

Authors: *A. M. FARRUGIA[1], A. S. COHEN[2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Anesthesiol. and Critical Care Med., Children's Hosp Philadelphia Univ. of Pennsy, Philadelphia, PA

Abstract: Traumatic brain injury (TBI) is caused by a physical blow to the head which results in an alteration of brain function. About 69 million people suffer from TBI annually, and it remains a leading cause of death and disability. Concussions, or mild traumatic brain injuries (mTBIs) account for roughly 70-90% of all TBIs. One well-characterized symptom of TBI is the resulting memory deficits. Specifically, TBI has been shown to decrease excitability in the area CA1 of the hippocampus. Our lab has previously shown that using the cannabinoid agonist WIN55,212-2 restores output in area CA1 in live mouse brain slices after mTBI. WIN55,212-2 is non-selective in its binding to cannabinoid receptors. Cannabinoid receptor type 1 (CB1) receptors are distributed on axons, terminals, and cell bodies in the central nervous system (CNS) making them an appropriate target for potential cannabinoid therapeutics after TBI. One CB1 agonist that has potential therapeutic benefit for TBI is tetrahydrocannabinol (THC). We use a behavioral test called contextual fear conditioning. Contextual fear conditioning is commonly used to examine an animal's hippocampal involved memory. Contextual fear conditioning assesses freezing behavior to examine the animal's memory to the context. Using contextual fear conditioning, we have found that there is a significant difference between sham and animals that received mTBI, with sham animals freezing significantly more. These results indicate that sham animals have better memory of the context than injured animals. We hypothesize that THC will increase freezing rates of injured animals to levels not significantly different than those observed in sham

animals. We will use 8-week-old C57BL/6 male mice. To produce mTBI, we use the lateral fluid percussion injury model, which utilizes a fluid pulse that increases intracranial pressure reproducing pathology of TBI. The pressure applied from the concussive force will be in the range of 1.4 to 1.6atm which constitutes a mTBI. Sham animals will receive all parts of the procedure except the actual concussive force. One-week post-injury/sham we will begin handling animals for one week to prepare them for contextual fear conditioning. We then will perform the contextual fear conditioning. Prior to conditioning and freezing assessment, we will inject mice intraperitoneally with THC at 10mg/mL. We show that sham and injured mice are significantly different in their freezing response during contextual fear conditioning. We expect that THC will increase freezing rates of injured animals to that of sham animals, indicating it has a potential to reduce the memory deficits associated with TBI.

Disclosures: **A.M. Farrugia:** None. **A.S. Cohen:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.18/Y15

Topic: C.10. Brain Injury and Trauma

Title: Pharmacological evaluation of clinically used drugs in a rat model of traumatic brain injury

Authors: M. KOJIMA[1,2], M. OKACHI[1], R. WATANABE[1], C. NISHIOKA[1], T. NATSUME[1], *A. HAMA[1], H. TAKAMATSU[1], M. OGAWA[2];
[1]NeuroResearch, Sapporo, Japan; [2]Hokkaido Univ., Sapporo, Japan

Abstract: A number of treatments have been suggested as therapeutics for traumatic brain injury (TBI), but thus far no clinically useful treatment exists. Rather than develop novel therapeutics, it is possible that drugs already approved for clinical use could have utility as treatments for TBI. The goal of the current study is to assess clinically used drugs as potential therapeutics for TBI. Under anesthesia, male Sprague Dawley rats underwent a controlled cortical impact (CCI) that mimics an acute TBI to the motor-sensory cortical hemisphere (3 mm posterior and 3 mm right to bregma). Overall balance, motor coordination and sensory motor reflexes were scored (Neurological Deficits Score) and hind limb motor coordination were assessed (Beam test). Following TBI, rats demonstrated moderate neurological deficits and deficits in hind limb coordination which persisted for at least 3 weeks after TBI, with no significant impairment 4 weeks after TBI. A significant ipsilateral brain lesion was observed in the ipsilateral cortex 4 weeks after TBI. The immunosuppressant, tacrolumus (FK-506), has shown neuroprotective effects in other rodent models of CNS injury. Glibenclamide, a drug used to modulate blood glucose in diabetic patients, decreases edema in rat models of CNS injury. Following TBI, rats (n=10 per group) were treated with either one intravenous (i.v.) dose of FK-506 (1 mg/kg) within 5 min. of TBI followed by subcutaneous (s.c.) injections (2 mg/kg/day) for 28 days,

intraperitoneal glibenclamide (10 μg/kg) within 5 min. of TBI followed by a continuous 28-day infusion (0.2 μg/hour/rat) via s.c. implanted Alzet pumps or vehicle, i.v. within 5 min. of TBI, followed by s.c. injections for 28 days. Glibenclamide improved neurological functioning 3, 7 and 14 days after TBI. Improved neurological functioning was observed 3 and 7 days after TBI with FK-506 treatment. The current study suggests that clinically used drugs could be of utility for the improvement of motor dysfunction following TBI. The exact mechanism of action of either drug on motor dysfunction following TBI remains to be explored. In addition, the effect of these drugs on brain lesions remains to be explored.

Disclosures: M. Kojima: None. **M. Okachi:** None. **R. Watanabe:** None. **C. Nishioka:** None. **T. Natsume:** None. **A. Hama:** None. **H. Takamatsu:** None. **M. Ogawa:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.19/Y16

Topic: C.10. Brain Injury and Trauma

Support: UPMC Children's RAC Graduate Fellowship
VA-I01-BX005291
Copeland Fund

Title: Investigating Neurogranin Signaling Changes in Experimental TBI and a Novel AAV-based Therapeutic Strategy.

Authors: *S. SVIRSKY[1,2], J. HENCHIR[2], S. W. CARLSON[2], C. DIXON[2];
[2]Neurolog. Surgery, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neurogranin, an emerging TBI serum biomarker due to its association with cognition, is a calcium-sensitive calmodulin-binding protein essential for modulating synaptic plasticity. We previously demonstrated controlled cortical impact (CCI) significantly reduces hippocampal neurogranin up to 4 weeks post-injury, though its role in modulating post-synaptic signaling after TBI is unknown. We hypothesize decreased neurogranin will be associated with aberrant post-synaptic signaling after CCI. Adult, male and female Sprague Dawley rats (250-350g, n=6 per injury/time-point/sex) received a sham/control or CCI injury (2.5mm depth, 4m/s) and ipsilateral hippocampal synaptosomes were isolated at 24 hours, 1, 2 and 4 weeks post-injury. Two-way ANOVA showed significant main effects of injury and sex across time in a protein-dependent fashion for hippocampal synaptic expression of neurogranin, phosphorylated-neurogranin (Ser36), phosphorylated-CaMKII (Thr286), suggesting altered post-synaptic signaling. To investigate neurogranin's role in the observed synaptic pathology, an adeno-associated viral vector was developed to selectively increase hippocampal neurogranin expression 4 weeks after CCI. A GFP-reporting T2A multicistronic vector design facilitated the identification of exogenously versus endogenously expressed neurogranin. Immunoblot and immunofluorescence

showed viral-mediated restoration of neurogranin expression after CCI compared to control. Co-immunoprecipitation confirmed calcium-dependent interactions between calmodulin with exogenous neurogranin. A significant positive correlation was observed between exogenous neurogranin and phosphorylated-CaMKII expression (R^2=0.2428, p=0.0377), confirming a downstream signaling effect. Ongoing studies seek to determine if neurogranin gene therapy ameliorates TBI-induced post-synaptic signaling and behavioral outcomes. This study furthers our understanding of mechanisms of cognitive dysfunction within the synapse sub-acutely after TBI.

Disclosures: S. Svirsky: None. **J. Henchir:** None. **S.W. Carlson:** None. **C. Dixon:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.20/Y17

Topic: C.10. Brain Injury and Trauma

Title: Pathophysiology of regeneration in traumatic spine injury, gene therapy targets for spine regeneration- current research and future directions.

Authors: *G. LANKA[1,2], D. D. SINGH[3];
[1]Andhra Med. Col. & King George Hosp., Jaipur, India; [2]Natl. Inst. of Med. Sci., Jaipur, India; [3]Amity Univ., Jaipur, India

Abstract: A spinal cord injury occurs due to trauma, compression, or disease, leading to tissue damage, disrupted neural pathways, and loss of function. This triggers an immediate acute inflammatory response where damaged cells release pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). These pro-inflammatory signals recruit immune cells like neutrophils and macrophages to the injured site, exacerbating the inflammatory response. The activated immune cells, in turn, release additional inflammatory factors and phagocytose cellular debris, contributing to the secondary injury phase. The secondary injury phase ensues, characterized by excitotoxicity due to excessive release of glutamate, causing neuronal cell death, and oxidative stress resulting from reactive oxygen species and free radicals, which damage cellular structures.

Disclosures: G. Lanka: None. **D.D. Singh:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.21/Y18

Topic: C.10. Brain Injury and Trauma

Support: DOD Grant: W81XWH-16-1-0555

Title: Improving glymphatic-lymphatic drainage eliminates post-traumatic brain edema

Authors: *R. HUSSAIN[1], J. TITHOF[2,3], W. WANG[1], H. HIRASE[4], D. H. KELLEY[3], J. A. CASTORENA-GONZALEZ[5], S. A. GOLDMAN[1], P. WEIKOP[4], M. J. DAVIS[6], M. NEDERGAARD[1,4];
[1]Univ. of Rochester, NY, Ctr. for Translational Neuromedicine, Rochester, NY; [2]Dept. of Mechanical Engineering, Univ. of Minnesota,, Minneapolis, MN; [3]Dept. of Mechanical Engineering, Univ. of Rochester, Rochester, NY; [4]Ctr. for Translational Neuroscience, Univ. of Copenhagen, 2200,, Copenhagen, Denmark; [5]Dept. of Pharmacology, Sch. of Medicine, Tulane Univ., New Orleans, LA; [6]Dept. of Med. Pharmacol. and Physiology, Sch. of Medicine, Univ. of Missouri, Columbia, MO

Abstract: Acute brain swelling (edema) is a significant cause of morbidity and mortality after head injury. Currently, decompressive craniectomy and osmotherapy are the only treatment options to counteract potentially fatal increases in intracranial pressure. We report here that post-traumatic surges in the stress mediator noradrenaline suppress glymphatic/lymphatic fluid transport in mice, resulting in tissue swelling and edema. We identify the cervical lymphatic system as the bottleneck for clearance of fluid accumulation after head injury. Traumatic brain injury resulted in a disturbance of rhythmic contractility, loss of entrainment, and impairment of CSF export through cervical lymphatics along with a simultaneous increase in central venous pressure. Inhibition of noradrenergic receptors normalized central venous pressure, restored cervical lymphatic flow, eliminated acute brain edema, and improved functional outcomes. Post-traumatic inhibition of adrenergic signaling also boosted lymphatic export of macromolecules/cellular debris, possibly explaining why the treatment sharply reduced neuroinflammation and accumulation of hyperphosphorylated tau protein. In sum, the high adrenergic tonus after traumatic brain injury and resulting brain edema is a critical determinant for functional recovery. Pan-adrenergic receptor inhibition improves glymphatic/lymphatic clearance of excess fluid and cellular debris and thus emerges as a novel therapeutic approach for treating acute traumatic brain injury.

Disclosures: R. Hussain: None. **J. Tithof:** None. **W. Wang:** None. **H. Hirase:** None. **D.H. Kelley:** None. **J.A. Castorena-Gonzalez:** None. **S.A. Goldman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sana Biotechnology, CNS2 Neuro. **P. Weikop:** None. **M.J. Davis:** None. **M. Nedergaard:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.22/Y19

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01AG068168
NIH Grant R56NS112207
NIH Grant R21NS102991
NIH Grant R21NS111174
NIH Grant R01AG078460

Title: Early effects of mesenchymal stromal cell extracellular vesicles on neuronal integrity and microglial morphology following cortical injury

Authors: *R. MCCANN[1], R. M. TATKE[2], Y. ZHOU[4], C. A. MOJICA[3], H. XIN[6], E. ZELDICH[7], D. L. ROSENE[5], M. MEDALLA[8], T. L. MOORE[9];
[1]Boston Univ. Grad. Program For Neurosci., Boston, MA; [3]Anat. & Neurobio., [2]Boston Univ., Boston, MA; [4]Anat. and Neurobio., [5]Anat. & Neurobio., Boston Univ. Grad. Program In Anat. & Neurobio., Boston, MA; [6]Neurol., Henry Ford Hlth. Syst., Detroit, MI; [7]Biochem., [8]Anat. & Neurobio., [9]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Damage from cortical injury can lead to functional disability that decreases quality of life. Therapeutic options are needed to improve recovery of function following injury. In our rhesus monkey model of cortical injury, we demonstrated that systemic treatment with bone marrow mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) facilitated full functional motor recovery after injury to primary motor cortex in the first 3-5 weeks after injury. Examination of the brains 16 weeks post-injury revealed a treatment-associated reduction in neuronal damage in the perilesional region. To further examine the effects of MSC-EVs now at an early recovery time point, brain tissue harvested at 6 weeks following injury were examined in a cohort of 8 female monkeys (treated n=4; vehicle n=4). In this cohort, treated monkeys recovered motor function 3-5 weeks after injury and protein biomarker assays of serum samples revealed a decreased systemic inflammatory markers along with evidence suggesting an increase in efficiency of clearing axonal damage from the lesion area. To further understand the mechanisms through which the MSC-EVs are acting, we assessed markers of neuronal integrity and microglia- the immune cells that mediate damage clearance- using immunohistochemistry in perilesional gray and white matter. Perilesional gray matter of treated monkeys showed greater density of microtubule associated protein 2 (MAP2) compared to vehicle monkeys. As a measure of dendritic plasticity following cortical injury, dendritic spine morphology and density of filled pyramidal neurons from the perilesional pre-motor cortex were assessed. Surprisingly, neurons from treated monkeys had significantly lower spine density compared to those in vehicle monkeys. However, this difference was driven by a specific reduction in the thin spine subtype. Since thin spines are thought to be transient spines with weaker connections, it is possible that MSC-EV treatment enhances clearance of unnecessary, perhaps damaged, weak spines. Assessments of the microglia marker, ionized calcium-binding adaptor molecule 1 (Iba1), in perilesional gray matter revealed an increase in the expression of homeostatic, ramified microglia. Additionally, we found a decreasing trend in the density of immune activation MHCII factor in the white matter of treated monkeys. Taken together, these findings support our hypotheses that MSC-EVs enhance microglial efficiency in damage clearance, allowing for

protection and maintenance of neural integrity of the perilesional area, leading to enhancement of recovery from cortical injury at the behavioral level.

Disclosures: **R. McCann:** None. **R.M. Tatke:** None. **Y. Zhou:** None. **C.A. Mojica:** None. **H. Xin:** None. **E. Zeldich:** None. **D.L. Rosene:** None. **M. Medalla:** None. **T.L. Moore:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.23/Y20

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS104117
NIH Grant NS1109221

Title: Elovanoids confer robust neuroprotection after traumatic brain injury in rats

Authors: ***N. G. BAZAN**[1], A. OBENAUS[2], A. ALAPATI[1], P. MUKHERJEE[1], L. KHOUTOROVA[1], L. BELAYEV[1];
[1]Louisiana State Univ. Hlth. Scienc Interdisciplinary Neurosci. Training Program, New Orleans, LA; [2]Univ. of California Irvine, Irvine, CA

Abstract: Traumatic brain injury (TBI) often leads to substantial cognitive impairments and permanent disability. The present study defines neuroprotection by very long-chain polyunsaturated fatty acid (VLC-PUFA) Elovanoid (ELV) precursors with C-32:6 and C-34:6 carbons following TBI. They displayed neuroprotective bioactivities *in vitro* neuronal injury and *vivo* experimental ischemic stroke models but have never been tested after TBI. Male Sprague Dawley rats (3-4 months old) were anesthetized with 3% isoflurane, mechanically ventilated, physiologically regulated, and subjected to a moderate right parieto-occipital parasagittal fluid-percussion injury (FPI) model. Rats were treated with ELV by intravenous (IV) and intranasal (IN) delivery. In addition, we examined if ELV can be detected in brain tissue after IN delivery. In series 1: ELV-IV (34:6, 300 µg/per rat) was administered IV one hour after FPI. In series 2: Elov-Mix-IN (32:6, 34:6, and acetyl form of ELVs) was administered intranasal (10 µg in 10 µl per nostril; total 20 µg per rat) at 1h and 24h after TBI. In both series, the behavior was evaluated daily for 14 days, followed by MRI on day 14. In series 3: ELV-IN at 1h and 24h after TBI, behavioral evaluation during 3 days, and LC-MS/MS was used to detect the ELV in the different areas of the brain. ELV-IV improved behavioral scores on days 2, 3, 7, and 14 by 20, 23, 31, and 34% and preserved hippocampal volume loss in the CA3 and DG compared to saline treatment. Whole brain tractography revealed that ELV-IV treatment resulted in increased numbers of cortical fibers at the injury site. Elov-Mix improved the total neurological score by 37, 45, 41, and 41% compared to saline treatment on days 2, 3, 7, and 14. T2WI abnormalities, including cortical thinning and enlarged ventricles, were smaller in ELV-IN-treated rats compared to the saline group. ELV-IN was detected in the ipsilateral cortex and striatum of ELV-IN-treated rats

on day 3. We have shown that the IV and IN administration of ELVs provides high-grade neuroprotection and can be selectively delivered to the brain.

Disclosures: **N.G. Bazan:** None. **A. Obenaus:** None. **A. Alapati:** None. **P. Mukherjee:** None. **L. Khoutorova:** None. **L. Belayev:** None.

Poster

PSTR472. Brain Injury: Innovative Therapeutic Strategies

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR472.24/Z1

Topic:

Title: Peripheral Nerve-Derived Stem Cell Spheroids induce functional recovery and repair after Traumatic Brain Injury in rats.

Authors: *Y. YU, Jr;
CHA university, Pocheon-si, Gyeonggi-do, Korea, Republic of

Abstract: Traumatic brain injury (TBI) often results in permanent functional impairment and places a socioeconomic burden on the patient's family and community. Recovery of neurological function after brain injury is accompanied by nerve regeneration and neuroplasticity. To minimize brain damage and induce regeneration, stem cell therapy is one of the promising treatments for brain damage, but clinical limitations including low viability and differentiation remain. Spheroids can enhance the function of these stem cells, so peripheral nerve-derived adult stem cells (PNSC), which have the ability to secrete nerve-specific activators and differentiate into neurons and glial cells at the same time, are applied as TBI animal models in the form of spheroids. In this experiment, 36 female Sprague Dawley rats were used as TBI animal models to investigate the therapeutic effect of PNSC spheroids. To construct the TBI model, the right parietal bone of the rat head was exposed through craniotomy and impact injury was induced using a stereotaxic impactor. Among the impactor conditions, the speed was 4.5 m/s, the depth was 2 mm, and the dwell time was set to 0.25 sec. Experimental groups consisted of G1: sham group (n = 8), G2: TBI group (n = 8), G3: TBI + PNSC spheroids 1 time injection group, G4: TBI + PNSC spheroids 3 times injection group. PNSC spheroids were intrathecally injected at a volume of $2x10^6$ cells/20 μl into the brain injury site from the day of TBI induction. To evaluate the neurological behavioral index of the experimental animals, the Rota Rod Test and mNSS test were performed on days 1, 3, 7, 14, 21, 28, 35, and 42 after TBI, respectively, and sacrificed on day 42. As a result of the analysis of the Rota-rod behavior test, the recovery of behavioral function was higher in G4 than in G3. In addition, for histological analysis, Hematoxylin&Eosin staining and Luxol Fast Blue staining were performed to confirm myelin regeneration in the rat brain injury area. Immunofluorescent staining was performed using various biomarkers such as GFAP found in glial cells of the CNS, CD31 which can detect angiogenesis, and IL-6 which can determine the degree of inflammation. Based on these experiments, we are trying to find out if PNSC spheroids can induce brain regeneration and help functional recovery in the experimental

group injected with TBI animal models. Implantation of these PNSC spheroids may represent a new treatment approach for patients suffering from TBI.

Disclosures: Y. yu: None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.01/Z2

Topic: D.02. Somatosensation – Pain

Title: Difference in thalamic astrocyte Ca^{2+} activities and sexually dimorphic nociceptive behaviors

Authors: *S. YANG[1], S. PARK[2], J. CHO[3], Y. HUH[4];
[2]Dept. of Brain and Cognitive Sci., [1]Ewha Women's Univ., Seoul, Korea, Republic of; [3]Brain Cognitive Sci., Ewha Womans Univ., Seoul, Korea, Republic of; [4]Catholic Kwandong Univ., Incheon, Korea, Republic of

Abstract: Nociception, the physiological process of sensing harmful stimuli, is regulated through the thalamocortical pain pathway. Moreover, astrocytic Ca^{2+} activity is thought to play important role in pain responses, as numerous studies have shown an increase in astrocytic Ca^{2+} in correlation with pain. Previous studies reported that females and males exhibit different nociceptive responses. Sexually dimorphic nociceptive responses may, in part, be mediated by differences in astrocytic Ca^{2+} activity in the thalamus. To investigate the relationship, we simultaneously recorded formalin-induced nociceptive behaviors and thalamic astrocyte Ca^{2+} signals using fiber photometry in freely moving mice. We found that the female mice exhibited greater nociceptive behaviors and greater number of thalamic astrocyte Ca^{2+} signals during 1st phase compared to male or ovariectomized female mice. To investigate whether increasing thalamic astrocyte Ca^{2+} is able to modulate nociceptive behaviors, we expressed ChR2 or control fluorophore (mCherry) in thalamic astrocytes and compared nociceptive behavioral differences during the formalin test with light stimulation. As expected, mice expressing ChR2 exhibited significantly greater nociception compared to the control. These findings suggest that increases in astrocytic Ca^{2+} in the thalamus contribute to enhance nociceptive responses. Overall, our results suggest that the difference in thalamic astrocyte Ca^{2+} activity may underlie sexual differences in nociception.

Disclosures: S. yang: None. **S. Park:** None. **J. Cho:** None. **Y. Huh:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.02/Z3

Topic: D.02. Somatosensation – Pain

Support: NIH Grant GM115384
NIH Grant NS121776

Title: Role of PFC to ACC pathway in regulating acute and chronic pain

Authors: G. SUN[1], E. ZHU[2], ***Q. ZHANG**[2], H. JEE[3], W. LIU[2], J. WANG[2];
[1]New York Univ., New York Univ., New York, NY; [2]NYU Sch. of Med., New York, NY; [3]NYU Grossman Sch. of Med., NYU Grossman Sch. of Med., New York, NY

Abstract: Pain perception and regulation involve not only cortico-subcortical pathways, but also complex cortico-cortical interactions. Recent studies have shown that two cortical areas, the prefrontal cortex (PFC) and anterior cingulate cortex (ACC), play important roles in pain modulation. Whereas PFC is known to project to the ACC anatomically, how this projection affects pain regulation remains unknown. Here, we studied the projection from the prelimbic PFC to the ACC in the context of acute and chronic pain in free moving rats by using a combination of optogenetics and time-lapse endoscopic calcium imaging. Our results revealed that ACC receives direct inputs from the prelimbic PFC, and that the activation of these inputs leads to a decrease in the nociceptive response among pyramidal neurons in the ACC. Furthermore, activation of this PFC-ACC projection reduced the aversive response to both acute and chronic pain, whereas inhibition of this pathway enhanced pain aversion. These results provide new insights into the cortical mechanisms of pain.

Disclosures: G. Sun: None. **E. Zhu:** None. **Q. Zhang:** None. **H. Jee:** None. **W. liu:** None. **J. Wang:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.03/Z4

Topic: D.02. Somatosensation – Pain

Support: MOST 110-2811-B-001-554

Title: Distinct output pathways emanating from the anterior paraventricular thalamus mediates separate aspects of pain-like behavior in murine chronic pain model

Authors: ***S. MINDAYE**[1,2], W.-H. CHEN[1], Y.-C. CHEN[1], C.-C. CHEN[1];
[1]Inst. of biomedical sciences, Academia Sinica, Taipei, Taiwan; [2]TIGP in Interdisciplinary Neurosci., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: The anterior paraventricular thalamus (PVA), anterior-midline thalamic nuclei, plays an indispensable role in the chronification of pain. PVA is connected with the central nucleus of the amygdala, a commune nod of pain, and this connection contributes to mechanical hyperalgesia development. However, cell-type specificity and the functional role of PVA in different aspects of pain-like behavior remain obscure. Anatomically PVA is connected to regions essential for pain perception indicating that the PVA might be a critical locus in the pain pathway. We used a combination of axonal tracing, behavioral tests, electrophysiology, in vivo calcium imaging, optogenetic, and chemogenetic approaches in mice aged 7 to 12 weeks to identify pain-responsive PVA neurons and investigate the downstream effects of PVA. In this study, we demonstrate that intra-plantar injection of formalin could activate PVA-VgluT_2^+ neurons. In addition, we identified PVA as a key brain region that mediates both nociceptive and emotional aspects of pain. Our data also showed that the PVA-VGluT_2^+ neurons project to the bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NAc). Notably, we discovered that PVA projections to the BNST and the NAc were anatomically and functionally segregated. Activation of efferent projections to BNST drives mechanical hypersensitivity, whereas activation of PVA efferent to the NAc elicits aversive behavior. This finding supports the idea that PVA plays an essential role in the nociceptive brain circuitry. Our result also provides an important insight into the mechanism of distinct components of pain response. Targeting PVA can be a potential target brain area for a therapeutic approach to reduce the suffering of patients with chronic pain.

Disclosures: S. Mindaye: None. **W. Chen:** None. **Y. Chen:** None. **C. Chen:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.04/Z5

Topic: D.02. Somatosensation – Pain

Title: Asymmetry of Pain-Induced Facial Grimacing

Authors: *A. S. ZUMBUSCH, P. SANCHEZ, J. FALCAREK-HOPE, L. YOFFE, E. NICKNER, G. FIRANESCU, S. SOTOCINAL, J. S. MOGIL;
Psychology, McGill University, Montreal, Montreal, QC, Canada

Abstract: Rationale: Facial expressions are evolutionarily adaptive for visually communicating an organism's state to others in its surroundings. Research shows that facial expressions relating to various experiences (e.g., fear, pleasure, etc.) in humans, non-human primates and other non-human animals are lateralized. That is, emotional expression is asymmetrical both in the observable facial output and in the associated neuroanatomy that governs their expression. As far as we know whether pain is similarly lateralized has never been assessed. **Objective:** We sought to investigate whether facial expression of pain as measured by the Mouse Grimace Scale (MGS) is also lateralized, and whether the pain model used alters the lateralization of grimacing.

Methods: Mice were given intraperitoneal injections of acetic acid (AA), a unilateral injection of complete Freund's adjuvant (CFA) into the hind paw, or a unilateral injection of carrageenan into the ankle and facial grimacing was video recorded. Still images of the mouse face were sampled every 2 minutes and were mirrored about the y-axis to create left-left and right-right facial chimeras. We hypothesized that grimacing would be lateralized such that the left side of the face would yield higher MGS scores than the right side. **Results:** Mice experiencing non-localized reflexive pain induced by AA had higher grimace scores on the left hemiface than the right. However, when the pain was localized to the left hind paw in the CFA model, grimacing was stronger on the ipsilateral (right) side of the face. **Future Directions:** We plan to assess the neurobiological substrate of hemifacial asymmetry of pain-induced facial grimacing by unilaterally inhibiting areas known to be associated with pain expression such as the insula and amygdala.

Disclosures: A.S. Zumbusch: None. **P. Sanchez:** None. **J. Falcarek-Hope:** None. **L. Yoffe:** None. **E. Nickner:** None. **G. Firanescu:** None. **S. Sotocinal:** None. **J.S. Mogil:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.05/Z6

Topic: D.02. Somatosensation – Pain

Support: NIDCR grant DE026749
NIH grant NS064022
NIH grant EY08098

Title: Infusion of estrogen receptor alpha agonist PPT into the central amygdala reduced orofacial zoster pain and increased GABA release in rats

Authors: *P. KRAMER[1], L. NGUYEN[2], M. UMORIN[2], P. R. KINCHINGTON[3];
[1]Texas A&M Univ. Col. of Dent., Dallas, TX; [2]Texas A&M Univ. Sch. of Dent., Dallas, TX; [3]Dept. of Ophthalmology and of Microbiology and Mol. Genet., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Background: Herpes zoster (HZ) or shingles results in orofacial pain. Previous studies in our lab suggested the central amygdala controls zoster pain. One means of controlling orofacial pain is through neurons projecting to the lateral parabrachial. Estradiol within the central amygdala has been shown to alter the pain response. From this information our lab hypothesized that estradiol would bind estrogen receptor alpha (ERα) and signal γ-aminobutyric acid (GABA) release to reduce orofacial pain. We tested our hypothesis by infusing the central amygdala of rats with estrogen receptor alpha agonist PPT (4,4',4''-(4-Propyl-[1*H*]-pyrazole-1,3,5-triyl)*tris*phenol) and measuring GABA release in the lateral parabrachial. In addition, orofacial pain was quantitated with and without infusion of PPT. Methods: Surgery was

performed on male Sprague-Dawley rats (300 gram) and a virus producing an engineered protein that will fluoresce upon binding GABA (iGABASnFR, Addgene) was infused in the right lateral parabrachial. After infusion an optical fiber was placed in the right lateral parabrachial. During this surgery an infusion cannula was placed within the central amygdala bilaterally. Four weeks after surgery the rats were divided (n=8 group) into virus and control groups that received MeWo cells containing varicella zoster virus (VZV); the virus responsible for zoster or MeWo cells lacking virus. Injections (100 µl) were in the left whisker pad and contained either 50,000 pfu of virus or control cells. Both these groups were further divided to infuse either vehicle or PPT into the central amygdala. The motivational and affective aspect of pain was then measured using Place Escape Avoidance Paradigm (PEAP) two weeks after whisker pad injection. During behavioral testing fluorescence (i.e., GABA release) in the lateral parabrachial was quantitated by the optical fiber. Results: Infusion of ERα agonist PPT into the central amygdala increased GABA release in the lateral parabrachial and decreased the orofacial pain response induced by VZV injection. Conclusion: Male rats show a reduced VZV pain response because estradiol interacts with ERα within the central amygdala to reduce orofacial pain by increasing GABA release within the lateral parabrachial. GABA in the lateral parabrachial inhibits ascending pain signals from the orofacial region.

Disclosures: P. Kramer: None. **L. Nguyen:** None. **M. Umorin:** None. **P.R. Kinchington:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.06/Z7

Topic: D.02. Somatosensation – Pain

Title: Distinct firing characteristics of neuron types in response to aversive stimulation in the frontal cortex

Authors: *B. BARABAS[1,2,3], D. MAGYAR[1,2,3], P. NAGY-PAL[2,3], T. ANDRASI[1,3], J. M. VERES[3], N. HAJOS[1,3];
[1]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Functional Neurosciences, Semmelweis Univ., Budapest, Hungary; [3]Network Neurophysiol., ELRN Intstitute of experimental medicine, Budapest, Hungary

Abstract: Cortical structures exhibit a high degree of organization, characterized by the diversity of pyramidal cells and interneurons. Despite significant knowledge of how neuron types are wired together, the orchestration of these neurons during behaviorally relevant stimuli *in vivo* remains largely unknown. In this study, we aimed to reveal the firing characteristics of different types of cortical interneurons and compare their firing properties to neighboring pyramidal cells in frontal cortex. We made visually-guided *in vivo* juxtacellular recordings in the superficial layers to detect the firing of 1) perisomatic region-targeting inhibitory neurons (n=85); 2)

dendrite-targeting inhibitory cells (n= 9); and 3) interneuron-selective interneurons (ISI, n=54) and their response to foot shocks in urethane-anesthetized mice (male-female, age P60 to P300). Pyramidal cell spiking upon aversive stimulation was recorded by a silicon probe and differentiated from interneurons based on their spike width and rate. Our findings demonstrate that each neuron type exhibits a characteristic response to foot shocks, altering their firing activity at specific time points. Among pyramidal neurons, three response groups were identified: 1) short excitation followed by inhibition, 2) inhibition and 3) no response. Excited pyramidal cells had the shortest latency response, followed closely by three interneuron types: neuropeptide Y-containing neurogliaform cells (NPY), vasoactive intestinal polypeptide/cholecystokinin-containing ISI (VIP/CCK-ISI), and chandelier cells (ChCs). These three interneuron types displayed firing that was initiated at a similar latency but differed in duration and intensity. Parvalbumin-positive basket cells (PVBCs) showed a dual response pattern, characterized either by pure inhibition lasting 1-3s or by inhibition interrupted by a short excitation. Cholecystokinin and type 1 cannabinoid receptor-expressing BCs (CCK/CB1+ BCs), as well as another subset of VIP-ISIs showed a prolonged activation occurring 1-2 s after the stimuli. The findings of this study indicate that pyramidal neurons in the frontal cortex receive both feedforward excitation and feedback inhibition in response to aversive stimulation. Importantly, our results determined that VIP-ISIs, NPY neurogliaform cells and ChCs are excited simultaneously by foot shocks. Thus, aversive stimuli partially dis-inhibit pyramidal cells by exciting VIP-ISIs but also elevate synaptic inhibition on the dendrites and axon initial segments of pyramidal cells, re-arranging the weight of GABAergic inputs along their different membrane compartments.

Disclosures: B. Barabas: None. **D. Magyar:** None. **P. Nagy-Pal:** None. **T. Andrasi:** None. **J. M. Veres:** None. **N. Hajos:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.07/Z8

Topic: D.02. Somatosensation – Pain

Title: Lysergic acid diethylamide (LSD) decreases pain aversion

Authors: *E. ZHU[1], S. MENNENGA[2], T. SIPPY[3], J. WANG[1];
[1]New York Univ. Langone Hlth., New York, NY; [2]Psychiatry Dept., New York Univ. Sch. of Med., New York, NY; [3]New York Univ., New York, NY

Abstract: Psychedelic drugs can alter or change perception, mood, and various cognitive processes. Recent clinical trials have demonstrated the therapeutic potential of psychedelics for treatment-resistant depression and anxiety. However, the effects of psychedelics on pain are not well understood. Here, we investigated the impact of lysergic acid diethylamide (LSD) on the cortical processing of pain in rat models. Using conditioned place aversion (CPA) and

conditioned place preference (CPP) assays, we found that intraperitoneal injections of LSD in rats can produce a persistent reduction in the aversive response to acute noxious stimuli. Furthermore, we show that the anterior cingulate cortex (ACC), a brain region known to regulate pain affect, likely mediates these anti-aversive effects. Not only does direct delivery of LSD into the ACC decreases pain aversion, but a selective 5-HT2A receptor antagonist in the ACC is shown to eliminate the anti-aversive effects of systemic delivery of LSD. These results suggest the pain-modifying potential for LSD and indicate the role of cerebral cortex for mediating such therapeutic effects.

Disclosures: **E. Zhu:** A. Employment/Salary (full or part-time):; Department of Anesthesiology, Perioperative Care and Pain Medicine, New York University Grossman School of Medicine, New York, NY, USA. **S. Mennenga:** A. Employment/Salary (full or part-time):; New York University Langone Health. **T. Sippy:** A. Employment/Salary (full or part-time):; New York University Langone Health. **J. Wang:** A. Employment/Salary (full or part-time):; Department of Anesthesiology, Perioperative Care and Pain Medicine, New York University Grossman School of Medicine, New York, NY, USA.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.08/Z9

Topic: D.02. Somatosensation – Pain

Support: NIH / NCCIH Grant R33AT009310

Title: Thalamocortical mechanism underlying real and imagined acupuncture

Authors: *Q. KONG, V. SACCA, K. WALKER, S. HODGES, J. KONG;
Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Background: Chronic pain is a significant public health issue that is notoriously difficult to manage. The thalamocortical circuitry has emerged as a crucial mediator in the modulation of chronic pain. While acupuncture and imagery interventions have demonstrated potential for pain management, the mechanisms underlying their analgesic effects remain unclear. This study aims to investigate the thalamocortical mechanisms underlying acupuncture and video-guided acupuncture imagery treatment (VGAIT), a combination of acupuncture and guided imagery. **Methods:** Twenty-four healthy participants were enrolled in the crossover study. We compared the different thalamocortical patterns associated with four interventions (real acupuncture, sham acupuncture, VGAIT, and VGAIT control) using the seed-based resting-state functional connectivity (rsFC) analysis. Three thalamic subdivisions - ventral posterolateral thalamus (VPL), mediodorsal thalamus (MD) and motor thalamus subregion (Mthal) - associated with somatosensory, limbic, and motor circuity was used as seeds. **Results:** Compared to sham acupuncture, real acupuncture altered the rsFC between the thalamus and default mode network

(DMN) (i.e., mPFC, PCC, precuneus, ANG, PHG), as well as the prefrontal, and somatosensory cortex (SI/SII). Compared to the VGAIT control, VGAIT demonstrated greater rsFC between the thalamus and key nodes within the interoceptive network (i.e., anterior insula, ACC, PFC, SI/SII), as well as the motor and sensory cortices (i.e., M1, SMA and temporal/occipital cortices). Furthermore, VGAIT demonstrated increased rsFC between the thalamus (VPL/MD/Mthal) and task-positive network (TPN), which could be further divided into the salience network (i.e., ACC and insula), and central executive network (i.e., DLPFC, SMG, and PoCG), when compared to real acupuncture. Further correlations between differences in rsFC and changes in heat or pressure pain threshold were also observed. **Conclusions**: These findings suggest that both acupuncture and VGAIT can modulate thalamocortical networks. Elucidating the underlying mechanism of VGAIT and acupuncture may facilitate their development, particularly VGAIT, which may be used as a potential remote-delivered pain management approach.

Disclosures: Q. Kong: None. **V. Sacca:** None. **K. Walker:** None. **S. Hodges:** None. **J. Kong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.K holds equity in two startup companies (MNT, BTT) and a patent on applying neuromodulation, but declare no conflict of interest.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.09/Z10

Topic: D.02. Somatosensation – Pain

Title: Cortical regions differently contribute to pain related behaviors

Authors: *G. WANG, Z. ZHOU, H. SHENG, H. LI, Y. ZHANG;
Inst. of Brain Sci., Fudan Univ., Shanghai, China

Abstract: Objective Pain is an unpleasant sensory and affective experience. Cortical areas associated with the sensory-discriminative properties and emotional-motivational aspects are different. Previous studies have implied important roles of specific cortical areas such as anterior cingulate cortex (ACC) and prefrontal cortex (PFC) in the affective processing and descending modulation of pain. However, the roles of cortical structure in modulating pain-related complex behaviors and the effects of cortical region lesions remain poorly understood. **Methods** The effects of cerebral cortex lesion on the behavioral responses to noxious stimuli were studied in Tra2b conditional knockout (cKO) “cortexless” mice and WT mice with lesions in specific cortical regions. The reflexive defensive responses were tested by *von* Frey & Hargreaves test, the affective motivational behaviors were tested by prolonged hotplate. Conditional place aversion (CPA) were used to test pain related aversion. **Results** (1) The affective motivational behaviors and pain-related aversion, but not reflexive defensive responses, are selectively impaired in cortexless mice. (2) Lesions of cortical sub-regions differently affect pain-related

behaviors. (3) Cortical regions have different responses to acute and sustained pain. **Conclusion** Cerebral cortex plays an important role in the processing of affective motivation behaviors and pain-related aversion to sustained pain. ACC and insular cortex (IC) are required for prolonged hotplate induced licking and jumping, and basolateral amygdala (BLA) is essential for pain-related place aversion.

Disclosures: G. Wang: None. **Z. Zhou:** None. **H. Sheng:** None. **H. Li:** None. **Y. Zhang:** None.

Poster

PSTR473. Pain: Central Processing

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR473.10/Z11

Topic: D.02. Somatosensation – Pain

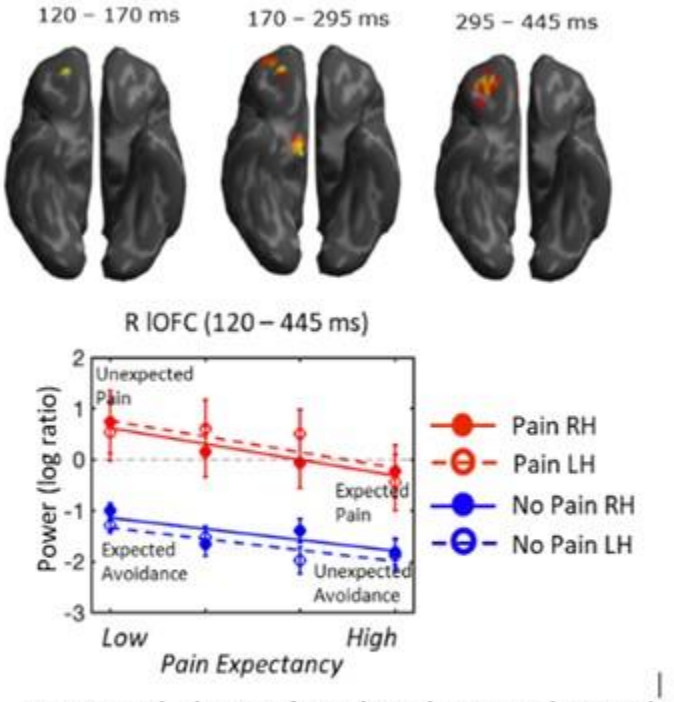
Support: NIH K01DA050804

Title: Magnetoencephalography correlates of human pain avoidance behavior

Authors: *R. GOPALAKRISHNAN[1], S. BAILLET[3], A. G. MACHADO[2], T. D. WAGER[4], M. ROY[3];
[2]Cleveland Clin., [1]Cleveland Clin., Cleveland, OH; [3]McGill Univ., Montreal, QC, Canada; [4]Dartmouth Col., Dartmouth Col., Hanover, NH

Abstract: Prediction error (PE) results when expected and observed sensory information diverge. Pain in an aversive PE that fosters reinforcement learning and avoidance behavior. While fMRI data support the involvement of PAG (Roy et al. 2014), the temporal-spectral dynamics of aversive PE are still largely debated. 27 healthy controls performed 2 blocks of instrumental pain avoidance task in a Magnetoencephalography (MEG) scanner. Participants chose between 2 options, while learning to avoid the choice with high probability of receiving a pain outcome. Task behavior was modelled as a function of pain outcome to estimate latent pain expectancies using a reinforcement learning model. Preprocessed MEG signals time-locked to outcome onset were source localized and time-frequency (TF) computed at each source. Normalized TF maps were entered into a linear regression model to identify significant source clusters, that satisfied axiomatic conditions (pain > no-pain, higher activity for low expected pain regardless of outcome) to define a PE (Rutledge 2010). Computational models confirmed the presence of 2 learning systems, one for received and avoided pain. Learning rates for received and avoided pain were similar for the first block, however, participants learned more from avoided pain than received pain in the second block. MEG activity from alpha frequency band indicated two distinct spatial clusters that satisfied the axioms at lateral orbito-frontal (OFC, 120 - 445ms) and ventral diencephalon (VDC, 170 - 295ms). Expected pain resulted in greater desynchronization compared to unexpected pain. Unexpected pain avoidance resulted in greater desynchronization compared to expected avoidance. Greater desynchronization during expected

pain and unexpected pain avoidance could indicate mobilization of neural resources associated with coping and learning strategies involving the cortico-striatal circuits through the OFC. These finding provide preliminary evidence into the complex spatial-temporal-spectral patterns of brain activity associated with expectancy effects on pain.



Top: Spatial clusters from lateral OFC and VDC that satisfied axiomatic conditions in the alpha band.
Bottom: Satisfied axioms portrayed using TF power estimates from lateral OFC (red vs blue – pain outcome vs pain avoided, solid vs dotted line – pain delivered to right vs left hand)

Disclosures: R. Gopalakrishnan: None. **S. Baillet:** None. **A.G. Machado:** None. **T.D. Wager:** None. **M. Roy:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.01/Z12

Topic: D.02. Somatosensation – Pain

Support: ETH Zurich Foundation - RESC (Pain-Sense)

Title: Pain-sense: an AI digital health tool to monitor physiological biomarkers and subjective predisposition to pain

Authors: *N. GOZZI[1], V. AURUCCI[1], A. CIMOLATO[1], G. PREATONI[1], F. CIOTTI[1], M. HUBLI[2], A. CURT[2], S. RASPOPOVIC[1];
[1]ETH Zurich, zurich, Switzerland; [2]Balgrist Univ. Hosp., zurich, Switzerland

Abstract: Chronic pain is a major health concern, impacting psychological health, functioning, and quality of life. However, its treatment is complex and is challenged by an interplay between biological, psychological, and social factors. Common pain treatments often rely on unspecific drug usage. This results in poor efficacy, low patients' satisfaction, and an extremely high number of medical examinations, placing a significant burden on the healthcare system. Designing individualized, targeted therapies requires understanding each subject's multidimensional pain experience, taking into consideration both the physical and emotional aspects involved. Unfortunately, the golden standard measurement for pain is yet self-reports, which are not capturing the complexity of pain and are intrinsically subjective. Today, there is growing interest in defining new metrics to measure pain, and machine learning (ML) has been proposed for this purpose. However, recent works mainly focused only on finding biomarkers of pain, i.e., objective physiological signature of pain, while disregarding the impact of psychosocial components. To this aim, we developed a telemonitoring tool to comprehensively qualify and quantify a patient's pain experience in ecological moments during multiple days. We collect psychosocial, clinical, and health information while continuously measuring physiological data, i.e., skin conductance, blood volume pulse, and heart rate, through a wearable device. We investigate through Artificial Intelligence (AI) how different subjective and objective factors are impacting pain perception. First, we identify, using explainable AI models, a painful state and discover the most important biomarkers in physiological signals. Then, we investigate how medications and sleep affect the perceived pain and these physiological biomarkers. Finally, using hierarchical AI models, we identify the subjective predisposition to pain, i.e., the mismatch subjects' self-reported pain and measured reliable physiological response. Our approach provides insights into the individual nature of the reported pain level, potentially opening the way to personalized therapies based on a comprehensive evaluation of everyone's pain experience.

Disclosures: N. Gozzi: None. **V. Aurucci:** None. **A. Cimolato:** None. **G. Preatoni:** None. **F. Ciotti:** None. **M. Hubli:** None. **A. Curt:** None. **S. Raspopovic:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.02/Z13

Topic: D.02. Somatosensation – Pain

Support: ETH ZURICH FOUNDATION - RESC (PAIN-SENSE)

Title: Multisensory intervention combining Transcutaneous Electrical Nerve Stimulation and Virtual Reality to decrease neuropathic pain

Authors: *G. AURUCCI, N. GOZZI, G. PREATONI, M. WAGNER, N. BRUNELLO, S. RASPOPOVIC;
ETH Zurich: Eidgenossische Technische Hochschule Zurich, ZURICH, Switzerland

Abstract: Chronic neuropathic pain represents a huge burden for society, with profound implications for physical and psychological individuals' well-being. The intrinsic multidimensionality of pain experience, together with the lack of objective pain biomarkers, prevents the efficacy of currently prescribed pain drugs, thereby resulting in high treatment dissatisfactions and deleterious side effects. In this view, novel non-pharmacological interventions are emerging. Among these, Transcutaneous Electrical Nerve stimulation (TENS) aims at targeting the sensory component of neuropathic pain via peripheral pain-relief mechanisms, while Virtual Reality (VR) immersive technologies have shown analgesic effects in shifting patients' attention from pain. However, despite the potential of these approaches in providing a holistic intervention encompassing diverse aspects of pain, both TENS and VR suffer from the inherent limitation of being employed in a non-specific way (imprecise nerve targeting and lack of VR personalized treatment) therefore limiting their efficacy. Moreover, their combination as a therapeutic tool is missing reliable clinical evidence. To these aims, we developed a multisensory intervention combining TENS and VR and tested its efficacy on a four consecutive days intervention on neuropathic pain patients. The therapy consisted of sessions with synchronous visuo-tactile stimulations combining specific nerves stimulation with virtual waves moving toward the feet of the subjects. We collected self-reported Neuropathic Pain Symptom Inventory (NPSI), unidimensional pain and physiological signals (electroencephalography (EEG) and data from a wearable device) to unravel both subjective and objective neurophysiological pain indicators validating the treatment effect. The combination of immersive technologies with selective transcutaneous stimulation induced a clinically relevant pain decrease (NPSI score) after the first days of intervention. More importantly, the analgesic effects lasted after one week, therefore suggesting a long-term effect of the proposed intervention. Alongside with subjective reports, EEG analysis showed therapy-induced changes over specific pain-related EEG power bands, objectively validating the therapy impact. Also, promising results are observable in the collected wearable physiological signals. Our results demonstrate the therapeutic potential of a multisensory intervention combining VR and TENS. The proposed platform paves the way toward a safe, non-invasive, and effective solution counteracting the excessive and deleterious utilization of pain medications.

Disclosures: G. Aurucci: None. **N. Gozzi:** None. **G. Preatoni:** None. **M. Wagner:** None. **N. Brunello:** None. **S. Raspopovic:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.03/Z14

Topic: D.02. Somatosensation – Pain

Title: Investigating the cortical activity associated with the impact of acute pain on working memory using EEG

Authors: *N. CIVITELLO[1], I. CASMEDES[1], M. LUERA[2], J. BARUAH[1], A. L. HARRIS BOZER[3];
[2]Home, [3]Tarleton State Univ., [1]Tarleton State Univ., Stephenville, TX

Abstract: Chronic pain affects about 100 million adults in the United States and has an annual cost of roughly $300 billion, outweighing the annual costs of heart disease, cancer, and diabetes. Evidence suggests that pain is highly interruptive for cognitive processes, such as memory, encoding, and retrieval. This study aimed to examine how cold-induced acute pain affects working memory in right-handed male and female participants. Participants between the ages of 18-30 were randomly assigned into either an acute pain stimulus (cold pressor task-CPT) pain group (n= 3) or the control group (n=4). Participants completed two rounds of a working memory task that measured their ability to both recall and recognize the last word of presented sentences. EEG data were recorded with iMotions software and a B-Alert 20-electrode system (10-20 electrode placement referenced to mastoids) after an impedance check in a double-walled and foam insulated sound attenuating chamber (Whisper Room). Matlab, Notepad++, and Cartool were used to filter (.05-50Hz) data, reject artifacts, and compute fast fourier transforms. Frequency band data (delta .05-3 Hz; theta 4-7 Hz; alpha 8-13 Hz; beta 14-30 Hz; and gamma 31-50 Hz) were extracted for all 20 electrodes. ANOVAs were run to compare cortical activity (power spectral density of all EEG electrodes) by group (pain/no pain). Electrodes Fp1, Fp2, and F8 are thought to represent cortical areas that are associated with pain perception and working memory. Previous pain research also suggests most changes in brain activity recorded from EEG result in a decrease in alpha power, but findings have been heterogeneous. This study expected that a decrease in alpha power, and possible increases in delta and beta power, would occur upon administration of cold-induced acute pain. ANOVAs indicated that there were no significant differences in the alpha band (8-13 Hz) across groups. The group without pain demonstrated significantly higher beta frequency band (13-30 Hz) activity for the electrodes T6 (p= .041), T3 (p= .024), O1 (p= .003), P3 (p= .006), Pz (p= .029), POz (p=.008), C3 (p= .048), O2 (p= .003). The group without pain demonstrated significantly higher gamma activity for the electrodes o1 (p= .014), P3 (p= .017), and POz (p= .014). This study is ongoing, but these results indicate the role of beta and gamma frequency oscillations in pain processing and working memory. These data suggest more research is necessary in understanding the specific electrophysiology of the effects of pain on working memory.

Disclosures: N. Civitello: None. **I. Casmedes:** None. **M. Luera:** None. **J. Baruah:** None. **A.L. Harris Bozer:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.04/Z15

Topic: D.02. Somatosensation – Pain

Support: CIHR Project Grant

Title: Characterization of altered nociceptive processing in fibromyalgia by means of Structural and Physiological Modeling (SAPM) analysis of functional MRI data.

Authors: *S. HASSANPOUR[1], H. ALGITAMI[2], M. UMRAW[2];
[1]Queen's Univ., Kingston, ON, Canada; [2]Queen's Univ. Ctr. For Neurosci. Studies, Kingston, ON, Canada

Abstract: Fibromyalgia (FM) is a chronic pain condition that affects a significant portion of the population. Prior research has suggested that individuals with FM display heightened. sensitivity to pain and signs of autonomic dysfunction. Therefore, the purpose of this study was to identify altered neural processes underlying pain sensitivity in FM by means of fMRI of the brainstem and spinal cord.
Pain regulation in the brainstem and spinal cord involves both reactive and continuous components that occur before, during, and after the application of a noxious stimulus. Evidence to date indicates that functional differences in FM are mainly in brain regions associated with motivating elements of pain processing, and important differences in nociceptive processing in brainstem and spinal cord regions have also been identified. We hypothesized that nociceptive processing is altered in FM compared to HC, in regions of the brainstem and spinal cord that are involved with autonomic regulation and with autonomic influence on descending pain regulation pathways, including the parabrachial nuclei (PBN) and nucleus tractus solitarius (NTS).
Existing fMRI data from the BS and SC were used from previous studies in our lab involving 30 female participants (15 FM and 15 HC). Data were obtained while a calibrated noxious heat stimulus was applied to the palm of the right hand (C6 dermatome), as well as periods before and after the stimulus. Participants were trained in how to rate their pain and were familiarized with the stimulation paradigm and could anticipate the pain. MRI data were analyzed using Structural and Physiological Modelling (SAPM) which is a novel connectivity analysis method developed in our lab. The SAPM provides a model of neural signaling that explains observed BOLD signal characteristics and includes information about inhibitory and excitatory signaling. We hypothesize that SAPM will provide supporting evidence for altered autonomic influence on descending pain regulation in FM, as demonstrated by the involvement of the parabrachial nuclei (PBN) and nucleus tractus solitarius (NTS).
The results demonstrate significant differences in brainstem/spinal cord network connectivity between the FM and healthy control groups The regions involved in these differences in connectivity included the locus coeruleus (LC), thalamus, NTS, PBN, and right dorsal region of the spinal cord in the C6 segment. The results reflect inhibitory and excitatory signaling differences that are likely involved in controlling arousal and autonomic function. For example, the LC and PBN are associated with pain modulation as well as autonomic homeostatic regulation.

Disclosures: S. Hassanpour: None. **H. Algitami:** None. **M. Umraw:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.05/Z16

Topic: D.02. Somatosensation – Pain

Title: Prospective clinical study for objective data collection in chronic pain patients during neuromodulation trial period

Authors: *S. KULKARNI[1], K. SCARFO[2], S. DEHGHAN[1], B. LAKIN[1], A. AHSAN[1], M. MANSOURI[1], D. PAGE[1];
[1]Applied Res. - Neuromodulation, Abbott Labs., Plano, TX; [2]Lifespan, Providence, RI

Abstract: Neuromodulation therapies are a well-established means for treating intractable chronic pain. In the current standard of care, patients and doctors assess whether the trial therapy is a success using a single pain-based survey question given at the end of the trial period. The purpose of this study is to assess whether: a) daily survey questions delivered via a digital platform can provide a better indication of therapy wash-in and wash-out rate, and b) objective sensor data from wearable devices can be used to supplement or replace the information provided by daily survey questions. The ultimate goal of this research is to develop a seamless and objective method for assessing therapy success in pain patients. In this study, chronic pain patients who were scheduled to undergo a trial period for a neuromodulation system were issued an Apple® iPhone and a Fitbit® smart watch. Subjects participated in a total of 4 in-clinic visits (3 study phases): A visit at least 7 days prior to their trial procedure (pre-trial phase), two visits, one at the day of trial implant and one at the end of the trial period (trial explant), and a final in-clinic visit at least 7 days after the end of their trial (post-trial phase). Patients were instructed to wear the smartwatch during the day and at night throughout all three study phases. Patients also responded daily to a series of survey questions (PROMIS-10, NRS, and Patient Pain Reduction) through a custom mobile application on the iPhone. Initial results from study participants demonstrate a between-patient average of 56% decrease in overall pain score during trial as compared to pre-trial and a 9% increase in pain score from trial to post-trial. The initial data plots suggest a multi-day wash-in and wash-out rate and ongoing data collection with a larger sample of patients is anticipated to help further study the observed trend in pain variation. In addition, the between-patient average satisfaction score for walking increased by 20% during trial as compared to pre-trial and further increased by another 17% during post-trial. Initial data from the Fitbit® device suggests trends toward improvement in both step count and sleep during the trial period. In conclusion, ongoing data collection with a larger sample of patients in the coming months is anticipated to help clarify these trends.

Disclosures: S. Kulkarni: A. Employment/Salary (full or part-time):; Abbott Laboratories. **K. Scarfo®:** 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.; Lifespan. **S. Dehghan:** A. Employment/Salary (full or part-time):; Abbott Laboratories. **B. Lakin:** A. Employment/Salary (full or part-time):; Abbott Laboratories. **A. Ahsan:** A. Employment/Salary (full or part-time):; Abbott Laboratories. **M. Mansouri:** A. Employment/Salary (full or part-time):; Abbott Laboratories. **D. Page:** A. Employment/Salary (full or part-time):; Abbott Laboratories.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.06/Z17

Topic: D.02. Somatosensation – Pain

Support: NIH Grant U44NS115111
NIH Grant R00NS119672
Medtronic
Boston Scientific
Abbott
Nevro

Title: Comparing Intraoperative Motor Responses Induced by High Resolution and Commercial Spinal Cord Stimulation Paddle Electrodes

Authors: *A. QUINTERO[1], D. BERWAL[1], I. TELKES[1], M. DIMARIZO[1], T. HARLAND[2], S. PANICCIOLI[3], B. L. MCLAUGHLIN[4], J. DALFINO[5], J. G. PILITSIS[1];
[1]Florida Atlantic Univ., Boca Raton, FL; [2]Albany Med. Col., Albany, NY; [3]NuVasive Clin. Services, San Diego, CA; [4]Micro-Leads, Inc., Somerville, MI; [5]Albany Med. Ctr., Albany, NY

Abstract: Chronic pain is a complex condition that affects about 20% of adults worldwide. Although spinal cord stimulation (SCS) therapy has been successful in treating a variety of chronic pain conditions, the physical constraints of current SCS methods have a limited impact on isolated lower back and distal lower extremity pain. This study aims to explore subject-specific intraoperative motor activity maps with respect to thoracic level motor responses using a high-resolution spinal cord stimulation (HR-SCS) paddle electrode versus commercial paddle electrode at thoracic levels T6-T10. We compared activity maps of HR-SCS paddles and commercial paddles in a cohort of 21 subjects (ranging from 29-78 years of age) with chronic axial low back and/or lower extremity pain. Top and bottom contacts in tripolar configuration were tested in 9 subjects and only bottom contacts were tested in 12 subjects. Epidurally evoked EMG responses at 6 mediolateral sites over the dorsal column were recorded. Stimulation gradually increased with a 0.5mA step size until a motor threshold was reached. 9 muscle groups were investigated per body side; abductor hallicus (AH), adductor magnus (ADD), biceps femoris (BF), gluteus maximus (GLUT), lower abdominals (LAB), quadriceps (QUAD), medial gastrocnemius (MG), tibialis anterior (TA) and upper abdominals (UAB). Our results show

consistent motor activity in the upper and lower abdominals regardless of paddle type. In all other muscle groups, subjects that reached motor thresholds at lower stimulation amplitudes demonstrated greater than 50% change in root mean square value (RMS) with respect to baseline (no stimulation) with HR-SCS and were considered “responders”. There was greater than 2x percentage change in muscle recruitment in ADD, AH, BF, GLUT, MG, QUAD and TA at thoracic levels T6-T8 (4 subjects) with HR-SCS compared to commercial paddle. We did not see any significant trends in age, sex, duration of illness, BMI, paddle location or MRI-based features among responders. Our sample included 14 females and 7 males. Our findings suggest the ability to optimize clinical benefits of current SCS therapy by using HR-SCS to reach dorsal root and dorsal horn targets of the spinal cord given the larger width (14.5mm) and thinner dimensions of the paddle electrode. These results may provide a more selective and therapeutic treatment opportunity for individuals with chronic isolated lower back and distal lower extremity pain compared to current commercially available systems.

Disclosures: **A. Quintero:** None. **D. Berwal:** None. **I. Telkes:** 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. **M. DiMarizo:** None. **T. Harland:** None. **S. Paniccioli:** None. **B.L. McLaughlin:** A. Employment/Salary (full or part-time):; Micro-Leads. **J. Dalfino:** 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.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.07/Z18

Topic: D.02. Somatosensation – Pain

Title: Investigating the Effects of Flotation Restricted Environment Stimulation Therapy on Neural Networks in Chronic Pain Patients via Functional Magnetic Resonance Imaging

Authors: *T. MCGAUGHEY, V. FINOMORE, Jr.;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Chronic lower back pain (CLBP) is one of the leading causes of disability in the United States. Traditional pain treatment paradigms focus primarily on the localized discomfort which stems from physical insult. However, there is an ever-growing body of literature that indicates there is a profound neural pathway that plays a central role in the transition from acute to chronic pain. This transition to chronic pain also comes with several hefty comorbidities like depression, anxiety, and increase risk of suicide. A promising intervention that has shown utility

in reducing anxiety and depression in healthy participants is Flotation Restricted Environment Stimulation Therapy (REST). Flotation REST flotation requires a participant to relax comfortably in a sensory deprivation tank. These tanks are specially designed to reduce auditory, visual, thermal, and tactile stimulation. While the mechanism is unclear on how Flotation REST imposes these positive benefits it induces significant neural network changes. Our central hypothesis is that flotation REST is beneficial for CLBP patients. Eleven participants underwent a resting state functional magnetic resonance imaging (fMRI) session before and after engaging in six flotation REST session. These scans were contrasted against nine control participants who underwent identical fMRI before and after six sessions in a zero-gravity chair, which was in a warm quiet dimly lit room to mimic the conditions of the float tank. All scans were taken on a 3T PRISMA MAGNETOM (Siemens Munich, Germany). Resting state fMRI scans were analyzed via independent component analysis through the CONN toolbox (www.nitrc.org/projects/conn). We found significant differences in resting state connectivity in the somatosensory and default mode networks across conditions in response to Flotation REST. These neural changes coupled with subjective alterations in pain show promise for flotation REST as a potential supplemental treatment for chronic lower back pain and demonstrates the importance of stress management in chronic pain treatment.

Disclosures: **T. McGaughey:** None. **V. Finomore:** A. Employment/Salary (full or part-time):; West Virginia University.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.08/Z19

Topic: D.02. Somatosensation – Pain

Support: The National Research Foundation of Korea (NRF) grant (No. 2021R1I1A3060828)
Basic Research Fund of the Korean Spinal Neurosurgery Society

Title: Assessment of Pain Severity Using Asymmetry of Quantitative Electroencephalography and Phase-Amplitude Coupling

Authors: *M. AHN[1], **D. GWON**[2], S. RYU[3], C. PARK[4], Y. HA[5];
[2]Computer Sci. and Electrical Engin., [1]Handong Global Univ., Pohang, Korea, Republic of; [3]Daejeon Eulji Med. Center, Eulji Univ., Daejeon, Korea, Republic of; [4]Electronics and Telecommunications Res. Inst., Daejeon, Korea, Republic of; [5]Yonsei Univ., Seoul, Korea, Republic of

Abstract: Chronic neuropathic pain (NP), associated with physical and psychological changes, has become a significant problem for people today and requires continuous management.

Currently, clinical monitoring of pain relies on vital signs such as blood pressure and oxygen saturation. Repeated experiences of severe pain induce dendrite changes in the brain, resulting in alterations in the power spectrum and asymmetric hemisphere. This study aims to utilize the asymmetry of quantitative EEG and phase-amplitude coupling (PAC) to perform regression analysis of pain severity. We used a public EEG dataset of 36 NP patients collected over a 5-min resting period. Pain severity was determined based on the actual pain score in the Brief Pain Inventory. The signal was denoised using independent component analysis and re-referenced by a common average reference. The band powers (BP) were calculated from theta (4-8Hz), alpha (8-12Hz), beta (12-30Hz), and gamma (30-50Hz), while the PAC was estimated by the modulation index between the phase frequencies; theta, alpha, low (12-17Hz) and high beta (18-30Hz) and the amplitude frequencies; low (30-50Hz) and high gamma (70-90Hz). Then regression analysis was conducted by linear (LR), 2nd order polynomial (PR), and support vector regression (SVR) on all combinations of features (n=16); the BP (n=8) of Fp1 and Fp2, the asymmetry of the BP (n=4), and the asymmetry of PAC (n=4). The model evaluation was conducted using leave-one subject-out cross-validation, and the adjusted R^2 was calculated. We found that asymmetry features, particularly beta (Fig 1A), gamma (Fig 1B), and low beta to low gamma PAC (Fig 1C) show significant negative correlations with pain scores while none of the BP yielded meaningful results. The adjusted R2 from the regression analysis is depicted in ascending order (Fig 1D). The PR yielded the highest performance with an adjusted R^2 of 0.996, followed by SVR with 0.122, and LR with -0.084. We verified the potential of using frontal EEG alone as biomarker for pain. These findings will be available to healthcare professionals in clinical practice.

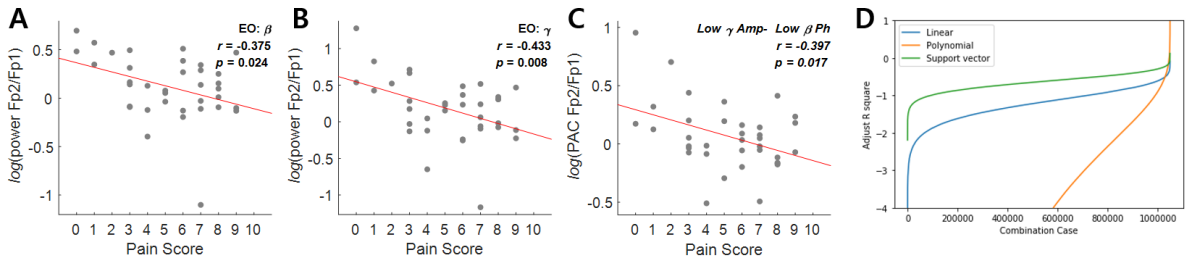


Figure 1. Results of Correlation analysis and Regression analysis.

Disclosures: M. Ahn: None. **D. Gwon:** None. **S. Ryu:** None. **C. Park:** None. **Y. Ha:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.09/Z20

Topic: E.05. Brain-Machine Interface

Support: NIH UH3 HEAL

Title: Quantifying pain location and intensity with multimodal pain body diagrams

Authors: *J. LIN, J. KWONG, R. LERICHE, T. A. WOZNY, A. SHAUGHNESSY, A. SCHMITGEN, P. SHIRVALKAR;
Univ. of California San Francisco, San Francisco, CA

Abstract: Pain is particularly difficult to capture and communicate due to its subjective, multidimensional nature spanning somatosensory, affective, and cognitive processes. Standard pain rating scales such as the numeric rating scale (NRS), visual analog scale (VAS), or McGill pain questionnaire (MPQ) are commonly used to quantify pain. However, these scales face response anchoring bias and often fail to capture complex pain experiences dispersed across body regions. In contrast, pain body diagrams (PBDs) visualize differential areas of pain. Previous versions of this tool were largely qualitative, but here we present a novel method that uses a pressure-hue transformation to visualize and quantify granular pain intensity and location data for 5 patients with chronic pain. Patients were instructed to apply different drawing pressure with a digital stylus on the PBD to output hues ranging from green to blue to red to represent mild to moderate to most painful regions, respectively. Three metrics from PBDs were extracted: (1) PBD mean intensity, which equals the sum of each pixel's hue value divided by the number of colored pixels, (2) PBD coverage, which equals the number of colored pixels divided by the total number of pixels on the body, and (3) PBD sum intensity, which equals the sum of all pixels' hue values. Using correlation analyses (n=609 PBDs), these PBD metrics were shown to be highly concordant with standardized pain metrics, including NRS, VAS, and MPQ. The PBD sum, coverage, and mean were significantly correlated with VAS and NRS scores in four out of five participants (Spearman's correlation $rs = 0.33\text{-}0.72$, $p < 0.004$) and to MPQ scores in three out of five participants (Spearman's correlation $rs = 0.38\text{-}0.53$, $p < 0.004$). Additionally, information theory analyses revealed PBD metrics contain greater entropy compared to the NRS, demonstrating less response anchoring within the PBD method (Tukey's t-test for individual comparisons $p < 0.05$). Furthermore, four out of five participants reported that the method was easy to use and accurately reflected their pain. Thus, PBDs implementing a pressure-hue transformation provide combined spatial and quantitative information that can longitudinally measure and track pain to comprehensively characterize a patient's pain experience.

Disclosures: J. Lin: None. **J. Kwong:** None. **R. Leriche:** None. **T.A. Wozny:** None. **A. Shaughnessy:** None. **A. Schmitgen:** None. **P. Shirvalkar:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.10/Z21

Topic: G.03. Motivation

Support: Department of Defense W81XWH-19-1-0734
Christopher and Dana Reeve Foundation

Title: Understanding the motivation to participate in a spinal cord epidural stimulation study: Perspective from the consumers, individuals with spinal cord injury

Authors: *B. UGILIWENEZA[1,2], E. ALVAREZ[2], A. N. HERRITY[3,2], C. RICH[5], K. BROTHERS[5], S. J. HARKEMA[3,2], C. HUBSCHER[4,2];
[2]Kentucky Spinal Cord Injury Res. Ctr., [3]Neurosurg., [4]Anatom. Sci. & Neurobio., [1]Univ. of Louisville, Louisville, KY; [5]Norton Children's Res. Inst. affiliated with the Univ. of Louisville Sch. of Med., Louisville, KY

Abstract: Spinal cord epidural stimulation (scES), FDA-approved for pain, has been investigated and has shown promises to improve cardiovascular, motor, bowel and bladder function and improve the quality of life in spinal cord injury (SCI). It is an invasive procedure involving an implant in the spinal cord that is still being evaluated for potential treatment. There are a limited number of centers that perform translational studies and participation requires relocation uprooting the individual from their community and a commitment to an intense daily rehabilitation program regiment. The aim of this study was to explore the motivation and expectations driving the decision of people with SCI to participate in a scES study. Semi-structured interviews were performed with 17 individuals with SCI (40±10 years old, 15±10 years since injury, 47% males, all cervical, AIS: 41% A, 35% B, 24% C) who enrolled in a scES study at the Kentucky Spinal Cord Injury Research Center (KSCIRC) at the University of Louisville (UofL). A thematic analysis approach was used within Dedoose Software. The analysis yielded 4 major themes. First and foremost, participants wanted to "Gain more Independence" which some expressed as gaining "*a little bit more freedom"* to *"experience the world a little bit more"*. They were hoping that this would be achieved through "Improvement in Function" with most citing internal autonomic functions such as bowel, bladder, sexual, cardiovascular, and thermoregulation, but also somatic functions such as hands and fingers control, trunk and leg muscle strength, voluntary movement, and standing. Any change in these areas would greatly improve their "Quality of Life". Despite the hope for improved outcomes, many participants expressed having managed expectations, acknowledging that individual unique attributes may lead to different benefits. Though participants had been told not to expect any change in function, they enrolled in the study because they believed that scES could induce *some* positive change and, *any* improvement was worth the study requirements. They also believed that even if there was no change at the end of the study, it was still important to participate for "Benevolence" to contribute to the advancement of research in the field of SCI and potentially help other people in the future. In summary, people with SCI participated in this scES research study with a cautious hope to improve their quality of life through improved function, but most importantly, they wanted to at least play a part in scientific breakthroughs aimed at helping the next person with spinal cord injury live an easier life.

Disclosures: B. Ugiliweneza: None. **E. Alvarez:** None. **A.N. Herrity:** None. **C. Rich:** None. **K. Brothers:** None. **S.J. Harkema:** None. **C. Hubscher:** None.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.11/Z22

Topic: D.02. Somatosensation – Pain

Support: NIH/NCCIH R01AT008563

Title: Modulation effects of transcranial direct current stimulation on frontal parietal network and its association with the placebo effect in healthy and cLBP cohorts

Authors: *V. SACCA, A. URSITTI, S. HODGES, N. TODOROVA, M. ZHU, S. REDDY, J. KONG;
Psychiatry, Massachusetts Gen. Hosp., Boston, MA

Abstract: Purpose: The aims of this study were to investigate: (i) how multi-session transcranial direct current stimulation (tDCS) at the right dorsal lateral prefrontal cortex (rDLPFC) can modulate the functional connectivity (FC) of the Frontoparietal network (FPN); and (ii) the association of the FPN connectivity changes with placebo analgesia in two independent cohorts: healthy controls and chronic low back pain (cLBP) subjects.
Methods: 81 healthy subjects were recruited and randomized into one of the three groups: (i) anodal at the rDLPFC and cathodal at the left orbitofrontal cortex (lOFC); (ii) cathodal at the rDLPFC and anodal at the lOFC; (iii) sham tDCS. 42 subjects were recruited and randomized in anodal and sham tDCS in the cLBP cohort. For both the cohorts, tDCS was applied at 2 mA for 20 min using the StarStim system for three consecutive days. Resting state fMRI scans were acquired pre- and post-tDCS on the first and third day. An expectancy manipulation model was applied to induce positive expectations using two inert creams applied on the right forearm, labeled as: lidocaine for inducing expectations of pain decrease, and neutral as a control. CONN toolbox was used for functional connectivity analysis. FPN was extracted using Independent Component Analysis (ICA). ANCOVA with age and gender as covariates was applied for group analysis.
Results: We found that anodal tDCS led to a non-significant increase of the placebo effect in comparison to sham tDCS in both healthy subjects and cLBP patients (cLBP cohort: $p= 0.19$; healthy cohort: $p= 0.68$). The effect sizes in the two cohorts were similar (Cohens $d= 0.39$ in cLBP participants; Cohens $d= 0.21$ in healthy subjects).ICA analysis showed that in comparison with sham tDCS, anodal tDCS led to an increased FC between the FPN and right operculum in the healthy subjects, while in the cLBP participants, anodal tDCS was associated with increased FC between the FPN and right precentral gyrus. Pearson partial correlation with age and gender as covariates showed that the FPN and right precentral gyrus increased FC was significantly correlated to the placebo effect ($r= 0.4$, $p< 0.001$) in the cLBP patients.
Conclusion: Our findings suggest that repeated anodal tDCS at DLPFC may be able to increase the placebo effect in healthy and cLBP populations and modulate the FC of the FPN.
AcknowledgeFunding: This study is funded by NIH/NCCIH R01AT008563.

Disclosures: **V. Sacca:** None. **A. Ursitti:** None. **S. Hodges:** None. **N. Todorova:** None. **M. Zhu:** None. **S. Reddy:** None. **J. Kong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JK holds equity in two startup companies (MNT, BTT) and a patent on applying neuromodulation, but declares no conflict of interest.

Poster

PSTR474. Central Mechanisms of Persistent Pain in Humans: Neuroimaging and Treatment

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR474.12/Z23

Topic: D.02. Somatosensation – Pain

Support: NCCIH/NIH R01 AT007176 to D.A. Seminowicz
Queen Elizabeth II Graduate Scholarship in Science and Technology

Title: Functional and structural magnetic resonance imaging of migraine and treatment outcomes

Authors: *C. L. CHEUNG[1], B. W. STEWART[4], S. S. MUNDH[2], D. A. SEMINOWICZ[3]; [1]Neurosci., [2]Schulich Sch. of Med. & Dent., [3]Med. Biophysics, Univ. of Western Ontario, London, ON, Canada; [4]Neural and Pain Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Migraine is both common and costly, with migraine patients also demonstrating functional brain abnormalities. However, insufficient relief and unpleasant side effects from migraine medications fuel a need for non-pharmacological interventions. Our lab conducted a randomized controlled trial on migraine patients and found meditation reduces headache days, but did not compare treatment responders (patients experiencing ≥ 50% headache day reduction) to non-responders. Therefore, this study will use the aforementioned trial data to determine if treatment response reduces migraine symptoms and restores normal brain function. We analyzed clinical data (headache frequency, headache intensity, headache disability, sleep quality, depression, and anxiety) and evoked pain and cognitive task fMRI data from male and female migraine patients randomized to either meditation (n = 50) or educational control (n = 48). Clinical outcomes were compared using a linear mixed effects model across four timepoints: baseline, 10 weeks (mid-intervention), 20 weeks (post-intervention), and 52 weeks (1 year follow-up). We found significantly reduced headache intensity at week 20 compared to baseline, with responders experiencing a significantly greater reduction than non-responders. We also found significantly reduced headache disability at week 10, week 20, and 1 year compared to baseline, with responders experiencing a significantly greater reduction than non-responders at week 20. Similarly, headache frequency was significantly reduced at week 10, week 20, and 1 year compared to baseline, with responders at week 20 and 1 year experiencing a significantly greater reduction than non-responders. In addition, when comparing a difficult cognitive task relative to an easy cognitive task, the left insula showed significantly decreased activity in responders at week 20 compared to baseline compared to non-responders. These results suggest

both meditation and treatment response decrease migraine symptoms, and that these reductions may be associated with altered cognitive-related activity in the posterior insula. Ultimately, our findings further our understanding of pain and cognitive interactions in migraine patients and more generally in acute and chronic pain.

Disclosures: C.L. Cheung: None. **B.W. Stewart:** None. **S.S. Mundh:** None. **D.A. Seminowicz:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.01/Z24

Topic: D.02. Somatosensation – Pain

Support: NIH NINDS 3R35NS105076-06S1

Title: Lipidated peptide targeted to Na_v/Ankryin interface attenuates pain-like behaviors in SNI model

Authors: *R. G. D. POWELL, II[1], J. SCHNEIDER[2], X. ZENG[1], C. J. WOOLF[1];
[1]Neurobio., Boston Children's Hosp., Boston, MA; [2]Bunker Hill Community Col., Bosotn, MA

Abstract: Neuropathic pain remains one of the most difficult disease states to adequately treat. Although advances have been made in understanding the pathophysiology underlying neuropathic pain, current first-line treatment options fail to offer lasting relief with minimal adverse effects. Nociceptive dorsal root ganglion (DRG) neurons remain a key contributor to neuropathic pain states due to plasticity in their signaling during injury. Following direct injury, DRG neurons can employ allostatic mechanisms that are crucial for proper repair, however in some cases, these neurons fail to fully revert to a relatively quiescent phenotype, resulting in neuropathy. The subsequent pain can be characterized by spontaneous pain, increased nociception to painful stimuli (hyperalgesia), and nociception when presented with a previously non-painful stimulus (allodynia). To circumvent the disconnect between the pathophysiology of neuropathy and pain treatment, we reevaluated neuropathic pain as a dysfunction of ion channel stabilization in peripheral neurons. Ankyrin-G is a scaffolding protein that facilitates interactions between cytoskeletal elements and membrane bound proteins; namely voltage gated sodium (Na_v) and potassium (K_v) channels, at the Nodes of Ranvier. We hypothesize that the interactions between Ankyrin-G and Na_v channels may hold potential as a unique target for pharmacological manipulation. Herein we describe the consequence of interrupting Na_v/Ankyrin-G interactions with a membrane-delimited peptide *in vitro* and *in vivo*. In cultured embryonic mouse DRGs the peptide was able to reduce sodium currents, action potential height, action potential number, and calcium influx in protein kinase A stimulated conditions. In the spared-nerve Injury model of neuropathic pain, the peptide was unilaterally injected into the ipsilateral hind paw of animals that underwent SNI. Following injection, the peptide precipitated a partial rescue of mechanical

thresholds in male and female mice after a single injection. This rescue was observed for 7 days following injection, at which point the peptide group was indistinguishable from the control group. Additionally, animals that received the peptide spent less time exhibiting pain-like behaviors in the acetone test and spent more time in colder and warmer areas in the thermal gradient ring when compared to scrambled controls. Altogether, these data suggest that the interaction between Na_v channels and Ankyrin-G is a contributing factor to the pathophysiology of neuropathic pain, and locally interrupting this interaction is sufficient in rescuing pain-like behaviors in mouse models of neuropathic pain.

Disclosures: R.G.D. Powell: None. **J. Schneider:** None. **X. Zeng:** None. **C.J. Woolf:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.02/Z25

Topic: D.02. Somatosensation – Pain

Title: 10khz scs restores mechanical and thermal processing in a rodent model of painful diabetic neuropathy

Authors: *K. LEE[1], D. LEE[2], D. WANG[1], Z. KAGAN[2], K. BRADLEY[2];
[1]Nevro Corp, Redwood City, CA; [2]nevro corp, Redwood City, CA

Abstract: A recent randomized controlled trial of 10 kHz spinal cord stimulation (SCS) in patients with painful diabetic neuropathy (PDN) has demonstrated significant and durable improvements in neurologic status, particularly sensory restoration of the foot (Petersen et al 2021, Argoff et al 2023). In order to gain further mechanistic insight into the improvements in sensory processing by 10kHz SCS, we analyzed the receptive field (RF) size and responses of spinal dorsal horn (DH) neurons to mechanical and thermal stimuli of the paw in a PDN rat model.For PDN-model, Rats were given a single effective dose of streptozotocin (STZ), while Naïve animals were injected with 0.9% saline. Four groups were studied: Naïve, STZ, STZ+ShamSCS, and STZ+10kHzSCS. Rats were anesthetized with urethane and a laminectomy was performed for DH neurons recording. We analyzed RF size and the response of DH neurons to vibrotactile stimulation and thermal sensation.Both the Naïve and STZ+10kHzSCS groups had significantly smaller RFs than the STZ Control and STZ+ShamSCS groups. In response to a 1 Hz vibration frequency, Naïve and STZ+10kHzSCS groups had higher peak power spectral density (PSD) of DH firing than STZ+ShamSCS, and PSD for the STZ+10kHzSCS group was statistically higher than for the STZ Control group. Response of DH neurons showed no significant differences between groups during the thermal stimulation, but the post-stimulus firing rates for the STZ+10kHzSCS group were significantly higher compared to STZ Control and STZ+ShamSCS.These data suggest that 10 kHz SCS amplified the DH response to large fiber (vibration) and small fiber (warm) signals and normalized the receptive field of the paw (acuity) in PDN rats. In diabetes, where peripheral sensory compromise from chronic

hyperglycemia can lead to loss of protective sensation, such 'tuned amplification' as provided by 10 kHz SCS in the central nervous system may compensate for the reduced functionality seen in the peripheral sensory systems.

Disclosures: **K. Lee:** A. Employment/Salary (full or part-time):; NEVRO CORP. **D. Lee:** A. Employment/Salary (full or part-time):; NEVRO CORP. **D. Wang:** A. Employment/Salary (full or part-time):; NEVRO CORP. **Z. Kagan:** A. Employment/Salary (full or part-time):; NEVRO CORP. **K. Bradley:** A. Employment/Salary (full or part-time):; NEVRO CORP.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.03/Z26

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS R01 NS109541
NIH/NINDS R61 NS123753

Title: Activation of beta-3 adrenergic receptors with the selective agonist mirabegron elicits multi-site mechanical hypersensitivity, grimace, and increased IL-6 levels in mice

Authors: *J. RICANO[1], J. CHEN[1], Y. WANG[1], A. G. NACKLEY[1,2];
[1]Anesthesiol., [2]Pharmacol. and Cancer Biol., Duke Univ. Sch. of Med., Durham, NC

Abstract: Mirabegron is an adrenergic receptor beta-3 (Adrb3) selective agonist that is currently FDA-approved for the treatment of overactive bladder, with common side effects including headache and multi-site body pain. Prior work in our lab has demonstrated that catecholamine activation of Adrb3 leads to chronic primary pain (CPP) through stimulating increased secretion of the proinflammatory cytokine interleukin-6 (IL-6) from adipocytes. The development of pain is blocked by pharmacologic inhibition or genetic knockdown of Adrb3. In this current study, we sought to evaluate the effects of the Adrb3 selective agonist mirabegron on evoked and spontaneous measures of pain as well as IL-6 production. We administered 2mg/kg of mirabegron or vehicle to C57BL/6 wild-type (n=12) or adipocyte Adrb3 conditionally knocked out (CKO) (n=12) mice. The mice were age-matched, with an equal number of males and females in each group. We measured mechanical allodynia and hyperalgesia 1, 3, 6, 24, 48, 72, and 96 hours after injection using von Frey filaments. Grimace was measured 24 hours after injection and analyzed using PainFace, an automated Mouse Grimace Scale. The operator was blinded to the conditions of the mice. A single dose of mirabegron produced mechanical allodynia at plantar sites, with wild-type mice experiencing mechanical allodynia as soon as 1 hour ($p<0.0001$) which persisted for 96 hours ($p=0.006$). Mirabegron also produced mechanical allodynia at abdominal and back sites, peaking at earlier 1-6 hour time points. Female, but not male, Adrb3 CKO failed to develop plantar allodynia ($p=0.0002$) and Adrb3 CKO exhibited delayed abdominal allodynia. There was a sharp increase in the Mouse Grimace Score of wild-

type mice that was not observed in Adrb3 CKO mice, with no significant differences between males and females. Treatment of mouse differentiated adipocytes with mirabegron also induced Adrb3-dependent increases in IL-6 levels. These results further support a role for Adrb3 in the onset of multi-site body pain and suggest that Adrb3 antagonists may be an effective therapeutic to alleviate CPP conditions. Our preliminary data also suggest that painful mirabegron-induced side-effects observed clinically may be mediated by adipocyte Adrb3 and its downstream effectors (e.g., IL-6).

Disclosures: **J. Ricano:** None. **J. Chen:** None. **Y. Wang:** None. **A.G. Nackley:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.04/Z27

Topic: D.02. Somatosensation – Pain

Support: NRF-2021R1C1C2005440
NRF-2020M3E5D9079744
NRF-2023R1A2C3002798

Title: Astrocytic GABA-mediated tonic excitation exacerbates pain signaling

Authors: *J. CHO[1], Y. JU[2], J. PARK[1], H. KIM[2], E.-B. HONG[2], C. LEE[3], H.-I. KIM[1], M.-H. NAM[2];
[1]Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Inst. for Basic Sci., Daejeon, Korea, Republic of

Abstract: Neuropathic pain is a debilitating condition characterized by mechanical allodynia, caused by the hyperexcitability of spinal dorsal horn neurons after nerve injury. Accumulating evidence supports that the loss of GABA-mediated inhibition, due to decreased expression of the K+/Cl- co-transporter (KCC2), leads to disinhibition of spinal neurons, ultimately resulting in pain hypersensitivity. However, the molecular mechanisms underlying the involvement of astrocytic GABA in hyperexcitation of spinal neurons remain poorly understood. In this study, we provide the evidence that tonic excitation by astrocytic GABA exacerbates pain signaling in a nerve ligation model. We demonstrated that reactive astrocytes tonically release an excessive amount of GABA, which paradoxically excites the neighboring dorsal and ventral horn neurons, leading to mechanical allodynia. This excitatory effect is attributed to changes in the depolarized reversal potential of GABA (E_{GABA}), which are caused by downregulation of KCC2 expression in spinal neurons. Moreover, 18F-FDG-microPET imaging revealed increased regional glucose metabolism in both the dorsal and ventral horns, indicating neuronal hyperexcitability. Notably, we also demonstrated that pharmacological blockade of astrocytic GABA synthesis through intrathecal administration of an MAOB inhibitor effectively alleviates the mechanical allodynia and restores glucose metabolism in the spinal cord. Collectively, our findings suggest that

modulating astrocytic GABA synthesis can be a promising therapeutic strategy for alleviating neuropathic pain.

Disclosures: **J. Cho:** None. **Y. Ju:** None. **J. Park:** None. **H. Kim:** None. **E. Hong:** None. **C. Lee:** None. **H. Kim:** None. **M. Nam:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.05/Z28

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS R03NS106166
NIH/NINDS R01NS109541

Title: Adipose-specific overexpression of miR-133a-3p reverses mechanical hypersensitivity in a rodent model of chronic primary pain conditions

Authors: *N. HERNANDEZ[1], J. CHEN[1], X. ZHANG[1], Y. WANG[1], B. P. CISZEK[2], M. KANKE[3], M. E. KLEIN[1], P. SETHUPATHY[3], A. G. NACKLEY[1];
[1]Anesthesiology, Ctr. for Translational Pain Medicine, Duke Univ. Sch. of Med., Duke Univ., Durham, NC; [2]WhiteCap Inst., Heber City, UT; [3]Biomed. Sci., Cornell, Ithaca, NY

Abstract: Chronic primary pain conditions (CPPCs), such as fibromyalgia and vestibulodynia affect >100 million Americans. The origin of CPPCs is linked to low activity of the catechol-O-methyltransferase (COMT) enzyme and corresponding increases in circulating levels of catecholamines that drive nociception and pain via activation of peripheral beta-adrenergic receptors (βARs). Here, we sought to identify pain-relevant miRNAs downstream of βAR activation and determine their functional effects in a 'high catecholamine' rodent model of CPPCs. In a cohort of female rats, separate groups received peripheral delivery of the β2- and β3AR antagonists ICI-118,511 (1.5mg/kg/day) and SR59230A (1.67 mg/kg/day) alongside sustained, systemic delivery of the COMT inhibitor OR486 (15mg/kg/day) or vehicle over 14 days (N=20). Results from RNA sequencing reveal that rats treated with OR486 exhibited decreased levels of miR-133a in circulation compared to controls, and this downregulation was prevented by peripheral delivery of ICI-118,511 + SR59230A. This genetic signature was replicated in cohorts of female and male mice with acute (N=32) and sustained COMT inhibition (N=23) as well as in a human cohort of vestibulodynia patients (N=75). To determine possible sources of miR-133a downregulation, we used qPCR to measure expression in peripheral (adipose, muscle) and central (spinal cord) tissues collected from the same CPPC mice used for plasma quantification. We found that miR-133a was downregulated in adipose at both time points, supporting our previous findings of a peripheral site of pain onset and maintenance. In the spinal cord, there was differential expression only at the 14-day time point, suggesting miRNA trafficking between adipose and the central nervous system. We then induced adipose-specific

overexpression of miR-133a in our CPPC mice to directly test its role in modulating pain. Compared to CPPC mice receiving a nonsense sequence, those treated with adipose-specific miR-133a exhibit significant increases in mechanical pain thresholds at multiple body sites, indicative of widespread analgesia (N=51). Further, miR-133a overexpression in primary dorsal root ganglion cultures had anti-nociceptive effects on capsaicin-induced phosphorylation of extracellular signal-regulated kinase (N=12) and capsaicin-induced calcium responses (N=32). These data provide the first link between miR-133a and CPPCs across species and support the use of peripherally-targeted miR-133a overexpression strategies for the treatment of CPPCs.

Disclosures: **N. Hernandez:** None. **J. Chen:** None. **X. Zhang:** None. **Y. Wang:** None. **B.P. Ciszek:** None. **M. Kanke:** None. **M.E. Klein:** None. **P. Sethupathy:** None. **A.G. Nackley:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.06/AA1

Topic: D.02. Somatosensation – Pain

Support: NIH Grant U18 EB029257

Title: Dual frequency spinal cord stimulation as a treatment for chronic pain robust to pain state and electrode lead migration

Authors: *K. LAMBERT[1], T. ZHANG[4], J. GILBERT[2], M. MOFFITT[5], W. M. GRILL[3]; [2]Duke Univ., [3]Biomed. Engin., [1]Duke Univ., Durham, NC; [4]Boston Scientific Neuromodulation, Boston Scientific Neuromodulation, Valencia, CA; [5]Boston Scientific, Valencia, CA

Abstract: Spinal cord stimulation (SCS) is an effective therapy for chronic pain, and there are tens of thousands of new patients implanted each year. While traditional SCS delivers tonic stimulation to achieve pain relief in ~60% of patients, more recent studies suggest improvements in efficacy with non-regular temporal patterns of stimulation. Using a validated biophysical model of the dorsal horn circuit, we designed and tested an optimized temporal pattern of SCS - termed dual-frequency SCS (dfSCS). In dfSCS, we target the dorsal column axons arising from pain center and surround sensory receptive fields with two different frequencies of tonic SCS. We searched for frequency pairs between 0 and 70 Hz that outperformed single frequency SCS (sfSCS) at the higher, lower, or average of the two dfSCS component frequencies, or a standard of 50 Hz. We chose pairs that exhibited robust performance across a range of distributions of center or surround dorsal column axons stimulated and across progressive pain conditions, represented as a loss of GABAergic and glycinergic inhibition. We tested dfSCS by implanting spared nerve injured (SNI) Sprague-Dawley rats (n=14) with paddle electrodes targeting the T12-T13 vertebral level. We verified our placement post-implantation and found that electrode locations were grouped into 3 placements: all contacts over T12, spanning T12-T13, and all over

T13. We measured paw withdrawal thresholds (PWT) during sham, dfSCS, and sfSCS at the higher dfSCS frequency, with increased thresholds relative to sham indicating less hypersensitivity and more effective stimulation. Responses to SCS were dependent upon final electrode position. Stimulation at T12/T13 or T13 increased PWT relative to sham, while stimulation at T12 mostly reduced PWT relative to sham. Across electrode locations, dfSCS decreased the number of rats whose PWTs worsened relative to sham by at least 30% (termed negative responder, n=2 vs n=6), and increased the number of positive responders (>30% increase relative to sham) compared to sfSCS (n=6 vs n=1). To account for pain progression, we also grouped rats by their pre-stimulation reduction in PWT, relative to pre-SNI baseline. Rats with <50% reduction in PWT were classified as being in mild pain, 50-70% reduction as in moderate pain, and >70% reduction as in severe pain. As predicted, across all pain states, dfSCS decreased the number of negative responders and/or increased the number of positive responders relative to sfSCS, indicating the potential of dfSCS to increase the percentage of patients that respond to therapy. Overall, dfSCS shows promise in alleviating evoked and spontaneous chronic pain more effectively than sfSCS.

Disclosures: K. Lambert: None. **T. Zhang:** A. Employment/Salary (full or part-time):; Boston Scientific. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on licensed patents on temporal patterns of spinal cord stimulation and receives royalty distributions from Duke University. **J. Gilbert:** A. Employment/Salary (full or part-time):; SPR Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on licensed patents on temporal patterns of spinal cord stimulation and receives royalty distributions from Duke University. **M. Moffitt:** A. Employment/Salary (full or part-time):; Boston Scientific. **W.M. Grill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on licensed patents on temporal patterns of spinal cord stimulation and receives royalty distributions from Duke University.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.07/AA2

Topic: D.02. Somatosensation – Pain

Support: Pronex Contract 014/2017; Protocol 46843.484.37488.23052016
Scholarship CAPES Finance Code 001

Title: Aspirin-triggered Lipoxin A4 Demonstrates Antinociceptive, Anxiolytic-like Effects, and Enhances Antinociception of CB1 and CB2 Receptor Agonists in Diabetic Rats

Authors: *M. V. FERREIRA[1], C. H. A. JESUS[2], J. P. B. COSTA[1], G. O. GUILHERME[1], B. LIEBL[1], W. VERRI, Jr.[3], J. M. ZANOVELI[1], J. M. CUNHA[1];

[1]Pharmacol., Univ. Federal do Paraná, Curitiba, Brazil; [2]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [3]Pathology, Univ. Estadual de Londrina, Londrina, Brazil

Abstract: Neuropathy is a prevalent complication of diabetes, causing spontaneous pain, hyperalgesia, and allodynia. Additionally, diabetes is associated with a higher likelihood of psychiatric disorders like depression and anxiety. Current pharmacological approaches may be ineffective in treating these complications, leading to the search for alternative therapies. Aspirin-triggered lipoxin A4 (ATL), a specialized pro-resolving mediator, exhibits various biological effects, including antinociception and modulation of type 1 cannabinoid receptors. This study aims to investigate the effect of ATL on the pain, depression, and anxiety associated with experimental diabetes and the effect of co-treatment ATL plus cannabinoid receptor agonists on mechanical allodynia. Diabetes was induced in adult male Wistar rats by streptozotocin (STZ; 60mg/kg; i.p.). ATL (0.3, 1, 3, 10, or 30 ng/rat; i.p.) or vehicle (VEH) treatment began 14 days after STZ and lasted until the 4th week. Mechanical allodynia was assessed by electronic Von Frey test (VFT) one day before STZ (baseline), and again at different time points after ATL treatment (alone or combined with intrathecal CB1 or CB2 receptor agonists; ACEA or JWH-133, respectively). In the 4th week after STZ, the open-field test (OFT), elevated plus-maze (EPM), and modified forced swimming test (MFST) were conducted.The experimenter was blind to the treatments, but not to the diabetic condition of the rats. All experimental protocols were approved by Institutional Ethics on Animal Experimentation (CEUA-BIO-UFPR #1418). When compared to NGL-VEH rats, DBT-VEH animals developed: 1) a reduction of the mechanical threshold on the VFT, peaking at the 27th day; 2) a reduction in the number of crossings in the OFT (29%); 3) a reduction in the time and entries on open arms in the EPM test (66% and 71%, respectively); 4) an increase in immobility time (25%), and also a reduction on swimming mean counts (83%) in MFST. Treatment with ATL (1 to 30 ng) reverses the mechanical allodynia of the DBT rats (18, 16, 12, and 21%, respectively). ATL treatment did not change the number of total crossings in the OFT. In the EPM test, ATL (10 ng) was able to increase entries (50%) and time (60%) on open arms. ATL was not able to change the parameters on the MFST. ATL treatment (3 ng) increased the antinociceptive effect of ACEA or JWH-133 (30 µg) in DBT rats (34 and 39%, respectively). These findings suggest that ATL has the potential in alleviating mechanical allodynia and anxious-like behavior associated with experimental diabetes. Although not fully explored in this study, our data indicate that ATL may directly or indirectly interact with both cannabinoid receptors.

Disclosures: M.V. Ferreira: None. **C.H.A. Jesus:** None. **J.P.B. Costa:** None. **G.O. Guilherme:** None. **B. Liebl:** None. **W. Verri:** None. **J.M. Zanoveli:** None. **J.M. Cunha:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.08/AA3

Topic: D.02. Somatosensation – Pain

Support: R21AG075419

Title: Spatially restricted delivery of AAVs to reduce nociceptor firing as a treatment for chronic pain

Authors: *L. SUN[1], G. CHAHYADINATA[2], B. JOHNSTON[4], J. NAM[4], A. BATTENBERG[4], S. BAZAREK[4], W. DING[4], L. YANG[4], D. M. DUBREUIL[3], A. H. HELD[2], M. ADLER[3], J. BROWN[4], K. EBERLIN[4], S. SHEN[4], B. WAINGER[5];
[1]Massachusetts Gen. Hosp., Cambridge, MA; [2]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [3]Neurol., Massachusetts Gen. Hosp., Boston, MA; [4]Mass Gen. Brigham, Boston, MA; [5]Mass. Gen. Hospital/Harvard Med. Sch., Boston, MA

Abstract: Chronic pain affects one quarter of adults, a third with substantial impairment of function, and costs over $500 billion annually in the US. Existing treatments have poor efficacy and limited therapeutic index. Additionally, the lack of effective and safe pain treatments has driven the opioid epidemic. There is strong human genetic evidence that reducing the excitability of the first-order pain-sensing neurons, termed nociceptors, will treat pain. Homozygous knockout of the NaV1.7 voltage-gated sodium channel, which is expressed predominantly in nociceptors, yields the rare syndrome of congenital insensitivity to pain. In contrast, gain of function mutations in the same channel cause severe pain syndromes including familial erythromelalgia. A large group of preclinical studies in mouse models also support the strategy of decreasing nociceptor excitability to treat pain. Instead of targeting sodium channels, we have developed a strategy based on the focal delivery of potassium channels using adeno-associated virus (AAV) vectors. This strategy is also supported by human genetic evidence, in that activating mutations of Kv7 channels are disease-modifying for patients with familial erythromelalgia. Potassium channel haplotypes have also been shown to mitigate labor-associated pain. To support the efficacy of this strategy, we perform a series of physiological and behavioral measurements in rodents. For physiological readouts, we use high-content optical imaging that combines optogenetic activation with a red-shifted calcium indicator readout in individual nociceptors. Using mice that express channelrhodopsin under the TrpV1 promoter, we demonstrate that in vivo injection of AAV-potassium channels yields a reduction in optical rheobase, the light threshold for nociceptor calcium flux activation, after the dorsal root ganglia are harvested and dissociated for in vitro analysis. Using several standard pain behavioral models, we show that AAV-potassium channels yield marked reduction in pain phenotypes compared to AAV-GFP controls. Our results validate the overexpression of potassium channels as a promising alternative for pain treatment and support the use of spatial restriction to achieve precision in AAV-mediated gene therapies. Our results show a promising methodology for spatially specific treatment of chronic pain and support for an exciting alternative to current pain treatments.

Disclosures: L. Sun: None. **G. Chahyadinata:** None. **B. Johnston:** None. **J. Nam:** None. **A. Battenberg:** None. **S. Bazarek:** None. **W. Ding:** None. **L. Yang:** None. **D.M. Dubreuil:** None. **A.H. Held:** None. **M. Adler:** None. **J. Brown:** None. **K. Eberlin:** None. **S. Shen:** None. **B. Wainger:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.09/AA4

Topic: D.02. Somatosensation – Pain

Support: DOD W81 XWH-20-10911
NIH R01 NS117340
VA I01 RX001475

Title: Alpha-7 Nicotinic Acetylcholine Receptors Regulate Nociceptive Sensitization and Immune System Activation after Fracture in Mice

Authors: *W. LI[1,2], X. SHI[1,2], T. WEI[3], T. GUO[3], W. S. KINGERY[3], D. J. CLARK[1,2];
[1]Dept. of Anesthesiology, Perioperative and Pain Med., Stanford Univ., Palo Alto, CA; [2]Anesthesiol. Service, Veterans Affairs Palo Alto Hlth. Care Syst., Palo Alto, CA; [3]Palo Alto Veterans Inst. for Res., Palo Alto, CA

Abstract: Background Both autonomic nervous system dysfunction and immune system activation are characteristic of chronic pain after limb injuries. Administration of the cholinergic agonist nicotine provides analgesia and limits immune system activation but has serious acute and chronic side effects; selective alpha-7 nicotinic acetylcholine receptor α7nAChR agonists with improved side effect profiles are now available. We hypothesized that α7nAChR administration would reduce nociceptive and immune changes after limb injury. **Methods** These experiments employed a well-characterized tibia fracture model of chronic post-traumatic limb pain in male C57BL/6 mice and a highly selective α7nAChR agonist, PNU-282987. Fracture mice were treated with vehicle or PNU-282987 at either a low (0.2mg/kg) or high (1mg/kg) dose once daily for 4 weeks. Hindpaw allodynia and hindlimb weight bearing were assessed at baseline and at 3 and 4 weeks after fracture. The assessment of adaptive immune responses included regional lymph node hypertrophy, germinal center formation, α7nAChR expression and autoreactive IgM production. Innate immune system activation focused on IL-1β and IL-6 generation in the skin of the fractured limb. **Results** Tibia fracture followed by cast immobilization resulted in both mechanical allodynia and hindpaw unweighting at both 3 and 4 weeks after tibial fracture. Analysis of popliteal lymph nodes demonstrated both hypertrophy and germinal center formation, which were sharply limited by PNU-282987 administration. Immunohistochemical studies showed that most α7nAChR protein was located on follicular B cells, and that expression was increased at the mRNA and protein levels after fracture. Analysis of hindpaw skin revealed a greater than 2-fold increase in IgM content in the fractured limbs which was abrogated by PNU-282987 administration as were the levels of both IL-1β and IL-6. Serum analyses demonstrated increased IgM-mediated reactivity against keratin 16, histone 3.2, GFAP, and NMDAR-β2 all of which were normalized in PNU-282987 treated mice. **Conclusions** Collectively, these data suggest that α7nAChR is involved in regulating nociception as well as the underlyng adaptive and innate immune responses in mice after tibial fracture. These novel findings suggest that well-tolerated α7nAChR agonists may be viable analgesics for the control of chronic pain after limb injuries.

Disclosures: **W. Li:** None. **X. Shi:** None. **T. Wei:** None. **T. Guo:** None. **W.S. Kingery:** None. **D.J. Clark:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.10/AA5

Topic: D.02. Somatosensation – Pain

Support: DoD grant 8W81XWH2110755
DoD grant W81XWH2110756

Title: Intravenous administration of MMP-9 monoclonal antibody relived diabetic neuropathic pain and improved mitochondrial function and neuropathy.

Authors: *Y. MATSUOKA[1,2], J. XU[2], K. LEE[4], X. GE[4], R.-R. JI[2,3];
[1]Anesthesiol., Rakuwakai Marutamachi Hosp., Kyoto, Japan; [2]Anesthesiol., [3]Neurobio., Duke Univ. Med. Ctr., Durham, NC; [4]The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Our previous study has shown that matrix metalloproteinase 9 (MMP-9) plays a central role in the development of neuropathic pain after nerve injury by regulating glial activation and neuroinflammation (Kawasaki et al, 2008). Clinical trials using small molecule inhibitors of MMPs have failed due to the lack of specificity and toxicity. By using functional selection, we developed a monoclonal antibody (mAb) that can effectively alleviate chemotherapy-induced neuropathic pain following intravenous injection (Lopez et al., 2019). Diabetes-induced peripheral neuropathy (DPN) may develop in 50% of diabetic patients and there is a lack of effective treatment for painful DPN. In this study, we tested the effects of the MMP-9 mAb in mouse models of DPN. DPN was induced by intraperitoneal injection of 150 mg/kg of streptozotocin (STZ) in CD1 mice, and MMP-9 mAb was administrated intravenously (IV) three times every other day 1 week after STZ injection. Mechanical and cold pain was assessed in von Frey filament and acetone tests. Seahorse XF analyser was used for mitochondrial respiration measurements.The results showed that IV injections of MMP-9 mAb significantly reduced mechanical allodynia, by increasing paw withdrawal threshold. We also observed accumulating effect of analgesia after the third injection. Mitochondrial function in the sciatic nerve was impaired in STZ mice, indicated by lowering basal respiration, ATP-linked respiration and maximal respiration. However, these deficits were significantly recovered by the treatment of MMP-9 mAb. STZ also resulted in a profound loss of intraepidermal neuronal fibers, which was also improved by the treatment of MMP-9 mAb. Our findings suggest that MMP-9 monoclonal antibody is not only highly effective in alleviating diabetic neuropathic pain but also can improve mitochondrial function and neuropathy (loss of epidermal nerve fibers) in diabetic animals.

Disclosures: **Y. Matsuoka:** None. **J. Xu:** None. **K. Lee:** None. **X. Ge:** None. **R. Ji:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.11/AA6

Topic: D.02. Somatosensation – Pain

Support: CIHR Banting Fellowship Grant 471896

Title: Cannabinol selectively functionally inhibits threshold sodium currents and reduces excitability in neurons of the dorsal root ganglion

Authors: *M.-R. GHOVANLOO[1], P. R. EFFRAIM[2], S. TYAGI[3], P. ZHAO[4], S. D. DIB-HAJJ[5], S. G. WAXMAN[4];
[1]Yale Univ., West Haven, CT; [2]Anesthesiol., Yale Univ., New Haven, CT; [3]Neurol., Yale Univ., West Haven, CT; [4]Neurol., Yale Univ., New Haven, CT; [5]Yale Sch. of Med. and VAMC, Yale Sch. of Med. and VAMC, West Haven, CT

Abstract: Cannabinol (CBN), a metabolite associated with Δ9-tetrahydrocannabinol that is not fully understood, has been proposed as a pain reliever. CBN interacts with endocannabinoid (CB) receptors, but it also affects non-CB targets such as different ion channels. We examined the impact of CBN on voltage-dependent sodium (Nav) channels both in HEK cells and in native dorsal root ganglion (DRG) neurons. Our findings indicate that CBN is a functionally-selectively inhibitor of Nav channels without structural-selectivity. CBN equipotently inhibits the maximum conductance (Gmax) and stabilizes channel inactivation. CBN also slows the recovery from slow-inactivated states and shifts the steady-state inactivation curves in the hyperpolarized direction, as the channels enter deeper and slower inactivated states. Recordings from a multielectrode array demonstrate that CBN decreases the excitability of DRG neurons. By utilizing our automated patch-clamp platform to perform voltage- and current-clamp analyses on freshly isolated DRG neurons, we were able to confirm these results. The inhibitory effects of CBN on Nav currents and DRG neuron excitability introduce a novel aspect to its mechanism of action, suggesting that this cannabinoid could be beneficial for treating neuropathic pain.

Disclosures: M. Ghovanloo: None. **P.R. Effraim:** None. **S. Tyagi:** None. **P. Zhao:** None. **S.D. Dib-Hajj:** None. **S.G. Waxman:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.12/AA7

Topic: D.02. Somatosensation – Pain

Support: NIH BRAIN R01AT011447

Title: Ascending Neural Circuits That Shape The Perception Of Visceral Pain

Authors: *Y.-T. CHENG[1], J. ROBBINS[1], S. JAYAKAR[1], R. KAWAGUCHI[2], B. TURNES[1], Z. ZHANG[1], D. YARMOLINSKY[1], C. GREENE[1], C. WOOLF[1];
[1]Neurobio., Children's Hosp. of Boston, Boston, MA; [2]Neurol., David Geffen Sch. of Medicine, Univ. of California Los Angeles, Los Angeles, Los Angeles, CA

Abstract: Visceral pain is a prevalent form of pain that afflicts diverse populations worldwide. It is triggered by the activation of nociceptors innervating internal organs, such as the gastrointestinal (GI) tract, which relay a sensory inflow that sensitizes the central nervous system, even following the remission of inflammation. Current treatments, such as opioids, are ineffective to treat visceral pain and exacerbate symptoms by producing constipation. A primary locus implicated in producing such maladaptive pain resides within the spinoparabrachial (SPB) circuit, which transmits ascending signals from the spinal dorsal horn to the parabrachial nucleus (PBN) and drives pain percepts and mood changes. The goal of this study is to dissect how the SPB circuit processes afferent input from an inflamed colon to drive visceral pain and alter behaviors. To understand how the SPB circuit encodes GI pain, we used context-dependent genetic labeling to characterize neurons activated by colonic inflammation. We first delivered Cre-dependent viruses encoding DREADDs into the PBN of $Fos^{CreERT2}$ mice. We then captured visceral nociception-related neurons (PBN^{VISC}) by feeding the mice with 3% DSS to induce colonic inflammation for 4 days, followed by 4-OHT injection to drive Cre expression of the DREADD modulators. Our results reveal that silencing or reactivation of PBN^{VISC} neurons following CNO injection was sufficient to modulate cutaneous sensitivity of the lower abdomen but had no effect on the hindpaw. To evaluate the effect of PBN^{VISC} neurons on visceral nociception, we infused low-dose capsaicin into the distal colon to induce acute GI discomfort. We found that silencing PBN^{VISC} neurons significantly reduced the capsaicin-induced jumping behaviors in mice. We then sought to examine the ability of PBN^{VISC} neurons to drive taste learning by pairing PBN^{VISC} neurons activation with sucrose consumption following a conditioning paradigm. We found that reactivation of PBN^{VISC} neurons produced taste aversion, such that the mice dramatically reduced their preference for sucrose after the training session. We next sorted PBN^{VISC} and PBN^{SOM} neurons and profiled their molecular identities by single-cell RNA sequencing. We observed a distinct combination of excitatory and inhibitory populations and unique types of neurons activated under different nociceptive conditions. Our ongoing work aims to image the population activity of PBN-projecting spinal neurons to define the evolving sensory coding that occurs during visceral pain. This study will help establish a circuit framework for therapeutic opportunities to disrupt and treat GI visceral pain.

Disclosures: Y. Cheng: None. **J. Robbins:** None. **S. Jayakar:** None. **R. Kawaguchi:** None. **B. Turnes:** None. **Z. Zhang:** None. **D. Yarmolinsky:** None. **C. Greene:** None. **C. Woolf:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.13/AA8

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01 NS112344

Title: Mechanism of central sensitization underlying nociplastic pain in female mice

Authors: *R. PARIYAR, J. WANG, J. CHUNG, J.-H. LA;
Neurobio., Univ. of Texas Med. Br., Galveston, TX

Abstract: Background: Nociplastic pain arises from 'altered nociception' without clear evidence of tissue or somatosensory system damage. Central sensitization is considered an important mechanism causing this altered nociception. Previously, we developed a novel murine model manifesting a nociplastic pain state that is maintained by ongoing nerve activity - specifically the afferents responsive to the TRPA1 agonist allyl isothiocyanate (AITC) - at the previous injury site in females. In this study, we investigated how the ongoing activity of AITC-responsive afferents maintains central sensitization underlying nociplastic pain in female mice. Considering that the vast majority of excitatory interneurons express somatostatin (SST) while the SST2A receptor is primarily expressed in GABAergic inhibitory interneurons (GABAn) in the dorsal horn, we hypothesized that ongoing inputs from AITC-responsive afferents excite SST-releasing excitatory interneurons (SSTn), which in turn suppresses GABAn through the SST2A receptor, ultimately causing disinhibition in the dorsal horn nociceptive circuitry.**Method:** Dorsal root-attached parasagittal spinal cord slices were prepared from transgenic mice expressing GCaMP6f in either SSTn or GABAn for *ex vivo* Ca^{2+} imaging of these neuronal activities. The dorsal root was electrically stimulated by a suction electrode to generate a range of afferent inputs, and AITC was locally applied to the dorsal root to selectively generate AITC-responsive afferent inputs. The afferent input-evoked Ca^{2+} signal was recorded at 2 Hz. The effect of intrathecal SST2A receptor antagonist on the nociplastic pain state in females was assessed by von Frey filament assay.**Results:** During the stimulation of AITC-responsive afferents, SSTn was activated, whereas GABAn activation by other afferent inputs was decreased. An SST2A receptor antagonist prevented this effect of AITC-responsive afferents on GABAn, while an SST2A receptor agonist mimicked it. Intrathecal injection of the SST2A receptor antagonist significantly inhibited mechanical hypersensitivity in the female nociplastic pain model.**Conclusion:** Our results support the hypothesis that ongoing activity of AITC-responsive afferents maintains central sensitization by activating SSTn in the dorsal horn and consequently suppressing GABAn *via* the SST2A receptor; this disinhibition results in altered nociception causing nociplastic pain in female mice.

Disclosures: R. Pariyar: None. **J. Wang:** None. **J. Chung:** None. **J. La:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.14/AA9

Topic: D.02. Somatosensation – Pain

Support: IPSEN studentship contract

Title: Recombinant botulinum neurotoxin A1 produces analgesia in a contusion model of spinal cord injury pain

Authors: J. BRANDON[1], M. KALINICHEV[2], A. VIGNAUD[2], *J. RIDDELL[1];
[1]Univ. of Glasgow, Glasgow, United Kingdom; [2]IPSEN, Les Ulis, France

Abstract: Neuropathic pain affects about 80% of patients with a spinal cord injury. This type of pain can be extremely debilitating and is difficult to control because current analgesic drugs are frequently ineffective. Here, we investigated whether a novel recombinant botulinum neurotoxin type A1 (rBoNT/A1; IPN10260, IPSEN Bioinnovation Limited) can prevent the development of neuropathic pain in a rat contusion model of spinal cord injury. Pain involving different modalities was investigated following administration of rBoNT/A1 by a peripheral (intraplantar) or central (intrathecal) route. Male Sprague-Dawley rats were subjected to a T3 spinal cord contusion injury (Infinite Horizon device, 200kdyn) and either rBoNT/A1 or vehicle administered at the time of injury. Animals were randomly assigned to one of three treatment groups: (1) 400pg/kg rBoNT/A1, delivered intrathecally using a 32G catheter (n=8) (2) 140pg/kg rBoNT/A1, given by intraplantar injection into the forepaw (n=8) and (3) 10µL vehicle (gelatine phosphate buffer; n=8) injected into the forepaw. rBoNT/A1 did not have an effect on sensation or motor function in naïve animals by either route. Assessment of pain-like behaviours and motor function was performed at regular intervals for 8 weeks post-injury, and both the testing and analysis performed blind to the treatment. Following injury, rats administered with vehicle developed signs of at-level pain. Testing of the forepaws showed allodynia to tactile and cold stimuli, as well as hyperalgesia to noxious mechanical (pinprick) and thermal stimuli. Increased sensitivity to tactile stimuli applied to the back just above the injury level developed in parallel. Animals treated with rBoNT/A1 showed less tactile and cold allodynia to forepaw stimulation than vehicle treated animals, as well as less thermal hyperalgesia. Responses to pinprick were similar in all treatment groups. The increased sensitivity to von Frey stimuli applied to the back was also less (by about 50%) in the rBoNT/A1 treated animals compared to the vehicle group. Similar results were observed for both the forepaw and intrathecal routes of rBoNT/A1 administration. The rBoNT/A1 had no effect on the recovery of motor function seen after spinal cord injury. Our results show that rBoNT/A1 reduces pain-like behaviours across several modalities in an animal model of spinal cord injury at safe, non-paralysing doses whether administered peripherally or centrally. This suggests that botulinum neurotoxin A1 could be a clinically relevant analgesic for the treatment of pain following spinal cord injury.

Disclosures: J. Brandon: None. **M. Kalinichev:** Other; Former IPSEN employee. **A. Vignaud:** A. Employment/Salary (full or part-time):; IPSEN employee. **J. Riddell:** 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.; PI for research grant.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.15/AA10

Topic: D.02. Somatosensation – Pain

Support: IPSEN Studentship Contract

Title: Botulinum neurotoxin type A1 produces multimodal analgesia in two models of peripheral neuropathic pain

Authors: *J. BRANDON[1], M. KALINICHEV[2], A. VIGNAUD[2], J. RIDDELL[1];
[1]Univ. of Glasgow, Scotland, United Kingdom; [2]IPSEN, Les Ulis, France

Abstract: There is a pressing need for better analgesics for the control of pain. Recent studies have shown that botulinum neurotoxin A may have analgesic properties, but the full range of pain modalities affected has not yet been studied. Here we investigated whether recombinant botulinum neurotoxin type A1 (rBoNT/A1; IPN10260, IPSEN Bioinnovation Limited) can reverse an established neuropathic pain state in two models of peripheral nerve injury. We investigated the range of pain modalities affected and compared the effect of different doses and routes of administration. Unilateral spinal nerve ligation (SNL; L5/6) was performed on male Sprague-Dawley rats, and rBoNT/A1 or vehicle administered by intraplantar injection into the hind paw on the nerve injured side four days after injury. SNL animals were randomly assigned to one of three groups: 1) 100 pg/kg rBoNT/A1 (a dose without effect on sensitivity to mechanical/thermal stimuli in naïve animals; n=8) 2) 120 pg/kg rBoNT/A1 (a dose producing a mild reduction in sensitivity to tactile stimuli in naïve animals; n=8) and 3) vehicle (n=8). Unilateral sciatic nerve transection and repair (SNT+R) was also performed in male Sprague-Dawley rats, and rBoNT/A1 or vehicle injected at the time of repair. Animals were randomly assigned to one of three treatment groups: 1) 100pg/kg rBoNT/A1 intraplantar (n=12) 2) Intraneural 140pg/kg rBoNT/A1 (n=12) and 3) Intraneural vehicle (n=12). Neither dose of rBoNT/A1 affected sensory responses in naïve animals. Pain-like behaviours in both models were assessed regularly for 8 weeks post-injury, and both testing and analysis performed blind to treatment. Animals were checked for autotomy several times daily. After rBoNT/A1 treatment, both nerve injury models demonstrated a reduction in tactile allodynia by at least 50% compared to the vehicle-treated group, but sensitivity to pinprick was not attenuated. Thermal hyperalgesia and cold allodynia were also reduced by rBoNT/A1 in the SNL model. Spontaneous pain indicators, including autotomy, elevated zero maze and conditioned place preference, also suggested rBoNT/A1 mediated analgesia in both models. Dynamic weight bearing results showed that rBoNT/A1 administration produced no motor deficits. Closely similar results were observed for both doses of the rBoNT/A1 used in SNL animals, and both routes of administration used in the SNT+R model. Our findings suggest that rBoNT/A1 has analgesic effects on several modalities of pain-like behaviour in animal models of neuropathic pain at safe,

non-paralysing doses. These results suggest that botulinum neurotoxin type A1 could be clinically relevant for the treatment of nerve injury pain.

Disclosures: **J. Brandon:** None. **M. Kalinichev:** Other; Former IPSEN Employee. **A. Vignaud:** A. Employment/Salary (full or part-time):; IPSEN Employee. **J. Riddell:** 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.; PI for Research Grant.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.16/AA11

Topic: D.02. Somatosensation – Pain

Support: NIH Grant RO1 DE031352
NIH Grant RO3 DE027780
NIH Grant T32 DE14318

Title: Macrophage Migration Inhibitory Factor Mediates Stem Cell Analgesia In Orofacial Pain

Authors: *J. D. MURILLO[1], P. CHANG[1], S. GANATRA[1], B. CHAPA[1], N. B. RUPAREL[2]; [1]UT Hlth. San Antonio, San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Cntr At San Antonio, Univ. of Texas Hlth. Sci. Cntr At San Antonio, San Antonio, TX

Abstract: Apical periodontitis (AP) is a common example of infection-induced pain that typically occurs when the dental pulp becomes infected. While root canal treatment has high success rates, persistent post-treatment pain occurs in 10-12% of patients. Currently available analgesic drugs have adverse effects, such as gastro-intestinal bleeding and risk of physical dependence. Therefore, identifying a novel class of analgesics that can prevent the development of persistent dental pain is of clinical relevance. Stem cell-induced analgesia is an emerging therapeutic that has demonstrated profound efficacy in animals and patients experiencing neuropathic pain. However, its effectiveness and mechanisms involved in treating dental pain is unknown. Our preliminary data indicates that i.v. injections of human stem cells of the apical papilla (hSCAP) can reverse mechanical allodynia in a model of AP. The objective of this study was to define mechanisms mediating hSCAP-induced anti-allodynia. Pulp exposures of the maxillary left 1st molars were done to induce AP. On day 21 post pulp exposures, homing of hSCAP was evaluated with immunohistochemistry. Here we demonstrate that hSCAP homed to periapical granulomas but not the medullary dorsal horn. RNA sequencing of periapical granulomas (site of infection) was conducted to determine factors responsible for hSCAP analgesia. RNA sequencing revealed hSCAP have a 133-fold increase in the expression of Macrophage Migration Inhibitory Factor (MIF). After identification of factors, conditioned media (CM) from hSCAP primed to periapical granulomas was collected to confirm release of

MIF from hSCAP. CM from primed hSCAP release MIF >5-fold compared to control CM. Furthermore, repeated intraoral injections of recombinant MIF reversed AP-induced allodynia. Additionally, MIF receptors CD74 and CXCR4 were found to co-localize on TRPV-1 positive neurons. Lastly, primed CM significantly attenuates capsaicin-evoked calcium from mouse trigeminal neurons and this effect is reversed by an anti-human MIF-Ab. Collectively, these data suggest a novel peripheral mechanism for human stem cell-induced inhibition of nociception due, at least in part, by MIF.

Disclosures: **J.D. Murillo:** None. **P. Chang:** None. **S. Ganatra:** None. **B. Chapa:** None. **N.B. Ruparel:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.17/AA12

Topic: D.02. Somatosensation – Pain

Support: R01NS102722
R01DE026806
R01DK118971
R01DE029951
R01DE029694
R01CA228525
R01CA231396
W81XWH1810431

Title: Nanoparticle Delivery Potentiates Anti-Nociceptive Effect of CGRP Receptor Antagonist in Oral Cancer

Authors: *N. HUU-TU[1], K. INOUE[2], V. CHOKSHI[2], R. SOTOODEH[2], M. PERERA[2], H. RHYU[2], J. KIM[2], C. SUH[2], N. NGUYEN[2], V. RUSINAK[2], P. LEWIS[3], N. PINKERTON[4], N. BUNNETT[5], D. ALBERTSON[6], B. SCHMIDT[7];
[1]NYU Translational Res. Ctr., New York, NY; [2]NYU Translational Res. Ctr., NEW YORK CITY, NY; [3]NYU Tandon Sch. of Engineering, Dept. of Chem. and Biomolecular Engineering., NEW YORK CITY, NY; [4]NYU Tandon Sch. of Engineering, Dept. of Chem. and Biomolecular Engin., NEW YORK CITY, NY; [5]Pain Res. Center, New York University, New York, NY 10010, USA, NEW YORK CITY, NY; [6]Dept. of Oral and Maxillofacial Surgery, Translational Res. Center, New York Univ. (NYU) Col. of Dentistry, New York, NY 10010, USA, NEW YORK CITY, NY; [7]NYU Col. of Dentistry, Translational Res. Center., NEW YORK CITY, NY

Abstract: Oral squamous cell carcinoma (SCC) is notoriously painful. Olcegepant, [OCP, a Calcitonin Gene-Related Peptide (CGRP) receptor antagonist], does not completely attenuate cancer nociception in mice with Human Squamous Cell Carcinoma (HSC-3) in the hind paw.

Delivery of OCP in nanoparticles (NPs) may provide more profound and longer duration anti-nociception than raw OCP. NPs loaded with OCP were administered to mice suffering from cancer-induced mechanical and thermal allodynia. To generate a xenograft oral SCC cancer model, we inoculated HSC-3 cells (JCRB0623, Japan) into the left hind paw of the *NU/J Foxn1^{nu}* mice (the Jackson Laboratories). After the mice developed cancer nociception, we injected raw OCP or the NP-encapsulated OCP into the cancer paw. Empty NPs or saline served as controls. To quantify cancer nociception development and evaluate the anti-nociceptive effect of the treatments, we used the paw von Frey filament and Hargreaves assays for mechanical and thermal allodynia, respectively. We recorded the anti-nociceptive effect of treatments at 1, 3, 6, 12, 24, and 48 hours post-treatment. In an *in vitro* experiment, NPs tagged with rubrene, a fluorescent, were applied to either HSC-3 cancer cells or cultured Schwann cells. Both HSC-3 and Schwann cells endocytosed NPs. NPs-loaded with OCP attenuated mechanical and thermal cancer nociception by 4.8 and 1.5 times, respectively, compared to raw OCP. The anti-nociceptive effect of NPs lasted 12 hours longer than raw OCP. These results demonstrate that NP drug delivery is a promising method for treating cancer pain.

Disclosures: **N. Huu-Tu:** None. **K. Inoue:** None. **V. Chokshi:** None. **R. Sotoodeh:** None. **M. Perera:** None. **H. Rhyu:** None. **J. Kim:** None. **C. Suh:** None. **N. Nguyen:** None. **V. Rusinak:** None. **P. Lewis:** None. **N. Pinkerton:** None. **N. Bunnett:** None. **D. Albertson:** None. **B. Schmidt:** None.

Poster

PSTR475. Treatments for Persistent Pain: Insight from Non-Human Models

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR475.18/AA13

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Opioids induce cortico-subcortical dysconnection correlating analgesia development in the awake mouse brain

Authors: J.-C. MARIANI[1], S. DIEBOLT[1], S. SCHULZ[3], T. DEFFIEUX[4], M. TANTER[4], ***Z. LENKEI**[2], A. KLIEWER[3];
[2]Inst. of Psychiatry and Neurosciences of Paris, [1]INSERM, Paris, France; [3]Universitätsklinikum Jena, Jena, Germany; [4]Physmed / Inserm U1273, Paris, France

Abstract: Opioid action on the brain, both for its therapeutic and abuse potential, is a target of high scientific interest. However, we still lack an understanding of the dynamics of large-scale brain effects induced by the activation of the μ-opioid receptor (MOP), the main central target of opioid drugs. Here, we have developed a robust multimodal functional ultrasound (fUS) imaging pipeline to measure the dynamics of opioid-induced changes in brain activation and functional connectivity (FC) patterns, through the intact skull, in awake and behaving mice. We report that the major opioid drugs morphine, fentanyl, methadone and buprenorphine lead to highly-reproducible, dose- and time-dependent reorganization of brain activation and FC patterns. These

effects are sensitive to induced tolerance or to pharmacological and genetic inactivation of the MOP. Local activation, as measured by perfusion changes, is rapid and correlated with hypermotility, while slower functional connectivity changes, displaying individual spatio-temporal profiles, parallel MOP phosphorylation and the development of analgesia. All the investigated drugs lead to a general opioid-specific dysconnectivity fingerprint: hippocampal and thalamic regions decrease connection to the somatosensory cortex and increase sub-cortical connections, while bilateral connectivity in the somatosensory cortex and hippocampus is preserved. Our results both suggest the delayed reorganization of inter-regional connectivity as an important brain effect of opioids and validate a new approach in the development of neuropsychiatric drugs, with the potential of identifying compounds with improved pharmacological profiles.

Disclosures: **J. Mariani:** None. **S. Diebolt:** A. Employment/Salary (full or part-time):; Iconeus. **S. Schulz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 7TM Antibodies. F. Consulting Fees (e.g., advisory boards); 7TM Antibodies. **T. Deffieux:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus. F. Consulting Fees (e.g., advisory boards); Iconeus. **M. Tanter:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus. F. Consulting Fees (e.g., advisory boards); Iconeus. **Z. Lenkei:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iconeus. F. Consulting Fees (e.g., advisory boards); Iconeus. **A. Kliewer:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.01/AA14

Topic: D.02. Somatosensation – Pain

Support: JSPS KAKENHI Grant Number JP23K10574

Title: Role of JNK in the morphological changes of injured C-fiber terminal via the phosphorylation of GAP43 in the dorsal horn of a rat neuropathic pain model

Authors: *H. YAMANAKA[1,2], K. KOBAYASHI[3], H. KANDA[1], K. NOGUCHI[2];
[1]Sch. of Pharm., [2]Lab. of Basic Pain Reserch, Hyogo Med. Univ., Kobe, Japan; [3]Dept. of Anat. and Neurosci., Hyogo Med. Univ., Nishinomiya, Japan

Abstract: Peripheral nerve injury produces neuropathic pain as well as phosphorylation of Jun amino terminal kinase (JNK) in dorsal root ganglia (DRG). In animal models of neuropathic pain, JNK inhibitors are known to alleviate pain at early time points after injury. Although the regulation of JNK can be a promising therapeutic target for the treatment of neuropathic pain, it

has not been reported the substrate of JNK in DRG neurons. Recent studies have revealed that the JNK phosphorylated S96 residue of Growth Associated Protein 43 (GAP43) in CNS neurons and in growth cones of the elongating neurites in vitro. It has been well known that peripheral nerve injury increased GAP43 in DRG neurons, which was transported to the peripheral and central axon terminals. Our previous studies have reported that spinal GAP43 was co-localized with L1-CAM positive axonal terminals, which showed the plastic changes such as hypertrophy of varicosities and increase of axo-axonic contacts. In this study, we confirmed the phosphorylation of JNK (pJNK) and examined the expression of phosphorylated GAP-43 at S96 (pGAP43) in the primary afferent of a rat neuropathic pain model. In contrast to the previous reports, the expression of pJNK in DRG significantly increased from 1day and continued to at least 30 days after injury. The expression of pGAP43 showed the specific pattern in the injured DRG. Nerve injury significantly up-regulated pGAP43 in the subsets (65%) of GAP43 positive neurons. About 96% of the pGAP43 positive neurons were co-expressed with pJNK. The limited population (15%) of pGAP43 were co-labeled with NF200, a marker of myelinated neuron in the DRG of neuropathic pain model. In the dorsal horn, pGAP43 positive terminals were mainly localized in laminae I-II of the dorsal horn ipsilateral to the injury. The pGAP43 immunoreactivities were observed in the L1-CAM-labeled axonal varicosities in the ipsilateral dorsal horn of neuropathic pain model. The delayed chronic intrathecal administration (7-14 days) of JNK inhibitor SP600125 was examined in the neuropathic pain model. The administration of SP600125 inhibited neuropathic pain behavior and suppressed the increase of pGAP43 labeled axon terminals. At the same time, the inhibition of JNK suppressed the hypertrophy of L1-CAM labeled varicosities in the dorsal horn. These data indicate that JNK regulates the morphological plasticity of L1-CAM-labeled C-fiber terminals via phosphorylation of GAP43. Injury-activated JNK may be a key mechanism of the morphological changes in the primary afferent which relay neuropathic pain signals to the dorsal horn circuits.

Disclosures: **H. Yamanaka:** None. **K. Kobayashi:** None. **H. Kanda:** None. **K. Noguchi:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.02/AA15

Topic: D.02. Somatosensation – Pain

Support: NRF-2017R1A5A2015391
NRF-2022R1A2C2092262

Title: Nlrp3 mediates antinociceptive effects of botulinum toxin a in rodent trigeminal neuralgia model

Authors: *D. AHN[1], J. SON[2], J.-S. JU[3], Y.-M. KIM[4], M.-J. JO[3], M. PARK[5], M. LEE[6];
[1]Dentistry, Kyungpook Univ., Daegu, Korea, Republic of; [2]Oral physiology, Kyungpook Natl.

Univ. of Dent., Daegu, Korea, Republic of; [3]Oral physiology, [4]Oral Physiol., Kyungpook Natl. Univ., Daegu, Korea, Republic of; [5]Dent. Hyg., Kyung-Woon Univ., Gumi, Korea, Republic of; [6]Dong-Eui Univ., Dong-Eui Univ., busan/ busanjin-gu, Korea, Republic of

Abstract: Although numerous studies have described botulinum toxin type A (BoNT-A) efficacy against trigeminal neuralgia, the underlying cellular mechanisms remain unclear. Here, we asked how the NOD-like receptor protein 3 (NLRP3) inflammasome and pro-inflammatory cytokines to contribute development of trigeminal neuralgia. Therefore, we have investigated cellular mechanisms that mediate the antinociceptive effects of BoNT-A in a rodent model of trigeminal neuralgia produced by demyelination of the trigeminal nerve root (TNR). Male Sprague-Dawley (weight: 250 - 270 g) rats were anesthetized with ketamine (40 mg/kg) and xylazine (4 mg/kg). The rats were mounted onto a stereotaxic instrument and an injection of lysophosphatidic acid (LPA; 1 nmol/3μl) was given into the TNR to produce demyelination. BoNT-A (3 U/kg) was diluted in 1 ml of saline and was injected subcutaneously into the most pain sensitive area. Significant mechanical allodynia was observed on postoperative day (POD) 1 and persisted until POD 130 after LPA injection into the TNR. LPA-induced demyelination increased expression of NLRP3 and pro-inflammatory cytokines levels (IL-1β, IL-6, IL-18 and TNF-α) in the trigeminal ganglion on POD 7 as compared with the sham group. Single or double treatments with BoNT-A (3 U/kg) led to significant prolonged antinociceptive effects. Furthermore, a single treatment with BoNT-A (3 U/kg) significantly suppressed the upregulation of NLRP3 expression and pro-inflammatory cytokines levels in the trigeminal ganglion. These findings indicate that treatment with BoNT-A produced antinociceptive effects in LPA-induced trigeminal neuralgia animal model through suppression of NLRP3 and pro-inflammatory cytokines in the trigeminal ganglion. This could be considered a new treatment strategy of BoNT-A for trigeminal neuralgia (supported by NRF-2017R1A5A2015391, NRF-2022R1A2C2092262 and by Hugel Inc.).

Disclosures: D. Ahn: None. **J. Son:** None. **J. Ju:** None. **Y. Kim:** None. **M. Jo:** None. **M. Park:** None. **M. Lee:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.03/AA16

Topic: D.02. Somatosensation – Pain

Support: National Research Foundation of Korea -NRF-2021R1F1A1055082
National Research Foundation of Korea-NRF-2022R1F1A1073652

Title: Gut microbiota and related metabolites are involved in the development of vincristine-induced peripheral neuropathy

Authors: S.-Y. YOON[1], *D. ROH[2];
[1]Daejeon Hlth. Inst. of Technol., Englewood, NJ; [2]Kyunghee Univ., Seoul, Korea, Republic of

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating adverse side effect of cancer treatment. Current understanding of the mechanisms underpinning CIPN is still limited and unclear. The gut is connected to the CNS by immunological mediators, lymphocytes, neurotransmitters, microbes and microbial metabolites. There are many reports that microbiome and related metabolites exert significant effects on neurological diseases. However, the potential contribution of microbiota and related metabolites in CIPN has not been well reported. In the present study, we elucidated the role of gut microbiota and metabolites in CIPN. The chemotherapeutic agent, vincristine, produces a robust painful neuropathy that results in mechanical allodynia and the loss of intraepidermal nerve fibers (IENFs). Vincristine-induced mechanical allodynia and loss of IENFs were significantly reduced in mice pretreated with antibiotics. We investigated metabolic changes in serum of mice treated with vincristine and antibiotics (vinc+antibio). Interestingly, high level of tryptophan metabolite was found in serum with vinc+antibio group. Moreover, pretreatment with tryptophan itself suppressed vincristine-induced mechanical allodynia and loss of IENFs. In conclusion, these results suggest that gut microbiota and related metabolites may be involved in the development of vincristine-induced peripheral neuropathy, and tryptophan could novel strategy for CINP management.

Disclosures: S. Yoon: None. **D. Roh:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.04/AA17

Topic: D.02. Somatosensation – Pain

Support: RX003621(SDH)
Center Grant B9253-C (SDH and SGW)
Grant from the Thomson Family Foundation (no grant number)
Gift from the Paralyzed Veterans of America (no gift number)

Title: NaV1.8 in small DRG neurons contributes to vincristine-induced mechanical allodynia.

Authors: *A. NASCIMENTO DE LIMA, H. ZHANG, L. CHEN, P. EFFRAIM, C. GOMIS PEREZ, X. CHENG, J. HUANG, S. WAXMAN, S. DIB-HAJJ;
Yale Univ., New Haven, CT

Abstract: Vincristine-induced peripheral neuropathy is a common side effect of vincristine treatment, which is accompanied by pain and can be dose-limiting. The molecular mechanisms that underlie vincristine-induced pain are not well understood. Our previous studies have shown that the tetrodotoxin-sensitive (TTX-S) voltage-gated sodium channel NaV1.6 in medium-diameter dorsal root ganglion (DRG) neurons contributes to the maintenance of vincristine-

induced allodynia. In this study, we investigated the effects of vincristine administration on excitability in small diameter DRG neurons and whether the tetrodotoxin-resistant NaV1.8 channels contribute to mechanical allodynia. Vincristine sulfate or saline was administered intraperitoneally in adult mice of both genders, and vincristine-induced allodynia were confirmed by behavioral tests. Electron microscopy on sciatic nerves dissected from mice after behavioral assessments showed no substantial axonal degeneration or apparent loss at the peripheral nerve level between vincristine-treated (VIN) and control groups. DRG neurons were isolated from animals of both groups for electrophysiological study. Current-clamp recordings demonstrated that small DRG neurons became hyper-excitable following vincristine treatment, with reduced current threshold and increased firing frequency. Nav1.8 currents were measured in the presence of extracellular TTX (1 μM) with a holding potential of -80 mV. Small DRG neurons from VIN mice showed increased current density and a hyperpolarizing shift in V1/2 of activation of NaV1.8 channels, which likely contributes to the hyperexcitability observed in neurons from VIN mice by current clamp recordings. Notably, vincristine treatment did not enhance excitability of small DRG neurons from NaV1.8 knockout mice, and the development of mechanical allodynia was delayed but not abrogated in those mice. Taken together, our data suggest that sodium channel NaV1.8 in small DRG neurons contributes to the development of vincristine-induced mechanical allodynia.

Disclosures: A. Nascimento de Lima: A. Employment/Salary (full or part-time):; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **H. Zhang:** Other; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **L. Chen:** Other; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **P. Effraim:** A. Employment/Salary (full or part-time):; Department of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **C. Gomis Perez:** A. Employment/Salary (full or part-time):; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **X. Cheng:** A. Employment/Salary (full or part-time):; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **J. Huang:** A. Employment/Salary (full or part-time):; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **S. Waxman:** A. Employment/Salary (full or part-time):; Department of

Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA. **S. Dib-Hajj:** A. Employment/Salary (full or part-time):; Department of Neurology, Yale University School of Medicine, New Haven, CT 06510, Center for Neuroscience and Regeneration Research, Yale University School of Medicine, New Haven, CT 06510, USA, Center for Rehabilitation Research, VA Connecticut Healthcare System, West Haven, CT 06516, USA.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.05/AA18

Topic: D.02. Somatosensation – Pain

Support: NS121946

Title: Mitophagy in Peripheral Sensory Neurons Contributes to the Resolution of Neuropathic Pain

Authors: *S. LEE[1], A. PRUDENTE[2], J. ROH[4], T. BERTA[3];
[2]Univ. of Cincinnati, [3]Anesthesiol., [1]Univ. of Cincinnati, Cincinnati, OH; [4]Col. of medicine, Gachon Univ. of Med. and Sci., Incheon, Korea, Republic of

Abstract: Mitophagy in Peripheral Sensory Neurons Contributes to the Resolution of Neuropathic Pain Sang Hoon Lee[1], Arthur Silveira Prudente[1], Jueun Roh[1,2], Temugin Berta[11] Pain Research Center, Department of Anesthesiology, University of Cincinnati Medical Center, Cincinnati, OH, United States[2] Gachon Pain Center and Department of Physiology, College of Medicine, Gachon University, Incheon, South KoreaPeripheral neuropathies resulting from chemotherapy or diabetes often manifest as neuropathic pain, which is difficult to treat with current analgesics. Our lack of understanding of the underlying mechanisms of peripheral neuropathy and resulting neuropathic pain hampers the development of new analgesics. While the accumulation of damaged mitochondria in peripheral sensory neurons is associated with neuropathic pain, the role of mitophagy (i.e., the endogenous mechanism for the disposal of damaged mitochondria by autophagy) in this disease is not fully understood. We hypothesized that mitophagy is an underlying mechanism of peripheral neuropathy and a novel analgesic target for the treatment of neuropathic pain. Here, we report that during the resolution of neuropathic pain (i.e., mechanical hypersensitivity) induced by the chemotherapeutic drug paclitaxel, mitophagy-related genes are upregulated. Furthermore, mice lacking expression of the serine/threonine kinase PTEN-induced kinase 1 (PINK1), a kinase involved in triggering mitophagy and presumably related to impaired mitophagy, exhibit prolonged paclitaxel-induced mechanical hypersensitivity. In contrast, boosting mitophagy in mice by delivering urolithin A, a mitophagy inducer derived from pomegranate juice, prevents both paclitaxel- and diabetic-

induced mechanical hypersensitivity. Together these findings demonstrate a previously undiscovered role of mitophagy in the resolution of neuropathic pain and validate mitophagy inducers as a new class of analgesics for the treatment of painful peripheral neuropathies.

Disclosures: **S. Lee:** None. **A. Prudente:** None. **J. Roh:** None. **T. Berta:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.06/AA19

Topic: D.02. Somatosensation – Pain

Support: NIH NS045594

Title: Functional effects of sympathectomy-induced sensory neuron apoptosis

Authors: *D. DE NARDIN LÜCKEMEYER, W. XIE, J. ZHANG, S. V. LACKEY, J. A. STRONG, J.-M. ZHANG;
Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Previously we have found that a localized "microsympathectomy" (mSYMPX), i.e. cutting the lumbar sympathetic postganglionic grey rami, led to death of sensory neurons in the adjacent DRG in rats and mice measured by propidium iodide labeling. Here, we studied possible functional consequences of sensory neuron cell death induced by mSYMPX. Adult mice of both sexes receiving mSYMPX or sham on the right side near L4 and L3 DRGs did not show significantly different behavioral responses to mechanical (von-Frey filaments) or thermal (cold, heat) stimuli and showed no motor deficits (rotarod). We next measured the number of L4 DRG neurons which were activated by mechanical stimulation via *in vivo* calcium imaging in GCaMP6s/Pirt-cre mice. We found that the number of neurons responding to paw poking with a series of von-Frey filaments was significantly reduced in the right (mSYMPX) L4 DRG compared to the contralateral side. The reduction was observed for both innoxious and noxious mechanical stimuli. On postoperative day 7, the slope of the (observed linear) relationship between number of cells responding and log force (mg) applied, was reduced from 36.2 on the contralateral side to 25.6 on the mSYMPX side ($p<0.003$). Preliminary data showed similar results at all examined time points (days 1, 3, 7 and 28). This early functional loss of DRG neurons was consistent with our observation that the expression of activated caspase-3, a cell death marker, was significantly elevated from 18 hours after mSYMPX, and with our previous finding that neuronal death (propidium iodide labeling) happened during the earliest days after mSYMPX. Further evidence of neuronal cell death included reduction in fluorescently labeled sensory neuron projections to the ipsilateral spinal cord, and downregulation of anti-apoptotic proteins after mSYMPX. Removal of the draining sciatic lymph node blocked effects of mSYMPX on neuron cell death, *in vivo* Ca imaging and apoptotic proteins. Our results suggested that DRG neuronal death induced by mSYMPX reduced the number of neurons which could

respond to mechanical stimulation and this effect requires the lymph node. However, in the absence of peripheral nerve injury, this mSYMPX-induced loss of functional neurons in DRG did not result in significant deficits in sensory or motor functions. We are now investigating how neuronal cell death contributes to our previous finding that mSYMPX markedly attenuated pain behaviors in several preclinical pain models (SNI, SNL, LID and chemotherapy).

Disclosures: D. De Nardin Lückemeyer: None. **W. Xie:** None. **J. Zhang:** None. **S.V. Lackey:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.07/AA20

Topic: D.02. Somatosensation – Pain

Support: CAPES
FAPESP
CNPq
CRID

Title: Astrocytes and myeloid cells role in paclitaxel-induced peripheral neuropathy

Authors: *B. LIMA ADJAFRE, L. ANDRADE, F. BONIFACIO, F. I. GOMES, R. GUIMARÃES, C. E. SILVA, F. CUNHA, J. ALVES, T. CUNHA;
Univ. de São Paulo, Ribeirao Preto, Brazil

Abstract: Neuropathic pain is a common side-effect of chemotherapy (CIPN) that can turn into a permanent disabilitywithout efficient treatment to resolve it. Astrocytes are support glial cells of the central nervous system(CNS) and are crucial to the development and maintenance of multiple CNS ailments but its role in CIPNremains elusive. To evaluate the astrocyte and immune cell compartment participation in development andmaintenance of CIPN we used *in vitro* (primary astrocyte and bone-derived macrophage cultures) and *in vivo*approaches (wild-type, $CCL2^{-/-}$, *Aldh1l1*$^{cre/ERT2}$-$TLR4^{flox/flox}$ and and *LyzM*^{cre}-$TLR4^{flox/flox}$ mice treated i.p. withpaclitaxel (PCX; 8 mg/kg) (CEUA FMRP – USP 011/2020). *In vitro* experiments using astrocytes obtainedfrom WT or $TLR4^{-/-}$ pups (4-days old), both stimulated with PCX (10 µM), TNF (10 ng/ml) or LPS (10ng/ml) showed an increase in chemokines (CXCL1, CCL2) and levels in PCX and LPS-treated WT cells, butnot in cytokines (IL-6, TNFa) levels. For macrophages, there was also an increase in cytokines levels.Interestingly, astrocytes and macrophages from $TLR4^{-/-}$ animals were unresponsive to both PCX and LPSstimulation, confirming the existing literature on TLR4 as a major receptor for paclitaxel. Since there was amodulation of CCL2 secretion *in vitro* after paclitaxel stimulation, we treated $CCL2^{-/-}$ mice, performedbehavioral experiments and observed that in the absence of this chemokine there is an increase in mechanicalpain threshold and a decrease in cold allodynia when compared to WT mice. A conditional knockout animalwas

generated by our group, deleting the expression of TLR4 gene specifically in astrocytes (*Aldh1l1*-expressing cells) and behavioral tests were also performed, but no difference in mechanical pain threshold orthermal allodynia was perceived. To evaluate the participation of myeloid cells from the periphery we alsoassessed behavioral responses after PCX treatment in *LyzM*^{cre}-TLR4$^{flox/flox}$, which lack the TLR4 expressionin neutrophils and macrophages. The animals were protected against CIPN, presenting a higher mechanicalpain threshold and a decrease in cold responses. We also performed a flow cytometry analysis in dorsal rootganglia which showed that, in WT animals, resident macrophages (CD45+CD11b+CX3CR1+CD64+) areincreasing in a time-dependent manner following CIPN. In conclusion, the data thus far show that althoughastrocytes can be activated *in vitro* by paclitaxel this feature can be irrelevant *in vivo* to the maintenance ofCIPN, whereas CCL2 involvement and peripheral leukocytes might have a more important contribution tothe development and maintenance of CIPN.

Disclosures: B. Lima Adjafre: None. **L. Andrade:** None. **F. Bonifacio:** None. **F.I. Gomes:** None. **R. Guimarães:** None. **C.E. Silva:** None. **F. Cunha:** None. **J. Alves:** None. **T. Cunha:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.08/AA21

Topic: D.02. Somatosensation – Pain

Title: Involvement of LRP1 in sensory neurons in mechanosensation

Authors: *A. CHARRON, F. CASTETS, A. MOQRICH;
Aix Marseille Université, CNRS, IBDM, UMR 7288, Marseille, France

Abstract: The low-density lipoprotein receptor-related protein-1 (LRP1) is an ubiquitous endocytic receptor belonging to the multifunctional member of the low-density lipoprotein (LDL) receptor family. LRP1 is a multi-functional protein involved in many biological processes as it has endocytic and signal transduction properties due to its interaction with numerous extracellular ligands and intracellular proteins. Recent evidence highlighted a role of LRP1 in pain sensation as deletion of LRP1 in microglia impairs the development of partial sciatic nerve ligation or PNL-induced tactile allodynia (Brifault, et al. Glia, 2021), and its inactivation in Schwann cells induced abnormalities in axon myelination that were associated with tactile allodynia, even in the absence of nerve injury (Orita, et al. The Journal of Neuroscience, 2013). These results suggest that depending on the tissue, LRP1 play a role in the mechanical sensitivity. Recently, in our lab, we show that TAFA4, a secreted protein highly enriched in C-LTMRs mediates it is painkilling effect through LRP1. Based on these data, we wonder whether sensory neurons' LRP1 plays a role in somatosensation. To address this question, we generated a mouse model in which we deleted LRP1 specifically in sensory neurons using the Adv cre tg mice (Zurborg, et al. Mol Pain, 2011). We show that deletion of LRP1 in sensory neurons leads

to mechanical hypersensitivity to light stimulus in naïve condition (tape test). Moreover, mice lacking LRP1 in sensory neurons develop long lasting mechanical hypersensitivity in the paw incision post-operative pain model, but not in the inflammatory (carrageenan) or the chronic constriction of the sciatic nerve (CCI) models. Our study shows that LRP1 plays a key role in touch sensation and suggest that LRP1-expressing neurons are selectively involved in a circuit that mediates incision-induced mechanical pain.

Disclosures: A. Charron: None. **F. Castets:** None. **A. Moqrich:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.09/AA22

Topic: D.02. Somatosensation – Pain

Support: MOST 111-2320-B-A49-011-MY3
MOST 109-2320-B-010-027-MY3

Title: Acidosis-related receptors involved in CCI-induced neuropathic pain by modulating neuron activation and axonal degeneration

Authors: *Y. CHIN, S.-P. DAI, W.-H. SUN;
Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Acidosis-related receptors involved in CCI-induced neuropathic pain by modulating neuron activation and axonal degeneration Yin Chin[1], Shih-Ping Dai[1] and Wei-Hsin Sun*[1 1] Department of Life Science & Institute of Genome Sciences, National Yang Ming Chiao Tung University, Taipei, TaiwanThe symptoms of neuropathic pain include persistent pain, local tissue acidosis, inflammation, demyelination and sensory hypersensitivity. Currently, the first-line therapies for chronic pain are often limited by short-term efficacy and unacceptable side effects. Therefore, elucidation of the mechanisms of neuropathic pain and identification of promising targeting genes may provide knowledge to develop long-lasting analgesia for chronic pain. The acid-sensing ion channel, ASIC3, as well as the proton sensing G protein-coupled receptor, TDAG8 and OGR1, are expressed in the nerve system and play roles in pain. To address the roles of these genes in neuropathic pain, chronic constriction injury of the sciatic nerve (CCI) was performed in mice with gene deletion, suppression or pharmacological block. It is intriguing to note that different genes play regulatory roles at distinct time points, suggesting a temporal modulation of chronic pain. TDAG8 affected pain in the early phase, while OGR1 and ASIC3 in the chronic phase. Compound muscle action potential (CMAP) was employed to evaluate axon loss or demyelination. CCI surgery induced neuron degeneration, resulting in interruption of neural conduction. Suppression of ASIC3 and OGR1 rescued the interruption. CCI surgery also enhance calcium signals in distinct population of dorsal root ganglion (DRG) neurons. Inhibition of TDAG8, ASIC3 and OGR1 reduced the increase in calcium

signals. In summary, these findings demonstrate that proton-sensing receptors temporally regulate neuropathic pain and one potential drug targets.

Disclosures: Y. Chin: None. **S. Dai:** None. **W. Sun:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.10/AA23

Topic: D.02. Somatosensation – Pain

Support: NIH 1RF1NS113883-01

Title: Effect of angiogenesis inhibitor Bevacizumab on spontaneous pain and sensory neuron cluster firing in injured DRG observed in the SNI model of neuropathic pain

Authors: W. XIE[1], J. ZHANG[1], D. DE NARDIN LÜCKEMEYER[1], S. V. LACKEY[1], *J. A. STRONG[1], X. DONG[2], J.-M. ZHANG[1];
[1]Anesthesiol., Univ. of Cincinnati, Cincinnati, OH; [2]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: We previously observed a unique cluster firing phenomenon in nerve injured DRG in the spared nerve injury (SNI) model of neuropathic pain. This sporadic spontaneous firing of spatially clustered DRG neurons contributes to spontaneous pain behaviors, which are very pronounced in this model. Furthermore, we found that cluster firing was closely associated with the movements of blood vessel in injured DRGs, which may activate Piezo2-expressing neurons in clusters. Cluster firing was decreased by blocking the Piezo2 channel and enhanced by increasing blood vessel movements with phenylephrine (systemic or local application) or by directly poking blood vessels with a glass micropipette to evoke a myogenic response. Angiogenesis after peripheral nerve injury has been described in recent studies. In the present study, we investigated whether angiogenesis triggered by peripheral nerve injury is involved in the abnormal functional coupling between sensory neurons and blood vessels in the injured DRG. Experiments were conducted in young adult mice of both sexes using the SNI model. To inhibit angiogenesis we used Bevacizumab, an anti-human VEGF antibody and FDA-approved anti-cancer drug (10 mg/kg, i.p. twice a week for 3 weeks) starting immediately after the SNI surgeries. Spontaneous pain behaviors were scored for SNI mice weekly after surgery for 4 weeks. We found that the pain scores in mice treated by Bevacizumab were much lower than in the saline control group. On day 21, the score decreased from 36.5 to 10.0 ($p<0.05$) and could no longer be increased by phenylephrine injection. For comparison, prior to SNI, pain scores were ~2. Following the last spontaneous pain scoring, *in vivo* calcium imaging was performed. The number of neurons participating in cluster firing increased after injection of the vasoconstrictor phenylephrine in vehicle treated mice, but failed to do so in Bevacizumab treated mice. The percentage of blood vessel pokes that evoked cluster firing was also reduced in the Bevacizumab

group (from 21% to 3%, $p<0.05$). The data suggest that the anti-VEGF antibody may reduce spontaneous pain behaviors by decreasing the abnormal functional coupling between DRG neurons and newly growing blood vessels. Effects on evoked mechanical pain responses are also being investigated.

Disclosures: W. Xie: None. **J. Zhang:** None. **D. De Nardin Lückemeyer:** None. **S.V. Lackey:** None. **J.A. Strong:** None. **X. Dong:** None. **J. Zhang:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.11/AA24

Topic: D.02. Somatosensation – Pain

Support: JSPS KAKENHI JP21J00759
JSPS KAKENHI JP23K06820
The Nakatomi Foundation
ONO Medic al Research Foundation
AMED-CREST 22gm0910011
Takeda Science Foundation 15668360

Title: Arachidonic acid-containing phospholipids in the dorsal root ganglion contribute to the pathogenesis of neuropathic pain

Authors: *S. YAMAMOTO[1,2], T. HASHIDATE-YOSHIDA[2], T. SHIMIZU[2,3], H. SHINDOU[2,4];
[1]Med. Inst. of Bioregulation, Kyushu Univ., Fukuoka, Japan; [2]Natl. Ctr. for Global Hlth. and Med., Tokyo, Japan; [3]Inst. of Microbial Chem., Tokyo, Japan; [4]The Univ. of Tokyo, Tokyo, Japan

Abstract: Neuropathic pain is characterized by debilitating chronic pain symptoms such as spontaneous pain, hyperalgesia, and allodynia, and is often caused by damage to the nervous system that results from cancer, chemotherapy, diabetes, and trauma. The emergence of transcriptomics or proteomics technology has provided beneficial resources cataloging mRNA and protein expressions in pathological conditions. However, these approaches do not provide information regarding metabolites such as lipids that are not directly encoded by genes. Recently, we carried out comprehensive lipidome analysis on mice with peripheral nerve injury (PNI) as a neuropathic pain model, and found that PNI increased arachidonic acid-containing phospholipids (ARA-PLs) in the dorsal root ganglion (DRG) but not in the bulk tissue of spinal cord. Therefore, we focused on the roles of ARA-PLs and their biosynthetic enzyme LPCAT3 (lysophosphatidylcholine acyltransferase 3, also called LPLAT12) in neuropathic pain. Using omega-6 fatty acids (such as arachidonic acid and linoleic acid)-deficient diet (L6D), we revealed that pre-feeding of L6D suppressed PNI-induced mechanical allodynia and ARA-PLs

upregulation. Next, we established cell type-specific *Lpcat3*-knockout mice; DRG neuron (*Advillin*^{Cre}), macrophage (*Cx3cr1*^{CreERT2}), and satellite glia (*Gfap*-Cre), and found that PNI-induced mechanical allodynia were attenuated in DRG neuron-specific and satellite glia-specific *Lpcat3* knockout mice. These results suggest that LPCAT3-mediated increase of ARA-PLs in DRG neurons and/or satellite glia contribute to PNI-induced mechanical allodynia. Focusing on lipidome alteration has great potential to deepen our understanding of pathogenesis of neuropathic pain.

Disclosures: S. Yamamoto: None. **T. Hashidate-Yoshida:** None. **T. Shimizu:** None. **H. Shindou:** 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.; ONO PHARMACEUTICAL CO., LTD..

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.12/AA25

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS R01 NS070711
NIH/NINDS R37 NS108278
NIH/NINDS F32 NS124833

Title: Peripheral TNF inhibition alleviates acute vaso-occlusive hypersensitivity in a mouse model of sickle cell disease.

Authors: *V. L. EHLERS, M. KONDA, S. J. ZORN, A. SRIRAM, A. D. MENZEL, C. L. STUCKY;
Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Debilitating pain is the most common complication for individuals with sickle cell disease (SCD). A mainstay of pain treatment for acute vaso-occlusive episodes (VOCs) is opioid therapy, which, when accessible by patients, is often accompanied by adverse side effects. To improve patient quality of life and access to effective treatment for acute pain episodes, novel peripheral targets that alleviate acute pain need to be identified. The monoclonal antibody Infliximab binds to tumor necrosis factor (TNF)-α and is used to treat other forms of pain that share hallmarks with SCD tissue inflammation and neuropathy, including arthritis and fibromyalgia. While monocytes from patients with SCD show increased secretion of TNF-α, whether TNF inhibition alleviates pain in SCD mouse models, and the mechanisms by which this occurs, remains unknown. The current experiments use evoked behavior assays, *in vitro* calcium imaging, and qPCR analysis to interrogate the role of TNF in SCD acute pain. We first demonstrate that systemic injection of Infliximab in SCD mice alleviates acute, VOC-induced

hypersensitivity, and that this effect persists over repeated VOC insults. Following acute VOC, dorsal root ganglia (DRG) neurons from Infliximab-treated SCD mice display reduced expression of *Trpv1* and *Tlr4*, two transcripts implicated in other pain conditions, relative to DRG neurons from vehicle-treated SCD mice. Additionally, DRG neurons from Infliximab-treated SCD mice show reduced calcium flux in response to the TRPV1 agonist capsaicin compared to DRG neurons from vehicle-treated SCD mice. Finally, markers of microglial reactivity and TNF signaling display differential expression in several brain regions of Infliximab-treated SCD mice following VOC, suggesting peripherally administered Infliximab is likely influencing inflammatory signaling at the level of the CNS. Future work will determine the role of Infliximab in SCD DRG neuron excitability following VOC using *in vitro* whole-cell patch clamp recordings. Together, these data suggest that TNF inhibition alleviates acute hypersensitivity in SCD, and this effect is due to altered neuronal function at the level of the peripheral and central nervous systems.

Disclosures: V.L. Ehlers: None. **M. Konda:** None. **S.J. Zorn:** None. **A. Sriram:** None. **A.D. Menzel:** None. **C.L. Stucky:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.13/AA26

Topic: D.02. Somatosensation – Pain

Support: NS113243

Title: New Tools for Studying Satellite Glial Cells in Mouse and Human Dorsal Root Ganglia

Authors: *A. S. PRUDENTE[1], R. TONELLO[1], J. ROH[1], Z. K. FORD[1], S. LEE[1], C.-K. PARK[2], J. A. STRONG[1], J.-M. ZHANG[1], S. DAVIDSON[1], T. BERTA[1];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Dept. of Physiol., Gachon Univ., Incheon, Korea, Republic of

Abstract: The specific function of satellite glial cells (SGCs), which surround sensory neurons in peripheral ganglia, remains understudied due to a lack of tools for their immunostaining, purification, and manipulation. Recent single-cell RNA sequencing (RNAseq) has revealed new molecular and functional features of SGCs. However, the approach is costly and has shallow sequencing, limiting its use and poor detection of genes with low expression. As SGCs are located uniquely around sensory neurons and are predicted to have pain-related functions, better tools are necessary for studying their function in health and disease. In this study, we identified transmembrane hepatocyte cell adhesion molecule (HepaCAM) as a highly expressed SGC-specific marker in the dorsal root ganglia (DRGs), which is also expressed in astrocytes in the central nervous system. We characterized a monoclonal antibody that allows for the immunostaining of SGCs in both mouse and human DRGs, as well as a cost-effective and bench-

friendly method for SGC purification based on immunopanning or magnetic beads. Using this antibody, we optimized a protocol and isolated highly pure mouse and human SGCs, and identified their respective RNAseq profiles. Although our RNAseq findings suggest that key features are conserved in mouse and human SGCs, we also identified some important transcriptional and functional differences. We anticipate that these resources will be valuable for future research on SGCs and identifying potential pain treatment targets in humans.

Disclosures: **A.S. Prudente:** None. **R. Tonello:** None. **J. Roh:** None. **Z.K. Ford:** None. **S. Lee:** None. **C. Park:** None. **J.A. Strong:** None. **J. Zhang:** None. **S. Davidson:** None. **T. Berta:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.14/Web Only

Topic: D.02. Somatosensation – Pain

Title: Carbonic anhydrase in the antinociceptive effect of diosmin in neuropathic pain

Authors: *D. ESTRADA-SANCHEZ, L. MACÍAS-ROSALES, M. RIVERA-HUERTA, R. BUSTAMANTE-GARCÍA, A. CARBALLO-VILLALOBOS;
Natl. Autonomous Univ. Mexico, CDMX, Mexico

Abstract: The study of new drugs for neuropathic pain is of great relevance, since, until now, there is no single treatment that works for all pain conditions. Regarding the search for new alternatives for the treatment of this pathology, the inhibition of carbonic anhydrase has been found as a new pharmacological target, since this metalloenzyme is also located in the peripheral nervous system and has an important role in the establishment of chronic pain (Szabolcs et al., 1989). Currently, in vitro studies have shown that flavonoids, such as diosmin, have an inhibitory effect on this metalloenzyme. The flavonoid diosmin is a compound little explored in the treatment of pain; previous studies suggest that diosmin produces antihyperalgesic and antiallodynic activity in neuropathic pain models. For this reason, the aim of this study was demonstrate the pharmacological potential of diosmin and to elucidate it´s possible mechanism of action through the inhibition of carbonic anhydrase, in a model of neuropathic pain in male Wistar rats, induced by chronic constriction injury of the sciatic nerve (CCI). At 15 post-surgery days, the termal (Hargreaves test) and mechanical (Von Frey electronic test) hyperalgesic response was evaluated at different times (0, 30, 60, 90 and 120 minutes), it should be noted that each treatment was made up of a group of at least 6 animals. The groups treated with pregabalin (reference drug) and diosmin presented an antinociceptive effect similar to that observed in the sham group, compared to the vehicle group in both tests. Treatment with diosmin significantly decreased both thermal and mechanical hyperalgesia induced by CCI in Wistar rats, and treatments with carbonic anhydrase inhibitors, acetazolamide and sulfanilamide also significantly decreased thermal hyperalgesia. With the results obtained, it was demonstrated that diosmin

produces antihyperalgesic effects and these are mediated in part by the inhibition of carbonic anhydrase by modifying the effect in the presence of inhibitors.

Disclosures: D. Estrada-Sanchez: None. **L. Macías-Rosales:** None. **M. Rivera-Huerta:** None. **R. Bustamante-García:** None. **A. Carballo-Villalobos:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.15/AA27

Topic: D.02. Somatosensation – Pain

Support: R01DE029493
R01DE029493
DoD W81XWH2210723

Title: Mitochondrial dysfunction, neuroinflammation, and neurodegeneration underlie pain associated with head and neck cancer perineural invasion

Authors: *Y. YE[1], M. D. SANTI[1], M. ZHANG[1], N. H. TU[1], T. XIE[2], K. ASAM[1], B. AOUIZERAT[1], M. AMIT[2], D. BOADA[3];
[1]Translational Res. Center, NYU Pain Res. Ctr., New York Univ., New York, NY; [2]MD Anderson Cancer Ctr., Houston, TX; [3]Wake Forest Univ., Winston-Salem, NC

Abstract: Perineuronal invasion (PNI), a process that defined by cancer spreading and invading to the nerve, leads to increased spontaneous pain, mechanical allodynia, function impairment in patients with head and neck cancer (HNC). The mechanisms of PNI-associated pain is poorly understood; in vivo models are lacking. The objective of the study is to develop a clinically-relevant animal model and explore the pathophysiology of pain associated with PNI.
Since infraorbital nerve (ION) is a common site for the study of trigeminal neuropathic pain and patients with PNI in the ION exhibit orofacial neuropathies, we produced a syngeneic mouse model of PNI by inoculating a mouse oral cancer line (MOC-2) into the ION. We measured mechanical hypersensitivities using the facial von Frey and mouse oral function with a validated gnawing assay-the dolognawmeter. Spontaneous pain was measured by the conditioned place preference assay and the open field test. To test the responses of trigeminal neurons to mechanical stimulation, we performed in vivo intracellular single fiber recordings in mice with PNI. We used multiplexed immunofluorescence staining to determine whether neuroinflammation is a characteristic of PNI. RNA-sequencing was performed using the mouse ION tissues as well as human HNSCC tissues.
Mice with PNI in the ION exhibited both evoked and spontaneous nociception, as well as impaired oral function. PNI resulted in a significant loss of mechanical sensitivities in trigeminal neurons. In the remaining mechanosensitive neurons, tactile afferents were desensitized, nociceptors exhibited a trend to be sensitized. The perineural niche was marked by immune cell

infiltration. Particularly, CD68+/F4/80- cells were present abundantly within the endoneural space of nerves invaded by tumor cells. Finally, our RNA-sequencing analysis confirmed neuroinflammation associated with PNI as well as changes in pathways associated with mitochondrial dysfunction. In addition, ION-PNI exhibit nerve degenerative features with perturbed pathways including Alzheimer's, Parkinson's, and prion diseases etc. Consistent with findings from our mouse studies, our transcriptomic data derived from patients with HNC, show a significant Gene Ontology (GO) term enrichment for Alzheimer disease, neurodegeneration and neuroinflammatory pathways in patients with PNI.
In conclusion, we developed a novel, syngeneic, anatomically relevant *in vivo* model that could be used to study mechanisms of PNI-induced pain. Our animal model and human studies identified neuroinflammation, neurodegeneration, and mitochondria function as key mechanisms underlie PNI associated pain in HNC.

Disclosures: **Y. Ye:** None. **M.D. Santi:** None. **M. Zhang:** None. **N.H. Tu:** None. **T. Xie:** None. **K. Asam:** None. **B. Aouizerat:** None. **M. Amit:** None. **D. Boada:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.16/AA28

Topic: D.02. Somatosensation – Pain

Support: Science KAKENHI Grant 21K17132

Title: Involvement of long noncoding RNAs (lncRNAs) containing ultraconserved regions expressed in primary sensory neurons in a rat neuropathic pain model

Authors: *T. ITO[1], M. MARUYAMA[2], H. IWASAKI[2], Y. MIYAGAWA[2], H. SUZUKI[2], S. MAEDA[1], A. SAKAI[2];
[1]Tokyo Med. and Dent. Univ., Tokyo, Japan; [2]Nippon Med. Sch., Tokyo, Japan

Abstract: Purpose: Damage to primary sensory neurons is a primary cause of peripheral neuropathic pain, in which gene expression changes in these cells play a significant role. Recently, long non-coding RNAs (lncRNAs) have emerged as key players in various biological functions, mainly by regulating gene expression through binding to nucleic acids and proteins. Among them, lncRNAs containing transcribed ultraconserved regions (T-UCRs) are characterized by complete sequence conservation of over 200 bases across humans, mice, and rats, implying their shared functional significance. Therefore, in this study, we investigated the involvement of T-UCRs expressed in primary sensory neurons in peripheral neuropathic pain. Methods: A neuropathic pain model was produced by complete ligation of the fifth lumbar (L5) spinal nerve in Sprague-Dawley rats. Pain behaviors were evaluated using a set of von Frey hairs and Plantar test. Comprehensive analysis of T-UCRs expressed in the L5 dorsal root ganglion was performed using a microarray. Full-length sequences of T-UCRs that exhibited an altered

expression was identified using a rapid amplification of cDNA ends (RACE). Expression changes after nerve injury was examined using a quantitative PCR. Expression distribution in the L5 dorsal root ganglion was examined using in situ hybridization. To specifically modulate T-UCR expression levels in primary sensory neurons, a serotype 6 adeno-associated virus vector was injected into the dorsal root ganglion.
Results and Discussion: Through comprehensive analysis, we identified several T-UCRs whose expressions were significantly altered in the L5 dorsal root ganglion. These T-UCRs were found to be persistently down-regulated from 4 days post nerve injury. Moreover, downregulation of T-UCR in primary sensory neurons of naïve rats caused mechanical
allodynia and thermal hyperalgesia, while upregulation relieved neuropathic pain. These findings suggest the involvement of T-UCR expressed in primary sensory neurons in the development of neuropathic pain. The functional analysis of this gene may provide insights into the molecular mechanisms underlying the pathogenesis of neuropathic pain.

Disclosures: T. Ito: None. **M. Maruyama:** None. **H. Iwasaki:** None. **Y. Miyagawa:** None. **H. Suzuki:** None. **S. Maeda:** None. **A. Sakai:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.17/BB1

Topic: D.02. Somatosensation – Pain

Support: 1F31NS125941-01
5R37NS108278-04

Title: Role of epidermal Piezo1 channel in paclitaxel-induced mechanical allodynia.

Authors: *O. ISAEVA[1], A. MIKESELL[1], A. MENZEL[1], A. SRIRAM[1], M. SCHULTE[2], C. L. STUCKY[1];
[1]Med. Col. of Wisconsin, Milwaukee, WI; [2]Versiti Blood Res. Inst., Milwaukee, WI

Abstract: Epidermal Piezo1 is highly expressed in rodent and human keratinocytes and plays a critical role in normal touch sensation in mice. However, whether epidermal Piezo1 signaling contributes to mechanical hypersensitivity in injury models is not known. In the present study, we evaluated the role of epidermal Piezo1 in sensitization to mechanical stimuli associated with chemotherapy-induced neuropathic pain (CIPN). We show that the treatment of mice with a chemotherapeutic agent, paclitaxel, induces robust behavioral mechanical allodynia. At the single keratinocyte level, paclitaxel treatment induces an increase in the calcium response of keratinocytes to mechanical stimulation, an increase in calcium response to Piezo1 channel agonist, Yoda1, in human and mouse keratinocytes and a decrease in the threshold of mechanically activated current recorded from mouse keratinocytes. Using epidermal cell-specific Piezo1 knockout mice (Keratin14_Piezo1cKO), we show that deletion of Piezo1 in keratinocytes

partially protected against paclitaxel-induced mechanical allodynia. We further evaluated the effect of paclitaxel treatment on Piezo1 activity in the heterologous cell system. We show that paclitaxel treatment results in a significant increase in pressure-induced Piezo1 activation and an increase in the conductance of single-channel Piezo1 current expressed in the HEK293T cell line. Our data suggest that paclitaxel treatment induces sensitization Piezo1 channel that may contribute to paclitaxel-induced mechanical allodynia.

Disclosures: O. Isaeva: None. **A. Mikesell:** None. **A. Menzel:** None. **A. Sriram:** None. **M. Schulte:** None. **C.L. Stucky:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.18/Web Only

Topic: D.02. Somatosensation – Pain

Support: TTUHSC Start Up

Title: The Role of Citrullination-Neutrophil Extracellular Trap in Diabetic Painful Neuropathy

Authors: V. THAKUR[1], K. MCGRATH[2], E. CHEN[2], *M. CHATTOPADHYAY[3]; [1]Mol. and Translational Med., [2]Paul L Foster Sch. of Med., Texas Tech. Univ. Hlth. Sci. Ctr. at El Paso, El Paso, TX; [3]Mol. and Translational Med., Texas Tech. Univ. Hlth. Sci. Ctr. - El Paso Campus, El Paso, TX

Abstract: Diabetic painful neuropathy (DPN) is one of the most detrimental complications of diabetes. Alterations in neuroinflammatory mediators play significant roles in the development of DPN. Infiltration of the neutrophils and monocyte/macrophages contributes significant role in the degenerative process of the distal sciatic nerve by forming neutrophil extracellular traps (NETs) under diabetic condition. Citrullination of histones due to increase in protein arginine deiminase (PAD) enzyme activity under hyperglycemia may promote NET formation, which can further increase the cytokine production by activating macrophages and proliferation of neutrophils. This study reveals that the increase in histone deacetylases (HDAC) are crucial in DPN and inhibition of HDAC using HDAC inhibitor FK228 would suppress NETosis and alleviate diabetic nerve degeneration and pain. FK228, also known as Romidepsin, is a potent FDA approved HDAC inhibitor for the treatment of cutaneous T-cell lymphoma however, the molecular mechanism of this drug is not completely understood. In this study, type 2 diabetic mice with pain were treated with intraperitoneal injections of FK228 at a dose of 1mg/kg, 2 times a week for 3 weeks. The results demonstrate that FK228 treatment can alter the expression of neutrophil elastase, extracellular or cell free DNA, the levels of citrullinated histone-3, PADI4, as well as growth associated protein-43 in the dorsal root ganglia, spinal cord dorsal horn neurons, sciatic nerve and foot skin of diabetic animals. The results also suggest that FK228-treatment can alleviate thermal hyperalgesia significantly along with changes in

expression of HDACs and expression of inflammatory mediators in diabetic animals. Overall, this study suggests that FK228 could offer an alternative treatment approach for DPN.

Disclosures: **V. Thakur:** None. **K. McGrath:** None. **E. Chen:** None. **M. Chattopadhyay:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.19/BB2

Topic: D.02. Somatosensation – Pain

Support: NIH R01 DK121131
NIH R01 DK118137

Title: Satellite glial cells mediate viscero-somatic cross-organ sensitization by facilitating spinal central sensitization

Authors: N. TIWARI, *L. QIAO;
Virginia Commonwealth Univ., Richmond, VA

Abstract: It is common in humans for pain to be perceived at a remote location away from the diseased organ. For example, a heart attack can cause neck, shoulder, and back pain. Patients with bowel diseases also often experience heightened sensitivity in the urinary bladder and somatic pain in the legs. The comorbidity is much more devastating. The underlying neural circuit and molecular mechanisms of cross-organ sensitization, especially viscera (e.g., colon) to somatic (e.g., hindpaw) cross-sensitization are not clear except that it involves spinal central sensitization. Following colonic inflammation, the level of calcitonin gene-related peptide (CGRP) in the dorsal horn of the spinal cord is increased to contribute to spinal central sensitization, however, CGRP levels in the injured colonic afferent neurons are decreased. We previously showed that CGRP levels are increased in the uninjured dorsal root ganglia (DRG) neurons within the injured spinal segments during colonic inflammation, therefore, we hypothesize that satellite glial cells (SGCs) that connect the injured and uninjured neurons within DRG have a role in spinal central sensitization and viscero-somatic cross-organ sensitization. In DRG, pain mediator brain-derived neurotrophic factor (BDNF) is generated by injured neurons and acts on TrkB.T1 in SGCs to facilitate SGC activation through calcium (Ca^{2+})-dependent pathway. Thus, we pursued SGC-targeted inhibition by using the Cre-loxP technique to delete TrkB.T1 specifically from SGCs ($TrkB.T1^{cKO}$) to examine the impact of TrkB.T1 deletion-mediated SGC inhibition on colonic inflammation-induced somatic hypersensitivity. In our study, colonic inflammation was induced by intracolonic instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Somatic mechanical and thermal sensitivity were assessed by hindpaw responses to graded von Frey filament stimulation or hot plate assay, respectively. Our results show that 1) TrkB.T1 in SGCs mediated BDNF-induced Ca^{2+} transients, and cAMP response

elements (CRE)-regulated luciferase activity; 2) TNBS-induced colitis increased hindpaw mechanical and thermal sensitivity in a sex-dependent manner ($n>5$, $p\leq0.05$); 3) TrkB.T1^{cKO} did not change baseline mechanical or thermal sensitivity but resulted in an attenuation of colitis-induced hindpaw mechanical hypersensitivity ($n>5$, $p\leq0.05$); 4) TrkB.T1^{cKO} did not impact colitis-induced thermal hypersensitivity ($n>5$, $p\leq0.05$). Together, these findings infer that SGCs participate in colon-to-hindpaw cross-organ sensitization that is mediated by spinal central sensitization, and SGCs can be a therapeutic target to mitigate widespread pain.

Disclosures: **N. Tiwari:** None. **L. Qiao:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.20/BB3

Topic: D.02. Somatosensation – Pain

Title: Trigeminal ganglia neurons TRAPped by facial brushing transduce mechanical allodynia in mice with trigeminal neuropathic injury

Authors: ***T. LI**[1], V. ARORA[1], S. KUMARI[1], M. J. CATERINA[2], Y. KIM[3], M. CHUNG[1]; [1]Dept. of Neural and Pain Sci., Univ. of Maryland, Baltimore, Sch. of Dent., Baltimore, MD; [2]Neurosurg., Johns Hopkins Sch. Med., Baltimore, MD; [3]Dept. of Oral & Maxillofacial Surgery, UT Hlth. San Antonio, San Antonio, TX

Abstract: *c-Fos* is an early response gene involved in gene transcription and tightly related toneuronal activity. In spinal dorsal horn neurons, activation of c-Fos has been used asa surrogate marker of neuronal activation. Recent studies suggest that Fos-mediatedgene regulation in primary afferents plays an important role in neuropathic pain.However, the contribution of c-Fos^{+} primary afferents to neuropathic pain remainsunclear. In mice with chronic constriction injury of infraorbital nerve (ION-CCI), facialbrushing induced a transient upregulation of *Fos* transcript in a subset of trigeminalganglia (TG) neurons. The majority of *Fos*+ neurons were colocalized with markers oflow threshold mechanoreceptors (LTMRs), such as *Ntrk2*, *Ntrk3*, and *Mafa*.Interestingly, a subset of *Fos*+ neurons were also colocalized with markers ofnociceptors, such as *Calca* and *Mrgprd*. To determine the contribution of theseneurons to mechanical allodynia, we adopted Targeted Recombination in ActivePopulations (TRAP) methods. Adeno-associated virus encoding Cre-dependenthM4Di, an inhibitory chemogenetic receptor, or mCherry was injected into TG of*Fos*^{CreER} mice. Two weeks after ION-CCI or sham surgery, mice with ION-CCI, but notsham surgery, showed robust mechanical allodynia. Tamoxifen was injectedintraperitoneally and facial brushing was delivered to TRAP brushing-activated TGneurons. In mice with AAV-hM4Di, systemic injection of clozapine-N-oxide (CNO)reduced mechanical allodynia, whereas CNO injection did not alter mechanicalallodynia in mice with AAV-mCherry. In mice with sham surgery, mechanical thresholdwas not altered in both AAV-hM4Di or AAV-mCherry groups. In contrast, in *Fos*^{CreER}mice with tamoxifen injection without

facial brushing, CNO injection did not influencemechanical sensitivity. Chemogenetic activation of the brushing TRAPped TG neuronsthrough hM3Dq, an excitatory chemogenetic receptor, increased the duration ofspontaneous wiping behavior in mice with ION-CCI. In conclusion, brushing stimulationinduced *Fos* expression in TG of mice with ION-CCI, and the inhibition of the brushingTRAPed TG neurons decreased mechanical allodynia, and the activation of theseneurons induced aversive behaviors. Therefore, *Fos* expression can be a usefulmarker of allodynia-mediating neurons in trigeminal neuropathy.

Disclosures: **T. Li:** None. **V. Arora:** None. **S. Kumari:** None. **M.J. Caterina:** None. **Y. Kim:** None. **M. Chung:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.21/BB4

Topic: D.02. Somatosensation – Pain

Support: The Korea Health Technology R&D Project (HI21C0572) through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare
The Pioneer Research Center Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning (2022M3C1A3090851)
The National Research Foundation (NRF) grant funded by the Korea government (MSIT) (2022M3C1A3081359)

Title: Severity of pain after nerve injury is not associated with structral feature of neuroma but with Ion channel distribution.

Authors: *J. KWON[1,2,3], J. KIM[3,4];
[1]Korea Univ., Seoul, Korea, Republic of; [2]Dept. of Hlth. Science, Grad. Sch., Transdisciplinary Major in Learning Hlth. System, Korea Univ., Seoul, Korea, Republic of; [3]Dept. of Hlth. Sci., Korea Univ. Grad. Sch., Seoul, Korea, Republic of; [4]Dept. of Hlth. and Envrn. Sci., Korea Univ. Undergrad. Sch., Seoul, Korea, Republic of

Abstract: Neuroma formation after partially or completely disrupted by nerve injury cause a painful condition. However, the underlying mechanisms of neuroma-associated pain are not fully understood. In addition, there were no studies about the association between the structural characteristics/molecular mechanisms within neuroma formation and pain severity. In the present study, we investigated the correlation between structural characteristics of neuroma and pain severity. We also investigated the role of alterations in different types of voltage-gated ion channel distribution with a prevalence of neuroma pain.To develop painful neuroma, the tibial nerve was completely transected and injured nerve was ligated in rats. Behavioral test was

quantified by using paw withdrawal threshold (PWT) test, Hargreaves test, and non-stimulus spontaneous pain test. Four months after nerve injury, the diameter of neuroma was measured, and correlations between the size of the neuroma and the pain severity were analyzed using Spearman's rank correlation test. To compare the fiber distribution in neuroma, sensory fiber was classified by labeling neurofilament (NF) with calcitonin gene-related peptide (CGRP), and the number of sensory filaments ($NF^+/CGRP^+$) was analyzed. The changes in the expression of axonal sodium channels (Nav1.7 and Nav1.8) and potassium channels (Kv1.2 and Kv1.4) were analyzed at day 7 and day 28 after nerve injury.We found that formation of neuromas was all present in rats with or without pain. In the present data, there was no significant association between size of neuroma and pain severity. They show any correlation between the number of sensory fibers and neuroma pain severity. However, the greater density of Nav channels was observed in painful neuroma compared to painless neuroma at both day 7 and day 28. Furthermore, Kv1.4 channel was more expressed in painless neuroma than in painful neuroma at day 7 although the expression of Kv1.2 channel did not significantly different between painful and painless neuroma. Our results suggest that severity of pain after nerve injury is not related to the structural features of neuroma, but to the difference in ion channels distribution. These findings suggest that changes in sodium channel expression could be a possible mechanism for painful neuroma.

Disclosures: **J. Kwon:** None. **J. Kim:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.22/BB5

Topic: D.02. Somatosensation – Pain

Support: P30GM122733

Title: Assessing the therapeutic potential of cannabichromene (CBC) for managing chemotherapy-induced neuropathic pain

Authors: *M. A. DE LEON[1], V. RAJE[1], W. GUL[1], M. ELSOHLY[1], H. M. HARRIS[2], N. M. ASHPOLE[1];
[1]BioMolecular Sci., Univ. of Mississippi, Oxford, MS; [2]Columbia Univ., New York, NY

Abstract: Chemotherapy-induced peripheral neuropathy is a debilitating adverse effect experienced by roughly 30-40% of patients receiving chemotherapy treatment. Unfortunately, current therapeutic strategies require long-term treatment with limited efficacy, often requiring opioid-based medications. Studies have suggested the potential use of cannabis-based medicines to alleviate neuroinflammation and subsequent pain. While multiple studies have explored the effects of delta^9-tetrahydrocannabinol and synthetic cannabinoids, a non-psychoactive cannabinoid would likely be a stronger candidate for drug development. Evidence indicates that

cannabichromene (CBC) has anti-inflammatory activity while devoid of psychoactive effects. Therefore, our current study evaluated the effectiveness of CBC and related derivatives against paclitaxel and cisplatin-induced neuropathic pain. To assess its impact against cisplatin-induced neuropathic pain (CINP), mice underwent a cisplatin dosing regimen (12-day period: alternating days of Ringers vehicle vs cisplatin injections). Increasing doses of CBC and a novel CBC derivative, CBC-Val-Hs, were administered two days following completion of cisplatin regimen. Mechanical sensitivity was assessed using an electronic von Frey (eVF) in which acute administration of CBC and CBC-Val-HS ablated the associated allodynia in doses greater than 10 mg/kg. To assess whether CBC could prevent the onset of neuropathic pain, CBC and cisplatin were co-administered to a cohort of mice in which mice that received 25mg/kg CBC showed significantly less tactile allodynia several days following cisplatin treatment when compared to mice that received vehicle. These data indicate that CBC can attenuate and reduce the extent of CINP. Ongoing studies are evaluating if CBC and CBC-Val-HS will also be effective in attenuating and preventing paclitaxel-induced neuropathic pain. For this, cohorts of mice are undergoing a paclitaxel dosing regimen (8-day period: four injections with one-day intervals) with and without co-administration of 10mg/kg CBC. In concordance with the use of the eVF to assess mechanical sensitivity, our lab is expanding in the use of non-invasive techniques to evaluate other pain behaviors within our models (e.g., the use of DeepLabCut to assess grimace and paw withdrawal). Together, these data suggest CBC is a promising potential therapeutic for preventing or alleviating neuropathic pain. Further studies are required to elucidate the effects of repeated dosing of CBC, sex-specific differences in pain and cannabinoid responsiveness, and determine the optimal therapeutic window of CBC.

Disclosures: **M.A. De Leon:** None. **V. Raje:** None. **W. Gul:** None. **M. ElSohly:** None. **H.M. Harris:** None. **N.M. Ashpole:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.23/BB6

Topic: D.02. Somatosensation – Pain

Support: NIH NIDDK R01 DK120824
NIH NIDDK U01 NS113873

Title: Functional, molecular, and morphological characterization of mouse glutamatergic myenteric neurons

Authors: *J. LIU, S. ZHANG, S. EMADI, T. GUO, L. CHEN, B. FENG;
Univ. of Connecticut, Storrs, CT

Abstract: The enteric nervous system (ENS) functions largely independent from the central nervous system (CNS) in regulating gastrointestinal peristalsis, i.e., coordinated motor

movements that require the interplay among functionally and morphologically distinct enteric neuron classes, including the intrinsic sensory neurons (intrinsic primary afferent neurons [IPANs]), descending and ascending interneurons, and excitatory and inhibitory motor neurons. We recently reported that a fraction (~2%) of neuron somata in the myenteric ganglia are positive for vesicular glutamate transporter type 2 (VGLUT2), indicating their potential neuromodulatory role of extrinsic primary afferents to relay information to the CNS via glutamatergic neural transmission. However, there is limited research focusing on the functional diversity of this specific subpopulation of VGLUT2-positive enteric neurons (VGLUT2-EN). In this study, we aimed to systematically characterize VGLUT2-EN using adeno-associated virus (AAV)-mediated sparse-labeling, single-cell mRNA sequencing (sc-seq), and GCaMP6f calcium (Ca^{2+})-imaging. Our sparse-labeling study revealed that the majority of VGLUT2-EN (18/20, 90%) exhibited Dogiel type I morphology, with approximately 65% of these cells identified as descending interneurons each projecting to 19-34 myenteric ganglia. Only 10% (2/20) of the VGLUT2-EN displayed Dogiel type II morphology, indicative of IPANs. By analyzing the marker genes obtained from our sc-seq data, we identified 47.69% of the VGLUT2-EN as descending interneurons and 9.23% as IPANs, consistent with the proportions from morphological analyses. Additionally, we conducted GCaMP6f recordings from individual VGLUT2-EN in an ex vivo preparation with flattened colorectum undergoing graded circumferential stretch, which revealed that most, if not all VGLUT2-EN were activated by colorectal stretch at the noxious level. The GCaMP6f responses to colorectal stretch was mostly inhibited by hexamethonium, a nicotinic acetylcholine receptor antagonist that blocks cholinergic neural transmission. Thus, we conclude that VGLUT2-ENs are mostly descending interneurons each projecting into multiple myenteric ganglia and that are activated indirectly by noxious colorectal stretch via cholinergic synaptic transmission. Overall, the current study sheds light on the potential role of VGLUT2-ENs in facilitating communication between the ENS and CNS via glutamatergic neural transmission to extrinsic afferents, and also highlights their potential as pharmacological targets for modulating visceral pain.

Disclosures: **J. Liu:** None. **S. Zhang:** None. **S. Emadi:** None. **T. Guo:** None. **L. Chen:** None. **B. Feng:** None.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.24/BB7

Topic: D.02. Somatosensation – Pain

Title: Phenotypic and functional models of chemotherapy induced peripheral neuropathy using scalable human induced pluripotent stem cell derived sensory neurons

Authors: *S. B. PAULSON[1], G. MCCABE[1], M. WINKLER[2], T. FINDLEY[3], I. GOLDBERG[3], K. YEUNG[3], O. H. U. SCHROEDER[2], P. WALSH[1], V. TRUONG[1];

[1]Anatomic Inc., Minneapolis, MN; [2]NeuroProof Systems GmbH, Rostock, Germany; [3]ViQi Inc, Santa Barbara, CA

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a common side effect of cancer treatment that results in damage to the peripheral nerves. This can significantly impact a patient's quality of life and can even lead to dose reductions or treatment discontinuation. In order to develop treatments and/or preventative measures for CIPN, there is a need for high-throughput, reproducible peripheral nerve disease models that give insight into the interplay between chemotherapeutics, axonal morphology, function, and potential neuroprotective compounds. In a phenotypic study, we treated human induced pluripotent stem cell-derived sensory neurons (hiPSC-SN) that are functionally and molecularly similar to primary DRG with four chemotherapy drugs (bortezomib, oxaliplatin, paclitaxel, and vincristine) in dose response with and without pre-treatment of the SARM1 inhibitor DSRM-3716. After 48 hours of treatment, axonal and mitochondria health was analyzed via beta-III tubulin (TUJ1) and tetramethylrhodamine, methyl ester (TMRM) staining. Paclitaxel altered soma morphology and axonal branching, while oxaliplatin showed minimal morphology changes. Both showed minimal mitochondrial membrane dysfunction. Bortezomib and Vincristine greatly degraded axons while also reducing mitochondrial activity. Vincristine-treated mitochondria exhibited a “fragmented” morphology. Using an AI-based high content analysis software, AutoHCS™ (ViQi, Inc.), images from the study were automatically detected and scored to identify dose-dependent phenotypic responses to the drugs and the neuroprotective effects of DSRM-3716. Here, it is demonstrated that DSRM-3716 could have a protective effect on sensory neurons treated with paclitaxel and oxaliplatin. To study how sensory neuron function could be affected by chemotherapeutics, sensory neurons were cultured on microelectrode arrays for 21 days. Vincristine induced hyperactivation after one hour of application. When co-treated with gabapentin after 48 hours, there was a rescue effect where spike train parameters returned to control levels. Together, these findings demonstrate the ability to phenotypically and functionally screen CIPN-related and potential neuroprotective compounds in human nociceptors in high throughput systems.

Disclosures: **S.B. Paulson:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **G. McCabe:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **M. Winkler:** A. Employment/Salary (full or part-time):; NeuroProof. **T. Findley:** A. Employment/Salary (full or part-time):; ViQi Incorporated. **I. Goldberg:** A. Employment/Salary (full or part-time):; ViQi Incorporated. **K. Yeung:** A. Employment/Salary (full or part-time):; ViQi Incorporated. **O.H.U. Schroeder:** A. Employment/Salary (full or part-time):; NeuroProof. **P. Walsh:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **V. Truong:** A. Employment/Salary (full or part-time):; Anatomic Incorporated.

Poster

PSTR476. Peripheral Mechanisms of Neuropathic Pain II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR476.25/BB8

Topic: D.02. Somatosensation – Pain

Support: NIH Grant PA-20-185

Title: The Characterization of GPR183 within Dorsal Root Ganglion During Chronic Pain States

Authors: *L. SALIH[1], A. MOUTAL[2], F. XU[3], D. SALVEMINI[4];
[1]St. Louis Univ., Saint Louis, MO; [2]Pharmacol. and physiology, St. Louis Univ., St Louis, MO;
[3]Dept. of Biol., [4]Pharmacol. and Physiological Sci., St. Louis Univ., Saint Louis, MO

Abstract: GPR183 is a recently deorphanized G-protein coupled receptor, activated by the oxysterol 7α,25-dihydroxycholesterol. GPR183 activation induced cold- and mechanical allodynia via activation of the mitogen-activated protein kinase (MAPK) and nuclear factor κB (NFκB) pathways. GPR183 transcript expression was increased in the spinal cord of rats with the chronic constriction injury (CCI) model of chronic neuropathic pain. We then investigated if GPR183 was expressed in rat dorsal root ganglion (DRG) and the function of GPR183 in these neurons. We found that GPR183 is primarily expressed within small and medium peptidergic neurons. However, treatment of cultured sensory neurons with the GPR183 ligand 7α,25-dihydroxycholesterol had no effect on intracellular calcium dynamics or on sensory neuron excitability. In chronic neuropathic pain, GPR183 protein expression increased within the CCI's injured DRG. Treatment with the selective GPR183 antagonist, SAE-14, reversed mechanical allodynia in rats with CCI. Indicating that GPR183 is tonically activated in chronic neuropathic pain to sensitize mechanical responses. Together, these results have begun the characterization of GPR183 expression and function in DRG sensory neurons. While the precise function of GPR183 in DRG remains unclear, we show that the receptor expression is increased in chronic neuropathic pain and that GPR183 inhibitors can hold a therapeutic value for chronic neuropathic pain.

Disclosures: L. Salih: None. **A. Moutal:** None. **F. Xu:** None. **D. Salvemini:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.01/BB9

Topic: D.02. Somatosensation – Pain

Support: RTI International Research and Development Funds

Title: Development of GPR139 antagonist probes

Authors: *A. DECKER, M. T. RAHMAN, H. CHAMINDA LAKMAL, C. JIN;
RTI Intl., RTP, NC

Abstract: GPR139 is an orphan G protein-coupled receptor (GPCR) extensively expressed in the medial habenula and locus coeruleus, brain regions implicated in drug dependence and opioid analgesia. A number of studies suggest that blocking GPR139 may have potential as a pain

treatment strategy by enhancing the analgesic efficacy of opioid medicines, thus necessitating a lower dose of opioids and thereby increasing opioid safety. In vitro studies demonstrated that GPR139 co-expresses with the mu opioid receptor (MOR) in the brain and negatively modulates MOR signaling. GPR139 activation was shown to promote MOR internalization, which leads to tolerance of opioid analgesics. In vivo studies showed that GPR139 knockout (KO) mice were more sensitive to the effects of morphine than wild-type mice and required a lower dose of morphine to effectively reduce pain response. While multiple synthetic GPR139 agonists are being developed to study receptor activation, there are only a few literature reports of GPR139 antagonists that have moderate potencies and poor drug-like properties. Thus, a potent GPR139 antagonist with good drug-like properties remains an unmet need for investigations into both the basic pharmacology and therapeutic potential of GPR139. This poster presents the design, synthesis, and pharmacological characterization of a series of novel GPR139 antagonists based on the JNJ-3792165 scaffold, a GPR139 antagonist reported in the literature. In vitro ADME analysis of selected compounds is also presented. This work provides critical structure activity relationship (SAR) information to guide further lead optimization, which will expedite the drug discovery research targeting GPR139.

Disclosures: **A. Decker:** None. **M.T. Rahman:** None. **H. Chaminda Lakmal:** None. **C. Jin:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.02/BB10

Topic: G.04. Emotion

Support: National Council for Scientific and Technological Development, CNPq - (429859/2018-0, 312747/2020-9)
Coordination for the Improvement of Higher Education Personnel, CAPES, Finance Code 001
L'ORÉAL-UNESCO-ABC for Women in Science

Title: Sex-dependent effects of maternal separation and social isolation on paclitaxel-induced nociceptive behavior in adult mice: Exploring the therapeutic strategy of 4-PSQ-loaded polymeric nanocapsules

Authors: *J. J. PALTIAN[1,2], C. A. R. DA FONSECA[2], A. P. B. WILLE[2], V. C. PRADO[3], D. ALVES[2], L. CRUZ[3], C. LUCHESE[2], B. J. KOLBER[4], E. A. WILHELM[2];
[1]Dept. of Neurosci., Univ. of Texas at Dallas, Richardson, TX; [2]Federal Univ. of Pelotas, Pelotas, Brazil; [3]Federal Univ. of Santa Maria, Santa Maria, Brazil; [4]Neurosci., Univ. of Texas at Dallas, Dept. of Neurosci., Richardson, TX

Abstract: Early-life stress reprograms biochemical stress response pathways, which may also have implications for the processing of chronic pain conditions, such as neuropathic pain. Cancer survivors treated with chemotherapeutic agents, such as paclitaxel (PTX), not only experience peripheral neuropathy but also suffer from prolonged stress. There is no specific treatment to alleviate peripheral neuropathy. The first aim of this study was to investigate the effects of maternal separation and social isolation (MSSI) on PTX-induced peripheral neuropathy (PIPN) in adulthood. The second aim was to evaluate the pharmacological effects of a promising candidate, 7-chloro-4-(phenylselanyl) quinoline-loaded polymeric nanocapsules (4-PSQ NC), on PIPN-induced behavior. Male and female Swiss mice aged 14 days were utilized for the study. Pups from the MSSI groups were housed with their mothers until the 14th postnatal day. On 15th day, these pups were housed in individual isolation cages for 6 h/day for 7 days. Thereafter, animals in this group remained in isolation until 8 weeks of age. Unstressed pups were reared under standard conditions. After this period, the animals' baseline response was assessed, followed by intraperitoneal (i.p.) administration of PTX (2 mg/kg) or 5% glucose solution (10 mL/kg, i.p.) once a day, for 3 days. Treatment with the 4-PSQ NC (1 mg/kg, intragastrically (i.g.)) or the unloaded formulation (10 mL/kg, i.g.) was performed at 48-hour intervals from day 6 to 18. Nociceptive response was evaluated on days 5, 11, and 17 of the experimental protocol. On day 19, the animals were euthanized to remove the adrenal glands. Our results indicate that MSSI affects baseline mechanical nociception, but not thermal nociception. After PTX exposure, both MSSI and unstressed male and female mice showed increased mechanical and thermal sensitivities. Male MSSI mice treated with PTX displayed a marked increase in mechanical and thermal sensitivities compared with unstressed mice. Interestingly, female mice subjected to MSSI and treated with PTX showed decreased thermal hypersensitivity compared to the unstressed group receiving PTX. This suggests that MSSI in female mice may actually increase resistance to PIPN in adulthood. Additionally, both male and female mice demonstrated an increase in relative adrenal gland weight, supporting the validity of the MSSI model. The study further demonstrates that alternate-day treatment with 4-PSQ NC reversed mechanical and thermal sensitivities in both MSSI and unstressed mice exposed to PTX. These findings highlight the potential of 4-PSQ NC as a therapeutic strategy to neuropathy induced by chemotherapy exposure.

Disclosures: J.J. Paltian: None. **C.A.R. da Fonseca:** None. **A.P.B. Wille:** None. **V.C. Prado:** None. **D. Alves:** None. **L. Cruz:** None. **C. Luchese:** None. **B.J. Kolber:** None. **E.A. Wilhelm:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.03/BB11

Topic: D.02. Somatosensation – Pain

Support: NIH Grant

Title: Oprm1 gene therapy for oral cancer pain

Authors: ***K. INOUE**[1], T. H. NGUYEN[2], J. DOLAN[2], S. YAMANO[3], S. YAMANO[3], B. L. SCHMIDT[2];
[1]NYU Dent. Translational Res. Ctr., New York University, Col. of Dent., New York, NY; [2]NYU Dent. Translational Res. Ctr., [3]Dept. of Prosthodontics, New York Univ. Col. of Dent., New York, NY

Abstract: Oral cancer patients often suffer from severe function-induced pain during eating, drinking and speaking. Quality of life is severely degraded in these patients. We seek to eliminate oral cancer pain with gene therapy. To improve non-viral gene transfer efficiency, we developed a novel non-viral hybrid vector - a cell-permeable peptide combined with a cationic lipids. *OPRM1* gene encodes the μ-opioid receptor (MOR), which is methylated and transcriptionally silenced in oral cancer patients. Nuclear factor-κB (NF-κB) represents a family of inducible transcription factors. which regulates a large array of genes. We hypothesize that forced expression *OPRM1* in oral cancer regulates the NF-kB pathway and causes secretion of endogenous opioids. To test this hypothesis, we generated a novel non-viral hybrid vector to transfect the *OPRM1* gene into oral cancer. We established different oral cancer models and demonstrated *OPRM1* re-expression following in vivo non-viral transfection with *OPRM1* plasmid DNA. Forced expression of *OPRM1* reduced the NF-kB signaling pathway. The in vivo studies showed that the mice with tongue cancer expressing *OPRM1* exhibited decreased nociception compared to control. Non-viral delivery of the *OPRM1* gene targeted to the cancer microenvironment has an analgesic effect. Non-viral gene delivery is a potential treatment for cancer pain.

Disclosures: K. Inoue: None. **T.H. Nguyen:** None. **J. Dolan:** None. **S. Yamano:** None. **S. Yamano:** None. **B.L. Schmidt:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.04/BB12

Topic: D.02. Somatosensation – Pain

Support: NIH Grant AD304-6218

Title: Racial differences in the response to a mindfulness intervention for chronic pain

Authors: J. J. POLCARI[1], **B. NEPHEW**[2], A. RODRIGUEZ[2], V. MELICAN[2], *J. KING[2], P. GARDINER[3];
[1]Worcester Polytechnic Inst., Winchester, MA; [2]Worcester Polytechnic Inst., Worcester, MA; [3]Cambridge Hlth. Alliance, Cambridge, MA

Abstract: Chronic pain is one of the most common reasons adults seek medical care in the US, with estimates of prevalence up to 40% and relatively higher rates in diverse populations. Mindfulness meditation has been associated with significant improvements in pain, depression, physical and mental health, sleep, and overall quality of life. Group medical visits are increasingly common and are effective at treating myriad illnesses including chronic pain. Integrative Medical Group Visits (IMGV) combine mindfulness techniques, evidence based integrative medicine, and medical group visits and can be used as adjuncts to medication, particularly in diverse underserved populations with limited access to non-pharmacological therapies. The objective of the present study was to assess the effects of race on the primary pain outcomes and evaluate potential relationships between race and additional patient characteristics in data from a randomized clinical trial of IMGV in socially diverse, marginalized patients suffering from chronic pain and depression. It was hypothesized that there would be racial differences in the effects of IMGV on pain outcomes. Our analyses identified significant racial differences in the response to IMGV. Black subjects had increased pain severity throughout the duration of the 21-week study but were less likely to respond to the pain intervention compared to White subjects. These results may be related to differential comorbidity rates, catastrophizing, and digital health literacy among these participant groups. To improve patient outcomes in similar studies, interactions between pain outcomes and these factors require further investigation to affect levels and trajectory of pain severity and enhance the response to complimentary interventions.

Disclosures: J.J. Polcari: None. **B. Nephew:** None. **A. Rodriguez:** None. **V. Melican:** None. **J. King:** None. **P. Gardiner:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.05/BB13

Topic: D.02. Somatosensation – Pain

Support: Department of Defense's Congressionally Directed Medical Research Program (Grant #: W81XWH2010277)

Title: Captopril loaded thermo-responsive hydrogel reverses mechanical hyposensitivity in the Lepr$^{db/db}$ type 2 diabetes model

Authors: *J. M. NICHOLS[1], C. V. CRELLI[2], H. PHAM[1], A. DHAYANI[2], C. GAFFNEY[1], F. CHERRY[1], L. LIU[2], P. GRACE[1], J. JANJIC[2], A. SHEPHERD[1];
[1]Symptom Res., MD Anderson Cancer Ctr., Houston, TX; [2]Duquesne Univ., Pittsburgh, PA

Abstract: Millions of people worldwide suffer from diabetic peripheral neuropathy (DPN). Recently, our lab has been exploring novel biomaterial/pharmaceutical combinations for the treatment of DPN. Previous works have shown that inhibition of angiotensin II can be effective

in treatment of neuropathic pain after injury to the sciatic nerve. Here we use a novel formulation of the angiotensin converting enzyme (ACE) inhibitor Captopril embedded in a thermo-responsive hydrogel to treat sensory loss associated with the Lepr$^{db/db}$ model of type 2 diabetes mellitus. We hypothesized that local administration of our Captopril-Hydrogel (Cap-HG) would inhibit the generation of angiotensin II by ACE and restore sensation to the distal limb of Lepr$^{db/db}$ mice with DPN. As part of our initial examination of safety and efficacy for the novel Cap-HG and drug free hydrogel (DF-HG) formulations, Cap-HG and DF-HG were tested *in vitro* with RAW 264.7 cells for effects on cell viability and inhibition of ACE activity prior to *in vivo* testing. Cap-HG and DF-HG were then administered to the hind feet of WT or Lepr$^{db/db}$ mice, and Von Frey tests were performed to determine the duration of the therapeutic effect. Following these tests, CAP-HG and DF-HG were injected into the foot pads of WT or Lepr$^{db/db}$ mice to examine *in vivo* safety and efficacy. As part of determining the safety of these hydrogels, we examined inflammatory infiltrates in the hind foot, and systemic blood pressure. To determine the effects of Cap-HG and DF-HG on the hind paws we analyzed the proteome profile of hind paw lysate and intra-epidermal nerve fiber (IENF) density from skin samples. From our *in vitro* testing we were able to conclude that Cap-HG is non-toxic as compared to free captopril at the same dose, while maintaining a similar inhibition of ACE activity. *In vivo* testing also showed that our Cap-HG was capable of reversing mechanical hyposensitivity when DF-HG was not. Importantly, this occurred in the absence of any significant inflammatory response in the hind foot or systemic drop in blood pressure, as measured by IVIS imaging and blood pressure cuff respectively. Proteome profiling of hindfoot lysates from uninjected, DF-HG injected, and Cap-HG injected showed a normalization of the proteome from Lepr$^{db/db}$ mice after Cap-HG injection; however, analysis of IENF density in the hind foot showed no significant difference compared to DF-HG injected mice. Taken together, the results from this study show the safety and efficacy of local therapeutic treatment with Cap-HG in DPN and suggest that the therapeutic potential of the treatment lies in the ability to alter the local microenvironment and not in the regrowth of IENFs.

Disclosures: **J.M. Nichols:** None. **C.V. Crelli:** None. **H. Pham:** None. **A. Dhayani:** None. **C. Gaffney:** None. **F. Cherry:** None. **L. Liu:** None. **P. Grace:** None. **J. Janjic:** None. **A. Shepherd:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.06/BB14

Topic: D.02. Somatosensation – Pain

Support: Harvard Medical School Charles Robert Broderick III Phytocannabinoid Research Grant

Title: High-content calcium imaging reveals potency of cannabidiol and other synthetic cannabinoids in blocking diverse classes of sensory neuron receptors

Authors: *G. V. CHAHYADINATA, J. NAM, A. BATTENBERG, B. WAINGER; Massachusetts Gen. Hosp., Boston, MA

Abstract: Cannabidiol (CBD), a principal non-psychoactive component of *Cannabis sativa*, has been approved to treat refractory seizures associated with Dravet syndrome, Lennox-Gastaut syndrome, and tuberous sclerosis. CBD's therapeutic potential has been hypothesized to include a wider range of neurological disorders, including pain. Its potency as an analgesic is tied to the modulation of molecular targets in the dorsal root ganglia (DRG), including voltage-gated channels, ligand-gated channels, and G-protein coupled receptors. However, CBD's targets remain poorly understood with only a small group of studies that each focus on a small number of specific receptors. Furthermore, despite promising in-vitro and in-vivo animal studies, the analgesic effect of CBD has not been replicated in well-powered clinical trials. To explore this discrepancy and assess CBD's effects on a wide range of sensory neuron receptors within broad groups of cells, we leverage the use of the APPOINT (automated physiological phenotyping of individual neuronal types) platform, a novel methodology that combines high-throughput single-cell calcium imaging, liquid handling, and automated analysis. We show that exposure to micromolar CBD concentrations (1-10uM) causes a dose-dependent inhibition of a large variety of ionotropic receptors (e.g. TRPV1, TRPA1, TRPM8, P2X3) and metabotropic receptors (e.g. BK, PGE2, SST, S1PR) associated with nociceptive signaling in dissociated murine DRG. CBD significantly reduces the percent of cells responding to these receptor agonists and the amplitude of responsive cells. Furthermore, CBD can inhibit extracellular and intracellular calcium flux, by blocking ryanodine receptors, Orai channels, and IP3 receptors. These findings have been validated through patch clamp experiments, which reveal profound effects of CBD in suppressing nociceptor excitability and blocking both voltage-activated sodium and potassium channels even at sub-micromolar concentrations. To expand the use of the APPOINT assay, we have also screened and identified other synthetic cannabinoids that effectively block calcium flux in sensory neurons. Altogether, our study brings a unique capacity to understand CBD's broad effects on various sensory neuron receptors as well as potential insight into the lack of effective studies in humans.

Disclosures: G.V. Chahyadinata: None. **J. Nam:** None. **A. Battenberg:** None. **B. Wainger:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.07/BB16

Topic: D.02. Somatosensation – Pain

Support: Dr John P. and Therese E. Mulcahy Endowed Professorship in Ophthalmology
Richard A. Perrit M.D. Charitable Foundation
K&P Scientific LLC

Title: Development of a novel therapeutic scFv antibody for the treatment of neuropathic pain.

Authors: *G. SANDHU[1], A. KUNAMNENI[4], K. N. WESTLUND[5,6], M. A. MONTERA[5], W. JESKE[2], J. FAREED[1], S. KAJA[3,2], R. DURVASULA[4];
[2]Mol. Pharmacol. & Neurosci., [3]Ophthalmology, [1]Loyola Univ. Chicago, Maywood, IL; [4]Infectious Dis., Mayo Clin., Jacksonville, FL; [5]Anesthesiol. and Critical Care Med., Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [6]Biomed. Lab. Res. & Develop., VA New Mexico Healthcare Syst., Albuquerque, NM

Abstract: Chronic neuropathic pain conditions like trigeminal neuralgia (TN) and sciatica lead to a serious decline in quality of life with partial or no improvement from currently available treatments. Despite their abuse potential, opioids remain the mainstay therapy for chronic neuropathic pain conditions. Microarray gene expression profiling in an established mouse trigeminal nerve constriction model of TN revealed a 4.0- and 2.7-fold increase in cholecystokinin-B receptor (CCKBR) expression in the trigeminal ganglia (TG) on day 3 and day 21, respectively, after trigeminal nerve injury. In the current project, we hypothesized that CCKBR overactivity contributes to nociceptive propagation in chronic neuropathic pain and that targeting CCKBR can serve as a novel efficacious non-opioid therapy in chronic neuropathic pain. To identify the mechanism of CCKBR-mediated nociception, firstly, we investigated the expression of the μ-opioid receptor (MOR) and CCKBR in rat and mice's lumbar dorsal root ganglia (DRG) and lumbar spinal cord using immunohistochemistry and found that they co-localized on a subset of neurons. Next, to demonstrate the functional antagonism of MOR by CCKBR, we selected the neuronal SH-SY5Y neuroblastoma cell line to conduct *in vitro* mechanistic studies. Immunocytochemistry revealed the colocalization of MOR and CCKBR in SH-SY5Y cells. Subsequently, we performed mechanistic studies in SH-SY5Y cells using fluorophore-based live-cell Ca^{2+} imaging. We demonstrated that activation of CCKBR increased intracellular Ca^{2+} while activation of MOR reduced it., Notably, the reduction in intracellular Ca^{2+} by MOR was opposed and overcome by the activation of CCKBR. Next, we developed an antibody approach as a non-opioid therapy by generating single-chain fragment variable (scFv) antibodies targeting CCKBR using the ribosomal display. The antibody selection was done based on high affinity, specificity, stability, and solubility enabling preclinical development for *in vivo* and *in vitro* efficacy studies. *In vivo*, a single intraperitoneal dose (given 3 wk or 7 wk after induction of trigeminal neuropathic chronic pain in mice) significantly reduced mechanical and cold hypersensitivity in both male and female mice through 10-12 wk of testing. Our data provide critical proof of concept that anti-CCKBR scFv can improve the behavioral functionality in a murine TN model and supports our hypothesis that CCKBR plays a role in nociceptive propagation in TN. Studies evaluating the efficacy of anti-CCKBR scFv antibodies in preclinical models for sciatica are currently underway.

Disclosures: G. Sandhu: None. **A. Kunamneni:** None. **K.N. Westlund:** None. **M.A. Montera:** None. **W. Jeske:** None. **J. Fareed:** None. **S. Kaja:** None. **R. Durvasula:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.08/BB17

Topic: D.02. Somatosensation – Pain

Support: NIH R01AT011517
University of Arizona Institutional Funds

Title: Identification and Localization of GPR63 and GPR153 as Novel Modulators of Opioid-Induced Antinociception in Chronic Pain

Authors: *A. PENA[1,2], E. GEVELHOFF[2], J. M. STREICHER[2];
[1]Neurosci., The Univ. of Arizona, Tucson, AZ; [2]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Chronic pain is a significant burden worldwide with variable etiologies complicating the development of pharmacotherapies for pain management. Treatments for pain disorders are currently dominated by opioid drugs which lose efficacy over time and yield various undesirable side effects. Thus, the need for identifying novel targets for modulating pain is critical. In this work, we performed a behavioral screening of four novel orphan G-protein coupled receptors - GPR63, GPR141, GPR150, and GPR153 - with poorly understood functions and no verified ligands to identify a potential role in modulating pain. Receptors were knocked down via *in vivo* transfection of targeted CRISPR-Cas9 DNA constructs in the spinal cords of adult male and female CD-1 mice. Another cohort of animals received a non-targeted universal negative control (NC) CRISPR construct. Acute pain was evaluated by tail flick over a two-hour time course following injection of 3.2 mg/kg morphine subcutaneously (SC). Chronic pain, modeled by chemotherapy-induced peripheral neuropathy (CIPN), was induced by 2 mg/kg paclitaxel via intraperitoneal (IP) injection resulting in the development of mechanical allodynia. This was followed by administration of 3.2 mg/kg morphine SC and a three-hour von Frey time course to evaluate changes in mechanical pain threshold. Receptor knockdown of all four candidates yielded no significant changes in the acute tail flick pain model. Knockdown of GPR63 and GPR153, however, completely blocked the analgesic effect of morphine in CIPN. All behavioral experiments were performed with n=20 as determined by power analysis, and equal amounts of male and female mice were used. These findings suggest that these receptors are not involved in direct neurotransmission of nociceptive pain but instead play roles in the neuropathology of chronic pain and/or in altering cellular responses to opioids in chronic pain. To further investigate this finding, we performed RNAScope *in situ* hybridization to colocalize RNA transcripts of mouse *Gpr63* and *Gpr153* with markers for nociceptive neurons, microglia, or astrocytes, all of which are known to contribute to the development of chronic pain. Together this work identifies completely novel pain modulators which could be exploited to develop new pain therapies.

Disclosures: A. Pena: None. **E. Gevelhoff:** None. **J.M. Streicher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Botanical Results, LLC, Teleport Pharmaceuticals, LLC.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.09/BB18

Topic: D.02. Somatosensation – Pain

Support: The College of Pharmacy, University of Minnesota

Title: Agmatine inhibits NMDA receptor-mediated calcium transients in spinal cord dorsal horn through nNOS attenuation.

Authors: T. XIE[1], R. E. SCHORN[2], C. D. PETERSON[1], K. F. KITTO[2], G. L. WILCOX[2], L. VULCHANOVA[2], ***C. A. FAIRBANKS**[3];
[1]Pharmaceutics, [2]Neurosci., [3]Pharmaceutics, Pharmacology, Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Previous research showed that intrathecally (i.t.) administered agmatine, an endogenous decarboxylated form of L-arginine that selectively blocks GluN2B-containing NMDARs, effectively attenuates and reverses tactile hypersensitivity without affecting motor coordination. Agmatine is also neuroprotective, which is mediated by neuronal nitric oxide synthase (nNOS) inhibition. This study aims to determine the mechanism of agmatine in spinal cord dorsal horn. We hypothesize that agmatine concentration- dependently attenuates NMDAR-mediated Ca^{2+} transients in the mouse spinal cord dorsal horn with GluN2B specificity through nNOS inhibition.
Female and male ICR mice (4-6 weeks, n=3-4 per sex) were perfused prior to laminectomy, and ex vivo spinal slices were incubated with Fluo-4. GluN2B conditional knock-down (GluN2B-KD) was achieved by intraspinal injections of AAV9-hSyn-GCaMP6s-cre and AAV9-hSyn-GCaMP6s-Δcre to C57 GluN2Bfl/fl mice. Ca^{2+} was imaged by two-photon microscopy and APV, ifenprodil, and agmatine were applied to spinal cord slices. Time-lapse of images were captured and the peak amplitude of fluorescence intensity were analyzed by Student's t-test and ANOVA. ICR mice (4-6 weeks, n=3-4 per sex) was i.t. injected with NMDA (0.3 nmol) and nociceptive response and thermal hyperalgesia (warm water immersion test; 49°C) were measured following i.t. agmatine and ICOS78201. Following agmatine incubation, NMDARs-mediated Ca^{2+} transients were concentration dependentlyattenuated, similar to APV and ifenprodil. In the GluN2B-KD animals, inhibition of NMDAR-mediated Ca^{2+} response by 100 μM ifenprodil (n=3 per sex, $P<0.01$) was significantly reversed, but not with incubation of 3.3 mM agmatine. When agmatine was co-applied with PSD95-nNOS inhibitor, IC87201, the attenuation of Ca^{2+} response by agmatine was significantly resolved (n=3 per sex, $P<0.01$). The alleviation of NMDA-induced thermal hyperalgesia by i.t. agmatine (1 nmol) was significantly reversed by IC87201 (0.01 nmol) pre-injection, while IC87201 (0.01 nmol) itself did not inhibit NMDA-induced thermal hyperalgesia (n=3 per sex, $P< 0.01$).
Agmatine concentration-dependently attenuated the NMDARs-mediated Ca^{2+} transients, suggesting that agmatine is an effective inhibitor of NMDARs in the spinal cord dorsal horn, which is consistent with theresults from electrophysiological and neuropharmacological studies. Inhibition of PSD-95-nNOS tethering by application of IC87201 reversed the inhibition of

NMDA-induced Ca^{2+} transients and thermal hyperalgesia consistent with agmatine's established inhibitory activity at nNOS.

Disclosures: **T. Xie:** None. **R.E. Schorn:** None. **C.D. Peterson:** None. **K.F. Kitto:** None. **G.L. Wilcox:** None. **L. Vulchanova:** None. **C.A. Fairbanks:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.10/BB19

Topic: D.02. Somatosensation – Pain

Title: A human plasma fraction alleviates cold allodynia in the mouse oxaliplatin-induced peripheral neuropathy model of pain

Authors: G. ZUNINO[1], T. BOLKVADZE[2], ***M. DUDEK**[2], S. BÄCK[2], V. KHEIFETS[1], T. BRAGGE[2], B. LU[1];
[1]Alkahest, Inc., San Carlos, CA; [2]Charles River Discovery Services, Kuopio, Finland

Abstract: Neuropathic pain (NP) is triggered by a lesion or disease of the central or peripheral somatosensory nervous system and is characterized by abnormal hypersensitivity to stimuli (hyperalgesia) and nociceptive responses to non-noxious stimuli (allodynia). Management of NP is challenging due to the heterogeneity of its etiology, symptoms, and underlying mechanisms, and more efficient approaches to treat and cure NP are urgently needed. Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting, adverse side-effect caused by oxaliplatin (OXP) and other chemotherapeutic agents and can be modelled in mice by repeated dosing of OXP. We utilized a manufacturing scale subfractionation approach to produce a therapeutically relevant human plasma fraction from healthy donors. This plasma fraction has been previously shown to provide benefits in CNS and peripheral phenotypes associated with diseases of aging. The objective of the study was to examine whether the plasma fraction can repair oxaliplatin-induced peripheral neuropathy (OIPN).
The study was performed on 60 male mice at the age of 7-8 weeks. Peripheral neuropathy was induced by six i.p. doses of 4.5 mg/kg OXP within a period of 3 weeks (D0 to D20). On D21 following the last OXP injection, cold allodynia was assessed by the acetone cooling test. Thereafter, a 7-day daily i.v. dosing of plasma fraction (150 µL/mouse) commenced and continued until D27. Responses of OIPN-related cool allodynia intensity to the treatments were tested on D34, D41, D48, and D55. Compound Muscle Action Potential (CMAP) and Nerve Condition Velocity (NCV) recordings were performed on D21 (pre-treatment), D42, and D56. At the study endpoint on D56, plasma, muscle and sciatic nerve samples were collected.
All OXP-treated groups displayed a robust model induction in the acetone cooling test (ACT) compared to Vehicle group on D21. Despite the highly significant cool allodynia prior to initiation of bioactive dosing, plasma fraction treatment led to significantly lower allodynia than the OXP-Vehicle group on D34, D41, D48, and D55. There was no difference in CMAP

amplitude between the groups. The NCV velocity was slower in the OXP-treated mice compare to Vehicle-treated mice on D21, D42 and D56, but no treatment effect was observed.
Taken together, plasma fraction treatment significantly reversed the OXP-induced cool allodynia compared to OXP Vehicle group. This data provides a therapeutically relevant approach to reverse CIPN. Additional subfraction of the plasma fraction combined with deep proteomic analysis to identify the bioactives contained within will inform and identify drivers of peripheral nerve rejuvenation.

Disclosures: **G. Zunino:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **T. Bolkvadze:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **M. Dudek:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **S. Bäck:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **V. Kheifets:** A. Employment/Salary (full or part-time):; Alkahest, Inc. **T. Bragge:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **B. Lu:** A. Employment/Salary (full or part-time):; Alkahest, Inc..

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.11/BB20

Topic: D.02. Somatosensation – Pain

Title: Acetaminophen inhibits diacylglycerol lipase A: implications for nociception

Authors: *A. STRAIKER, M. DVORAKOVA, K. MACKIE, J. BILLINGSLEY, H. B. BRADSHAW;
Indiana Univ., Bloomington, IN

Abstract: Introduction: Acetaminophen is one of the most commonly used medications for pain and fever relief in the world. First synthesized in 1877, its mechanism of action remains unclear. As a consequence, even though acetaminophen liver toxicity causes ~500 deaths each year in the US alone, it has not been possible to design safer alternatives. The endocannabinoid system is an endogenous signaling system consisting of cannabinoid receptors, lipophilic ligands known as endocannabinoids and the enzymatic machinery to produce and break down these lipids. Cannabinoid receptors are widely distributed in the CNS and elsewhere in the body and have been reported to affect pain and inflammation. Endocannabinoids have been proposed to have a role in acetaminophen action. **Methods:** In this study, we tested for an interaction between acetaminophen and endocannabinoid signaling in autaptic hippocampal neurons, a well-characterized model of endogenous neuronal cannabinoid signaling as well as lipase activity assays and a hot plate test for nociception. **Results:** We now report that acetaminophen inhibits endocannabinoid production in these neurons, doing so at concentrations (as low as 10uM) that fall within the range achieved clinically when acetaminophen is used to treat pain. In lipase activity experiments we find that acetaminophen inhibits the activity of diacylglycerol lipase α

(DAGLα) but not DAGLb. This gave rise to the counterintuitive hypothesis that DAGLα *inhibition* may be antinociceptive. In a hot plate test we confirm that the analgesic effects of acetaminophen require CB1 and intriguingly we find that DAGL inhibition by RHC80267 (20mg/kg) is antinociceptive in WT but not CB1 knockout mice. **Conclusions:** Based on these findings we propose 1) that DAGLα may play a counterintuitive role in some forms of nociception and 2) a novel mechanism for the antinociceptive actions of acetaminophen whereby acetaminophen inhibits a DAGLα/CB1-based circuit that plays a permissive role in at least one form of nociception.

Disclosures: A. Straiker: None. **M. Dvorakova:** None. **K. Mackie:** None. **J. Billingsley:** None. **H.B. Bradshaw:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.12/BB21

Topic: D.02. Somatosensation – Pain

Support: NINDS UG3 NS123964 (RCL)
NINDS R21 NS105880 (RCL)
NINDS 3R21NS105880-01S1 (RCL)
DoD W81XWH-19-1-0525 (RCL)

Title: Replication-defective HSV carbonic anhydrase-8 non-opioid analgesic prolongs afterhyperpolarization in small rat primary afferents via activation of kv7 channels

Authors: *C. SARANTOPOULOS[1], M. B. KANDEL[1], G. Z. ZHUANG[1], Y. KANG[1], M. MARZULLI[2], W. F. GOINS[2], J. C. GLORIOSO[2], R. C. LEVITT[1];
[1]Anesthesiol., Univ. of Miami, Miami, FL; [2]Dept. Microbiol. Mol. Genet, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Treating chronic pain sufferers with safe, efficacious non-opioid analgesics is challenging, but of high priority. Using a novel JDNI8 replication-defective HSV-1 viral vector, expressing carbonic anhydrase 8 (CA8) analgesic peptide (vHCA8), we show robust DRG transduction of primary afferent neurons via intra-articular knee joint injection, which produces profound, prolonged analgesia. To investigate vHCA8 analgesic mechanism-of-action we examined excitability parameters of small rat DRG neurons. Electrophysiologic parameters in neurons infected with wild-type vHCA8 vectors (vHCA8WT) were compared with controls, -which were those infected with inactive null-point mutant (S100P) (vHCA8MT) and non-infected cells-, using the same recording technique. Whole-cell current-clamp recordings were obtained from small (<30 μm) DRG neurons, selected by vHCA8-GFP green fluorescence, and depolarized by brief square current command pulses, 48 hours after neurons were dissociated and infected with vHCA8. There were no changes in resting membrane potential, amplitude or

duration of the action potential between vHCA8WT and controls. vHCA8WT, as compared to controls, significantly prolonged the afterhyperpolarization (AHP) duration (419±187ms vs 232±148 ms, P=0.006) and enhanced AHP peak amplitude (-9.3±4.9 mV vs -6.2±3.4 mV, P=0.003). These changes in AHP by vHCA8WT were completely reversed by the selective Kv7 blocker, XE-991. Other K^+ channel blockers (eg, apamin, iberiotoxin, glibenclamide) failed to reduce AHP in vHCA8WT infected neurons. Our results are consistent with vHCA8 attenuating neuronal excitability via activation of Kv7 voltage-gated potassium channels known to be important analgesic therapeutic targets.

Disclosures: C. Sarantopoulos: A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS UG3 NS123964. **M.B. Kandel:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS UG3 NS123964. **G.Z. Zhuang:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS UG3 NS123964, NINDS R21 NS105880. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT (61/847,405), Shareholder Adolore Biotherapeutics, Inc. **Y. Kang:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **M. Marzulli:** A. Employment/Salary (full or part-time):; University of Pittsburgh School of Medicine. **W.F. Goins:** A. Employment/Salary (full or part-time):; University of Pittsburgh School of Medicine. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT (62/532,182), Shareholder Adolore Biotherapeutics, Inc. **J.C. Glorioso:** A. Employment/Salary (full or part-time):; University of Pittsburgh School of Medicine. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT (62/532,182), Shareholder Adolore Biotherapeutics, Inc. **R.C. Levitt:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NINDS UG3 NS123964, NINDS R21 NS105880, DoD W81XWH-19-1-0525. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor of PCT (61/847,405), Shareholder Adolore Biotherapeutics, Inc.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.13/BB22

Topic: D.02. Somatosensation – Pain

Support: Department of Pharmacology
Department of Dermatology Goltz professorship
College of Pharmacy

Title: Agmatine-based analog inhibits spinal NMDAr-mediated EPSCs with apparent subunit selectivity

Authors: *L. CAYE[1], C. D. PETERSON[2], C. A. FAIRBANKS[3], G. L. WILCOX[4];
[1]Pharmacol., [2]Col. of Pharm., [3]Pharmaceutics, Col. of Pharm., [4]Neuroscience, Pharmacology, Dermatol., Univ. of Minnesota, Minneapolis, MN

Abstract: The N-methyl-D-aspartate receptor (NMDAr) serves as an effective therapeutic target for non-opioid analgesics. Previous research has shown that agmatine, a decarboxylated form of L-arginine, produces antinociception in vivo, largely mediated by antagonism of NMDA receptors containing NR2B subunits located in the spinal cord dorsal horn. We developed a strategically substituted analog of agmatine (SSA3) with improved biopharmaceutical features that produces analgesia in pre-clinical models of chronic pain. However, the molecular mechanisms of this analog have yet to be studied. We therefore characterized the efficacy and subunit selectivity of this compound at the NMDA receptor in the dorsal horn of mouse spinal cord slices.
Methods: We evaluated SSA3 antagonism of spinal NMDArs as measured by a decrease in the excitatory postsynaptic current (EPSC) amplitude and duration. Male and female *$Na_V1.8$-ChR2*-expressing mice were used for this experiment in order to selectively activate $Na_V1.8$-expressing nociceptive afferents. Anesthetized mice were sacrificed and 400 µm transverse spinal cord slices were taken from the lumbar spinal cord. After obtaining a whole cell configuration, neurons were voltage-clamped at +40 mV to relieve Mg^{++} blockade and 470nm blue light pulses (10 ms) were shone through a 60x objective onto the root entry zone and the resulting NMDAr-mediated EPSCs were recorded. Blue light pulses were delivered every 30 seconds and a 5 second voltage clamp recording was taken after each one. Once a baseline responsiveness was established, the tissue was subsequently incubated for 3 minutes at a time with increasing concentrations of SSA3. Pharmacological blockers NBQX, picrotoxin, and strychnine were present in the control and drug solutions to isolate NMDAr-mediated EPSCs.
Results: SSA3 effectively reduced the amplitude of blue light-evoked NMDA EPSCs in a concentration-dependent manner over a range from 0.3 mM to 10 mM, consistent with that seen with agmatine (Waataja et al., J Neurophysiol., 2019). SSA3 also concentration-dependently reduced EPSC duration as indicated by the decay constant Tau. This reduction in EPSC duration is consistent with NR2B over NR2A subunit selectivity.
Conclusion: The results of this study support the hypothesis that SSA3 shares a mechanism of action with agmatine. The improved biopharmaceutical features of SSA3 together with this subunit selectivity suggest that SSA3 may be a therapeutically useful compound.

Disclosures: L. Caye: None. **C.D. Peterson:** None. **C.A. Fairbanks:** None. **G.L. Wilcox:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.14/BB23

Topic: D.02. Somatosensation – Pain

Support: NIH - R01 DK115478
NIH - F32 DK128969
BWF - 1022337

Title: The contribution of amygdala calcitonin gene-related peptide (CGRP) receptors on the development of persistent bladder pain

Authors: *L. LEWTER[1,2], U. CHATTERJEE[1], E. SCHMITZ[1], A. NOFAL[1], B. KOLBER[1,2]; [1]Neurosci., The Univ. of Texas at Dallas, Richardson, TX; [2]Ctr. for Advanced Pain Studies, Richardson, TX

Abstract: Chronic pain is a significant public health challenge, affecting hundreds of millions worldwide. Most pain research focuses on somatic pain. However, visceral pain (e.g., bladder pain) is also a serious health issue and is currently understudied. Within the brain, the central amygdala (CeA) is an important contributor to the pathology of chronic pain. Findings from both human and rodent studies have revealed left versus right brain differences in the amygdala in pain modulation. The amygdala in the brain's right hemisphere has been shown to increase pain outside of the body and in internal organs, including the bladder. Previous work in our lab shows that calcitonin gene-related peptide (CGRP) has divergent functions in the left and right CeA in a mouse model of acute bladder pain. However, the influence of CGRP receptors (CGRP-Rs) on the development of bladder pain is unknown. For this study, we sought to determine the contribution of CGRP-Rs on the hemispherical and temporal changes of the CeA as bladder pain transitions from acute to chronic/persistent pain. We used a mouse model of bladder pain (100 mg/kg cyclophosphamide, 3 days) to conduct physiology, histology, behavior, and *in vivo* calcium imaging experiments 2-21 days post-injury (DPI). Urinary bladder distension and visceromotor responses (UBD-VMR) and bladder histology (hematoxylin and eosin staining) were used to measure bladder pathology progression. We administered a CGRP antagonist - $CGRP_{8-37}$ (or vehicle) into the right or left CeA before abdominal von Frey (mechanical sensitivity) in male and female mice. Data show that $CGRP_{8-37}$ attenuated the development of bladder nociception in the abdominal von Frey assay when administered in the right CeA but not the left. Additionally, gradient-index (GRIN) lenses were implanted into the right and left CeA of *Calcrl*-Cre mice to measure the neural activity of CGRP-R positive cells over time. Preliminary data indicate that spontaneous neural activity of CGRP-R cells was similar in both hemispheres across time. However, the stimulus-evoked activity of CGRP-R cells was increased in the right CeA compared to the left CeA. Collectively, these data support the study of cell-specific manipulation of the right amygdala to produce pain relief. Focusing on the contributions of CGRP receptors in visceral pain modulation will provide insight into the underlying

mechanisms contributing to bladder pain. These data, in turn, will lead to the development and advancement of effective central nervous system-targeted therapies for chronic bladder pain.

Disclosures: L. Lewter: None. **U. Chatterjee:** None. **E. Schmitz:** None. **A. Nofal:** None. **B. Kolber:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.15/BB24

Topic: D.02. Somatosensation – Pain

Support: NIH R01 DK120824
U01 NS113873

Title: Alleviating visceral pain by spatially and temporally synchronized low-frequency stimulation of dorsal root ganglia

Authors: *L. CHEN, S. ZHANG, E. WOON, B. FENG;
Univ. of Connecticut, Storrs, CT

Abstract: Background: Visceral pain is the cardinal complaint of many functional disorders associated with visceral and pelvic floor organs. It is contributed to by heightened peripheral drive from sensitized C-fiber afferents. We previously reported that dorsal root ganglion (DRG) stimulation efficiently blocks C-fiber transmission at 20-50 Hz, which is the optimal blocking frequency (OBF) to attenuate afferent drive from C-fiber afferents. The OBF for thinly myelinated Aδ-fibers is 20-100 Hz, with significant overlap with the OBF for C-fibers. To achieve selective C-fiber transmission block, we developed a multisite DRG stimulation protocol for delivering temporally and spatially synchronized stimulations (TSSS), each operating at frequencies below the OBF for blocking C-fibers. Methods: Male C57BL/6 mice (8-12 weeks, 25-35 g) were used in this study. Stainless steel wire electrodes were sutured to the oblique abdominal musculature for recording the electromyogram (EMG) of the visceromotor response (VMR) evoked by colorectum distension (CRD) delivered from a distending balloon inserted intra-anally. The bilateral L6 DRGs were carefully exposed to allow the insertion of a microfabricated bilateral stimulating electrode array into the bilateral L6 foramen for delivering the TSSS. A telemetric stimulating and recording system were used to record the EMG signals and to deliver TSSS to both L6 DRGs via a total of 10 electrode pairs. The five electrode pairs on one DRG deliver trains of 5 Hz stimulation (0.2 ms pulse width) each with a relative temporary delay of 40 ms, which collectively form a TSSS of 25 Hz stimulation at a focal region in the DRG. The VMR to CRD recorded in urethane-anesthetized mice was used as a readout of colorectal afferent transmission, which is mostly contributed to by C-fibers. The VMR to CRD was recorded before, right after, and 30 minutes after the delivery of TSSS. Results: The urethane anesthesia protocol enables robust recordings of VMR to CRD, which was suppressed

by 80% immediately after TSSS to bilateral L6 DRG. The VMR returned to the pre-stimulus level 30 minutes after the cessation of TSSS. In contrast, concurrent stimulations from five pairs of electrodes (each at 5 Hz) with no temporal delay did not efficiently suppress the VMR to CRD. Conclusion: We provided proof-of-concept that TSSS with each stimulation below the OBF for C-fibers enables reversible blockage of colorectal afferent transmission. The low-frequency stimulation (5 Hz) at each site on the DRG likely mitigates the off-target blocking effects on Aδ-fibers.

Disclosures: L. Chen: None. **S. Zhang:** None. **E. Woon:** None. **B. Feng:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.16/BB25

Topic: D.02. Somatosensation – Pain

Support: College of Pharmacy, University of Minnesota

Title: Development of agmatine-based NMDAr antagonists for the treatment of post-operative pain

Authors: *C. PETERSON[1], L. CAYE[2], K. KITTO[3], G. L. WILCOX[4], C. FAIRBANKS[1]; [2]Mol. Pharmacol. and Therapeut., [3]Neurosci., [1]Univ. of Minnesota, Minneapolis, MN; [4]Dept Neurosci, Pharmacol, Dermatol, Univ. Minnesota Med. Sch., Minneapolis, MN

Abstract: NMDA antagonism is a well-established non-opioid clinical strategy to reduce pain with limited side effects. Agmatine, decarboxylated l-arginine, is an NMDA antagonist with established pre-clinical and clinical efficacy in reducing pain but may be limited by pharmacokinetic and pharmacodynamic parameters. To this end, we developed a series of agmatine-based compounds and characterized their analgesic efficacy in a mouse model of post-operative pain. Methods: We designed and synthesized strategically-substituted agmatine compounds (SSAs) with an intent to assess their efficacy in a model of post-incisional hyperalgesia. Female and male ICR mice (21-30 g) were given an incisional injury to the plantar surface of the hindpaw, then received an intravenous injection of either vehicle control or SSA3 prior to recovery from isoflurane anesthesia. Morphine sulfate (MS) was delivered as a positive control either as a single agent or in combination with SSA3. von Frey mechanical thresholds were assessed prior to injury and following recovery from isoflurane anesthesia. In addition, open field activity was recorded prior to and following the surgical incision, and assessed to evaluate alterations in locomotion, sedation, and anxiety behavior in both the incised mice and an additional, non-injured control cohort.
Results: We observed that increasing doses of the NMDAr antagonist SSA3 were effective in reducing mechanical hypersensitivity following incisional injury of the paw. Contralateral hindpaws did not display altered thresholds, indicating a lack of a motoric effect. Additionally,

open field analysis did not show any alteration from vehicle control in ambulatory distance, ambulatory time, or duration in the inner zone of the apparatus in the injured subjects. Non-injured subjects also evaluated for open field activity did not display altered ambulatory distance, ambulatory time, or duration in inner zone. Taken together, these data support the efficacy of SSA3 to reduce mechanical hypersensitivity following surgical incision.

Disclosures: C. Peterson: None. **L. Caye:** None. **K. Kitto:** None. **G.L. Wilcox:** None. **C. Fairbanks:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.17/CC1

Topic: D.02. Somatosensation – Pain

Support: F32DE0300031
K99DE031802 - 01A1
R01NS106301
R01DA044481

Title: Mechanistic enhancement of motor cortex stimulation-induced antinociception

Authors: *N. MERCER LINDSAY[1,2], S. HAZIZA[1], T. M. BAER[1], M. J. SCHNITZER[1], G. SCHERRER[2];
[1]Stanford Univ., Palo Alto, CA; [2]The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Motor cortex stimulation (MCS) reduces pain experience in humans suffering from chronic pain; however, antinociceptive efficacy is inconsistent across patients. Prior work suggests that opioidergic signaling in descending pain pathways, notably in the rostral ventromedial medulla (RVM), mediates pain reduction after MCS. Using mice as a model system, we sought to identify stimulation protocols that enhance MCS analgesic efficacy by detailing response properties of key neurons in the stimulation site (i.e., motor cortex) and in a downstream circuit responsible for modulating pain (i.e., RVM) during and following MCS treatment. To maximize translatability, we investigated the analgesic mechanisms engaged by the non-invasive stimulation technique most commonly used to manage chronic pain, transcranial magnetic stimulation (TMS). We built a device that induces transient magnetic fields across a focal ~2 mm diameter volume but with similar magnitudes to those created by clinical TMS instruments. After performing TMS of the motor cortex in mice with an infraorbital nerve constriction, a model of trigeminal neuropathic pain, we observed a dose-dependent decline in pain behaviors for one week. Next, we identified cortical neuron types activated by TMS that transmit this activity to downstream pain circuits. We used fiber-optic voltage-sensing to track the aggregate transmembrane voltage dynamics of layer 5 pyramidal (L5) neurons during single-pulse TMS. We found that L5 neurons responded bi-phasically after each TMS pulse, with a

brief discharge followed by a sustained depolarization. Further, selective inhibition of motor cortical L5 neurons prevented MCS-induced antinociception. Next, to probe the contribution of endogenous opioid signaling in descending pain control circuits to MCS-induced antinociception, we performed intracranial injections of either an opioid receptor antagonist or a dual enkephalinase inhibitor into the RVM prior to MCS. Remarkably, we found that these agents bidirectionally modulated the magnitude and duration of MCS-induced antinociception. Lastly, to identify neural responses induced by different MCS protocols, we used Neuropixels electrophysiological probes to record the activity of thousands of RVM neurons. Together, our data reveal that MCS activates L5 neurons, which recruit a downstream opioidergic circuit in the RVM to induce antinociception.

Disclosures: N. Mercer Lindsay: None. **S. Haziza:** None. **T.M. Baer:** None. **M.J. Schnitzer:** None. **G. Scherrer:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.18/CC2

Topic: D.02. Somatosensation – Pain

Support: R34DA046635 from NIH / NIDA

Title: Effects of transcutaneous auricular vagus nerve stimulation associated changes of functional connectivity of brainstem nuclei in chronic low back pain

Authors: *T. LI, S. HODGES, N. TODOROVA, S. REDDY, M. ZHU, A. URSITTI, J. KONG; Dept. of Psychiatry, Massachusetts Gen. Hosp., Boston, MA

Abstract: Background: This study aims to investigate the modulation effects of transcutaneous auricular vagus nerve stimulation (taVNS) on the brainstem vagus nerve pathway in patients with chronic low back pain (cLBP). **Methods:** 71 cLBP patients were recruited and randomly assigned to either four weeks of taVNS or transcutaneous greater auricular nerve stimulation (tGANS, applied on the earlobe). Resting-state functional magnetic resonance imaging (fMRI) data were collected at baseline and after the last treatment. Seed-based functional connectivity (FC) analyses were performed using the CONN and SUIT toolbox with the nucleus tractus solitarius (NTS), raphe nucleus (RN), and locus coeruleus (LC) as seeds. **Results:** 51 patients (taVNS: n=25; tGANS: n=26) completed the study. Within-group comparisons showed a significant reduction of symptoms in both groups, illustrated by patients' pain bothersomeness and pain interference (PI) scores. Between-group comparisons revealed no significant findings. rsFC analysis results: **1) NTS seed:** taVNS led to a decreased NTS rsFC with the bilateral insula, left thalamus, and bilateral anterior cingulate cortex (ACC), and an increased NTS rsFC with the bilateral occipital middle cortex. tGANS led to a significant decrease in NTS rsFC with the right medial orbital prefrontal cortex (mOPFC), left medial prefrontal cortex (mPFC), right thalamus,

and right insula, and a significant increase in NTS-bilateral occipital cortex rsFC. The comparison of the bilateral NTS rsFC changes ('pre' - 'post') between taVNS and tGANS treatments showed a decrease in NTS rsFC with the right thalamus, right ACC, and right postcentral gyrus (PoCG) in the taVNS group; **2) LC seed:** Following taVNS treatment, LC rsFC significantly decreased with the left mOPFC, right hippocampus (HPC), and right insula. tGANS led to a decreased LC rsFC with the right insula and increased LC rsFC with the right PoCG. taVNS treatment decreased LC rsFC with the left PoCG, left precentral gyrus (PreCG), right prefrontal cortex (PFC), and right angular gyrus (AG) as compared to the tGANS group; **3) RN seed:** taVNS treatment increased RN rsFC with the bilateral PoCG and left insula; tGANS increased RN rsFC with the left precuneus and decreased RN rsFC with the left ventrolateral prefrontal cortex (vlPFC); taVNS treatment produced greater rsFC increases between the RN and the right PoCG, left putamen, and right Rolandic operculum, as compared to the tGANS group. **Conclusion:** Our findings suggest that both taVNS and tGANS can reduce back pain and modulate FC of the key regions on the brainstem vagus nerve pathway.

Disclosures: T. Li: None. **S. Hodges:** None. **N. Todorova:** None. **S. Reddy:** None. **M. Zhu:** None. **A. Ursitti:** None. **J. Kong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J.K has holds equity in two startup companies (MNT, BTT) and a patent on applying neuromodulation, but declares no conflict of interest.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.19/CC3

Topic: D.02. Somatosensation – Pain

Support: NIH grant UG3NS115718
NIH grant R01NS070814
NIH grant R21NS101954
NIH grant R01NS054791
NIH grant R01NS117761
NIH grant R01NS110598

Title: Orally active positive allosteric modulators of human Mas-related G protein-coupled receptor X1 as novel therapeutics for neuropathic pain

Authors: A. UNIYAL, I. BERHANE, C. ZHANG, Q. ZHENG, N. HIN, A. G. THOMAS, J. LIU, Q. HUANG, X. CUI, Q. PENG, B. S. SLUSHER, S. N. RAJA, X. DONG, T. TSUKAMOTO, ***Y. GUAN**;
Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Objective and rationale: Mas-related G protein-coupled receptor C (MrgprC, human MrgprX1) is expressed specifically in small-diameter primary sensory neurons and is a promising new analgesic target. Positive allosteric modulator (PAM) has the advantage over the orthosteric agonist in terms of safety and selectivity by promoting spatial and temporal GPCR signaling dependent upon endogenous ligand availability. Here, we aim to develop new orally active human MrgprX1 PAMs and determine their efficacy and safety in mouse models of neuropathic pain. **Methods:** We have generated a BAC-transgenic mouse line in which MrgprX1 is expressed under the control of the mouse MrgprC promoter. We then crossed this line into the Mrgpr $^{-/-}$ background, to generate MrgprX1:Mrgpr$^{-/-}$ mice (MrgprX1). In this way, only human MrgprX1 is expressed in mouse primary sensory neurons. We also identified a submicromolar MrgprX1 PAM, *6-(tert-butyl)-5-(3,4-dichlorophenyl)-4-(2-(trifluoromethoxy)phenoxy)thieno[2,3-d]pyrimidine* (BDTTP). **Results:** In-vitro assays demonstrated that BDTTP has an EC_{50} of 0.1 µM with half-lives of >30 min and >60 min in mouse and liver microsomes, respectively. Oral pharmacokinetic studies in mice suggested that BDTTP is orally available with a spinal cord-to-plasma ratio of 13%. C_{max} in the spinal cord is more than 40-fold greater than the EC_{50} value of BDTTP. In-vivo efficacy studies showed that BDTTP (100 mg/kg, p.o.) inhibited heat hypersensitivity (Hargreaves test) in humanized MrgprX1 mice, but not in Mrgpr$^{-/-}$ mice, after nerve injury. The peak effects were observed at 2-hour post-administration. Spontaneous ongoing pain behavior (flinching, licking, and shaking) after nerve injury was also inhibited by BDTTP in MrgprX1 but not in Mrgpr$^{-/-}$ mice. An acute in-vivo toxicity study using single-time administration of the compound (100 mg/kg, p.o.) did not elicit any behavioral abnormalities in MrgprX1 mice (e.g., sedation, itch scratching, agitation). The open-field and rota-rod tests suggested that BDTTP did not produce any CNS-associated side effects (impaired locomotor or motor coordination). **Conclusion:** Our study suggests that orally active MrgprX1 PAMs could pave the way for the development of a novel class of effective non-opioid pharmacological agents for the management of neuropathic pain with minimal side effects.

Disclosures: A. Uniyal: None. **I. Berhane:** None. **C. Zhang:** None. **Q. Zheng:** None. **N. Hin:** None. **A.G. Thomas:** None. **J. Liu:** None. **Q. Huang:** None. **X. Cui:** None. **Q. Peng:** None. **B.S. Slusher:** None. **S.N. Raja:** None. **X. Dong:** None. **T. Tsukamoto:** None. **Y. Guan:** 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.; Medtronic. inc, TissueTech. inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); TissueTech. inc.. F. Consulting Fees (e.g., advisory boards); Medtronic,. inc..

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.20/CC4

Topic: D.02. Somatosensation – Pain

Support: 75N95019D00026

Title: In vivo PK, side effect profile, efficacy, and abuse liability assessment of pregabalin in rats

Authors: *E. A. DUGAN[1], M. A. VARNEY[1], D. BUDAC[1], M. URBAN[1], Q. CHANG[1], S. A. WOLLER[2], S. IYENGAR[2], T. HANANIA[1];
[1]PsychoGenics, Inc., Paramus, NJ; [2]NIH/NINDS, Rockville, MD

Abstract: In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated pregabalin through the tiered approach established to profile potential novel analgesics. First, pharmacokinetic studies were conducted to guide dosing, select the route of administration, and to determine the time course, supporting subsequent behavioral studies. Following administration by oral gavage, plasma levels increased over the first hour, giving peak levels of 60 µM. Pregabalin had a plasma half-life of around 4.5 hours and the brain exposure was 8 to 10 µM at 1 hour following oral administration. Next, the modified Irwin (n=4) and rotarod tests (n=10) were conducted to evaluate potential neurologic, physiologic, and fine motor effects that may impact outcome measures in the pain models. In the modified Irwin test, pregabalin affected body position, induced slight sedation, and delayed visual placement following administration of higher doses but did not affect rotarod performance. Pregabalin was profiled in several pain models, including plantar incision (n=10), L5/L6 spinal nerve ligation (SNL; n=10), chemotherapy-induced peripheral neuropathy (CIPN), and MIA osteoarthritis (OA) models. Pregabalin decreased both spontaneous guarding behaviors and increased paw withdrawal threshold in a dose-dependent manner in the plantar incision pain model (Brennan et al. 1996). In the SNL model (Kim and Chung, 1992), pregabalin reduced mechanical allodynia behavior. Assessment of pregabalin in the CIPN and OA models is ongoing, and results will be presented at the meeting. The abuse liability potential of pregabalin was assessed using both the intravenous self-administration (SA) model (Chang et la., 2015) and the conditioned place preference model (Kimmel et la., 2000). Both tests showed that pregabalin does not cause rewarding behavior in the rat. The results of these studies demonstrate an example of the tiered approach within the PSPP program for evaluation of novel non-opioid, non-addictive treatments for pain.

Disclosures: **E.A. Dugan:** A. Employment/Salary (full or part-time):; Psychogenics. 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.; CRO. **M.A. Varney:** A. Employment/Salary (full or part-time):; Psychogenics. 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.; CRO. **D. Budac:** A. Employment/Salary (full or part-time):; Psychogenics. 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.; CRO. **M. Urban:** A. Employment/Salary (full or part-time):; Psychogenics. 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.; CRO. **Q. Chang:** A. Employment/Salary (full or part-time):;

Psychogenics. 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.; CRO. **S.A. Woller:** A. Employment/Salary (full or part-time):; NINDS. **S. Iyengar:** A. Employment/Salary (full or part-time):; NINDS. **T. Hanania:** A. Employment/Salary (full or part-time):; Psychogenics. 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.; CRO.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.21/CC5

Topic: D.02. Somatosensation – Pain

Title: Past-oriented v. future-oriented reappraisal for thermal pain modulation

Authors: *I. LALENA, J. C. THOMPSON;
Psychology, George Mason Univ., Fairfax, VA

Abstract: Affective neuroscience has extensively investigated the effects of cognitive reappraisal and other emotion regulation strategies on pain perception and management. Few studies, however, have focused on possible temporal aspects of cognitive reappraisal involved in pain. In this study, we followed a novel model of reappraisal, proposed by Vlasenko (2021), based on temporal anchoring of cognitive change: past-oriented versus future-oriented. We administered acute thermal pain stimulation to the left forearm of human subjects (n=32), aged 18 to 25 (M = 19.27, SD = 1.69). We conducted a paired samples t-test and found no significant difference between pain ratings of past-oriented (M = 5.45, SD = 2.44) and future-oriented (M = 5.71, SD = 2.24) reappraisal conditions; $t(31) = -0.4740$, $p = 0.6388$. The comparison of only the first past-oriented (M = 5.27, SD = 2.53) and first future-oriented (M = 6.02, SD = 2.43) reappraisal trials also yielded no significant difference in pain ratings, $t(15) = -0.8769$, $p = 0.3944$. To check for a possible order effect, we conducted a 2 × 2 repeated measures ANOVA, examining potential interactions between Reappraisal Type (past-oriented, future-oriented) and Order (past-future, future-past). There was no significant main effect of Reappraisal Type, $F(1,15) = 1.3066$, $p = 0.27092$, or Order, $F(1,15) = 0.43114$, $p = 0.52138$, nor was there a significant interaction between Reappraisal Type and Order, $F(1,15) = 0.45685$, $p = 0.50939$. Additionally, we conducted paired samples t-tests to compare pain ratings of both past-oriented reappraisal and future-oriented reappraisal to baseline pain ratings. We found no significant difference between pain ratings of the past-oriented reappraisal condition (M = 5.45, SD = 2.44) and baseline pain ratings (M = 5.28, SD = 1.87); $t(31) = 0.3471$, $p = 0.7308$. There was also no significant difference between pain ratings of the future-oriented reappraisal condition (M = 5.71, SD = 2.24) and baseline pain ratings (M = 5.28, SD = 1.87); $t(31) = 0.7607$, $p = 0.4526$. These

findings suggest that temporal aspects of cognitive reappraisal might not play a role in regulating emotions or pain management during acute thermal pain stimulation.

Disclosures: I. Lalena: None. **J.C. Thompson:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.22/CC6

Topic: D.02. Somatosensation – Pain

Support: Duke University Anesthesiology Research Fund

Title: Auricular electrostimulation protects against neuroinflammation, pain, and cognitive decline via resolvin-mediated microglial signaling in mice

Authors: *W. HE[1], S. BANG[2], R.-R. JI[2,3];
[1]Ctr. for Translational Pain Medicine, Dept. of Anesthesiol., Duke Univ. Med. Ctr., Durham, NC; [2]Duke Univ., Durham, NC; [3]Dept. of Neurobio. and Cell Biology, Duke Univ. Med. Ctr., Durham, NC

Abstract: As a nonpharmacological treatment, auricular electrostimulation (aES) has been shown to protect against inflammation, migraine, and cognitive dysfunction, yet the underlying mechanisms remain unclear. In the present study, we investigated the protective effects of aES on lipopolysaccharide (LPS)-induced neuroinflammation and associated comorbidities, including hypothermia, hyperalgesia, and cognitive dysfunction. We used CD1 mice in this study, as they are more resistant to LPS-induced septic death than C57BL/6 mice. Intraperitoneal injection of LPS (1 or 10 mg/kg) caused a rapid reduction in rectal temperature at 2 hours, and this hypothermia lasted for 24 hours. Notably, aES, given before the injection of LPS, effectively prevented the development of hypothermia and suppressed the LPS-induced systemic inflammation (serum increases in IL-1β and IL-6). The dorsal vagal complex (DVC), which encompasses the area postrema (AP), the nucleus of the solitary tract and the dorsal motor nucleus of vagus nerve (DMV), is a major integrative center for vagal regulation. LPS induced microglial reaction (microgliosis) in the DVC at 24 hours, but aES prevented this microgliosis. Interestingly, LPS decreased the CSF levels of resolvin D2 (RvD2), whereas aES treatment could partially reverse this decrease. At 14 days of LPS injection, we also observed hyperalgesia and cognitive dysfunction, as well as microgliosis in the hippocampus. Strikingly, all these dysregulations were reversed by aES treatment or intrathecal treatment of RvD2 or microglial inhibitor minocycline. Our findings suggest that aES plays a protective role against LPS-induced inflammation (e.g., cytokine increase in blood) and neuroinflammation (e.g., microgliosis in the DVC and hippocampus), thus conferring protection against LPS-induced sickness behaviors (hypothermia, hyperalgesia, and cognitive dysfunction). Mechanistically, aES may achieve these

benefits via producing RvD2 and regulating central microglial signaling in the DVC and the hippocampus. Currently, we are investigating the sex differences in the aES-mediated effects.

Disclosures: **W. He:** None. **S. Bang:** None. **R. Ji:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.23/CC7

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS118504
NYSCF
HHMI
The McKnight Endowment Fund for Neuroscience
The Brain Research Foundation

Title: A cell atlas of the amygdala enables synergistic pharmacology against pain unpleasantness

Authors: ***D. BERG**[1], D. LEE[2], M. B. CHEN[1], A. TASSOU[2], N. MERCER LINDSAY[1], X. JIANG[1], Y. KE[1], J. KRZESKI[2], S. QUAKE[1], M. SCHNITZER[1], G. SCHERRER[2];
[1]Stanford Univ., Palo Alto, CA; [2]The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Pain is a complex experience with sensory, emotional and cognitive dimensions. We recently reported the discovery of a discrete ensemble of neurons in the basolateral amygdala (BLA) that encodes, and is causally responsible for, pain negative emotions (i.e., the unpleasant quality of pain) (Corder, Ahanonu et al., Science, 2019). To determine the molecular identity of nociceptive amygdalar neurons, we profiled the gene expression of individual neurons active during nociception using single-cell RNA-sequencing (scRNA-seq). We identified 17 major neuron types and 28 subtypes of amygdalar neurons. We established a comprehensive amygdalar neuron atlas by mapping the spatially resolved patterns with which the marker genes defining cell types are organized. Additionally, we integrated our scRNA-seq results with published literature regarding the function and cell types of amygdalar neurons to guide the discovery of amygdalar analgesic drugs. Next, we searched for G protein-coupled receptors (GPCRs), which are highly druggable biological targets, enriched on amygdalar neuronal types with functional relevance to pain processing. We tested agents engaging these GPCR targets in several pain assays to determine their potential clinical utility and identified multiple amygdalar analgesics with antinociceptive activity. Considering our prediction that the tested drugs modulate multiple pain-related pathways, we derived a combination strategy of three drugs that synergize to enhance analgesia for acute pain affect and a preclinical model of orofacial chronic pain. Together, our findings establish the molecular structure of the nociceptive amygdala and identify highly druggable targets for the development of analgesics against pain unpleasantness across pain types.

Disclosures: **D. Berg:** None. **D. Lee:** None. **M.B. Chen:** None. **A. Tassou:** None. **N. Mercer Lindsay:** None. **X. Jiang:** None. **Y. Ke:** None. **J. Krzeski:** None. **S. Quake:** None. **M. Schnitzer:** None. **G. Scherrer:** None.

Poster

PSTR477. Non-Opioid Treatments

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR477.24/CC8

Topic: D.02. Somatosensation – Pain

Support: NSTC110-2320-B-039 -023 -MY3

Title: The role of TRPM8 in opioid and endocannabinoid analgesia

Authors: *Y.-H. CHEN, D. PHAM;
China Med. Univ., Taichung, Taiwan

Abstract: The Transient Receptor Potential Melastatin 8 (TRPM8) channel is a temperature-sensitive channel with significant physiological and pathological implications. Until now, there has been limited evidence to support the link between TRPM8 and analgesic efficacy. The present study aims to explore the role of TRPM8 in opioid and endocannabinoid analgesia in mice. Male adult C57BL/6 mice and TRPM8 knockout mice aged 6 to 8 weeks were used. To induce intraplantar inflammation, complete Freund's adjuvant (CFA) was applied to the intraplantar surface of the hind paw. The Von-Frey test was performed after a 60-minute acclimation period to measure mechanical hyperalgesia. In the Complete Freund's adjuvant (CFA) mouse pain model, intraperitoneal injection of morphine (10 mg/kg) produces analgesic effects. If TRPM8 is activated by oral administration of menthol, it was found to increase the analgesic effects of morphine. However, this increase in analgesic effect was not observed in TRPM8 knockout mice. In TRPM8 knockout mice, the level of mechanical allodynia in the CFA mouse pain model is comparable to that in wild-type mice. In TRPM8 knockout mice, morphine still produces analgesic effects, but this analgesic effect is significantly reduced compared to wild-type mice. Next, we tested the analgesic effects of the CB1 receptor agonist and observed the influence of modulation on analgesia. WIN 55,212-22, a CB1 receptor agonist, produces analgesic effects when intraperitoneally injected (1.5 mg/kg) in the CFA mouse pain model. This analgesic effect can be suppressed by pre-treatment with AM251. Pre-treatment with the TRPM8 inhibitor AMTB slightly enhances this analgesic effect. In TRPM8 knockout mice, WIN 55,212-22 still produces analgesic effects, but the analgesic effect is significantly greater than in wild-type mice. Additionally, this analgesic effect can be suppressed by pre-treatment with AM251. This result suggests that TRPM8 activation enhances the analgesic effects of morphine, while TRPM8 gene knockout diminishes the analgesic effects of morphine. Inhibiting TRPM8 or TRPM8 gene knockout enhances the analgesic effects of WIN 55,212-22. TRPM8 plays an important role in the opioid and endocannabinoid analgesia.

Disclosures: **Y. Chen:** None. **D. Pham:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.01/CC9

Topic: D.03. Somatosensation – Touch

Support: NIH Grant 1R15DE027844

Title: Tooth loss-induced alterations to subcortical structures in the naked mole-rat

Authors: ***N. J. HITE**[1], M. M. SKINNER[2], D. K. SARKO[3];
[1]Molecular, Cellular, and Systemic Physiol., [3]Anat., [2]Southern Illinois University, Sch. of Med., Carbondale, IL

Abstract: Globally, complete tooth loss affects nearly 7% of the population, impacting approximately 23% of older adults. Surveys consistently show correlations between tooth loss and cognitive measures, yet scarce information exists on the impact of tooth loss on subcortical structures underlying these measures. Animal studies predominantly show the degradation of hippocampal neurons yet fail to explain how tooth loss-induced plasticity of primarily somatosensory projections alter subcortical structures such as the hippocampus. In the current study, we examined the long-term impact of tooth loss by analyzing the lateralization of neuroanatomical plasticity following surgical removal of the lower right incisor in adult naked mole-rats compared to sham-operated animals. Subordinate male and female animals were used, with sex incorporated into analyses whenever possible based on sample size. Brains were perfused and extracted one year following surgery. Histological, histochemical, and immunohistochemical processing allowed visualization of neuronal populations within subcortical structures including, but not limited to, hippocampal subregions, thalamic nuclei, and amygdala nuclei. Notably, we found that tooth extractions induced contralateral CA1 cell count reductions, supporting findings from mouse models of aging and tooth loss that showed CA1-specific atrophy. Our data reveal the impact of tooth loss on subcortical neuroplasticity and help to expand our understanding of the impact of tooth loss by incorporating an ideal animal model for studies of tooth loss, the naked mole-rat. These results provide a foundation for elucidating somatosensory neuroplasticity effects on limbic structures, with broader implications for resulting deficits in cognitive and affective neural processes and behaviors.

Disclosures: **N.J. Hite:** None. **M.M. Skinner:** None. **D.K. Sarko:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.02/CC10

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: The ventral posterior lateral (VPL) thalamic nucleus does not serve as a substrate for rapid lower jaw-to-forepaw reorganization in the anterior forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI)

Authors: *R. S. WATERS[1], J. W. TSAO[2], L. WANG[3];
[1]Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]NYU Langone, New York, NY; [3]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Introduction: Forelimb deafferentation results in immediate lower jaw-to-forepaw reorganization in the anterior forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI), but not in the posterior FBS. It is unknown whether rapid lower jaw-to-forepaw reorganization also occurs in the ventral posterior lateral (VPL) thalamic nucleus, but investigating this possibility is crucial. A positive outcome would indicate that VPL likely serves as a subcortical source for the newly observed rapid lower jaw input in the deafferented FBS. To this end, we examined VPL immediately after deafferentation of the brachial plexus for new input from the lower jaw while simultaneously assessing lower jaw reorganization in FBS.
Methods: Anesthetized rats with intact forelimbs were used to identify the digit representation in VPL and FBS. A microelectrode was inserted into VPL to record single and multi-unit responses. Mechanical and electrical stimulation applied to forepaw and lower jaw skin surfaces was used to elicit responses. Once the digit representation in the VPL was identified, the electrode was secured in place. Similarly, the digit representation in the anterior FBS was identified, and the electrode was fixed accordingly. Lidocaine, a 2% solution, was then injected into the brachial plexus to induce deafferentation. Digit representations in both VPL and FBS were reexamined for the presence of lower jaw responses. Microstimulation was used to investigate connectivity between recording sites in VPL and FBS. IGOR-Pro software was used for signal processing. Electrolytic lesions were used to recover recording/stimulating sites in FBS and VPL.
Results: a) In rats with intact forelimbs, all sites within the digit representation in VPL and FBS responded exclusively to input from the forepaw. b) Following lidocaine administration in the brachial plexus, cells within the digit representation in VPL and FBS became unresponsive to input from the forepaw. c) However, cells within the anterior FBS, but not in the VPL, became immediately responsive to new input from the lower jaw. d) Most importantly, sites within the unresponsive digit representation in VPL project to the newly responsive lower jaw sites in the anterior FBS.
Conclusions: Our findings indicate that forepaw VPL does not provide a source for the new lower jaw input in the deafferented anterior FBS. However, it is worth noting that other investigators reported lower jaw reorganization in hand VPL of monkey months to years after forelimb deafferentation; a similar delayed reorganization in rat may allow VPL to provide a substrate for lower jaw-to-forepaw reorganization in the posterior FBS.

Disclosures: **R.S. Waters:** None. **J.W. Tsao:** None. **L. Wang:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.03/CC11

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: The lower jaw barrel subfield (LJBSF) in rat primary somatosensory cortex (SI) provides a likely sole source of new lower jaw input in the anterior forepaw barrel subfield (FBS) that follows forelimb deafferentation

Authors: *L. WANG[1], A. L. DE JONGH CURRY[2], J. W. TSAO[3], R. WATERS[1];
[1]Anat. & Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [2]Biomed.Engin, Univ. of Memphis, Memphis, TN; [3]NYU Langone, New York, NY

Abstract: Introduction: Forelimb deafferentation leads to immediate lower jaw-to-forepaw reorganization in rat forepaw barrel subfield (FBS) in primary somatosensory cortex (SI). The new lower jaw input is localized in the anterior region of the FBS. Based on existing anatomical evidence, one source of the new input comes from the neighboring lower jaw barrel subfield (LJBSF). Because lower jaw responses are recorded immediately after forelimb deafferentation, the lower jaw input is likely already present in the FBS in intact forelimb rats but blocked from expression. Injection of bicuculline methiodide (BMI), a $GABA_A$ blocker into the anterior FBS of intact forelimb rats immediately unmasks the lower jaw input. In this study, we ablated the LJBSF pathway to the FBS and abolished the previously unmasked lower jaw input in the anterior FBS.
Methods: Adult rats under anesthesia, with intact forelimbs, were used for this experiment. In order to map the physiological representation of the forepaw and lower jaw skin surfaces, as well as the border region between these two subfields, a microelectrode was inserted into the FBS and LJBSF and used to record single and multi-unit responses that were evoked through mechanical and electrical stimulation of the forepaw and lower jaw skin surfaces. Following mapping, one or more sites in the FBS was selected for iontophoresis of bicuculline methiodide (BMI), aimed at unveiling the previously unexpressed lower jaw input. BMI iontophoresis was repeated and sites within the FBS were remapped to determine the presence of lower jaw input. Electrolytic lesions (ranging from 4 to 8) were systematically placed along the border of the LJBSF to disrupt the pathway between the LJBSF and FBS. Signal processing was conducted using IGOR-Pro software. To visualize the barrel fields, staining with cytochrome oxidase was performed. Additionally, lesions were placed at selected recording sites within the FBS and LJBSF to reconstruct the electrode penetrations and validate their accuracy.
Results: a) In forelimb intact rats, FBS neurons express input exclusively from the forepaw, b)

Iontophoresis of BMI in the anterior FBS of intact forelimb rats results in the immediate expression of new lower jaw input, and c) Ablation of the pathway from the LJBSF to the FBS abolishes the previously unmasked lower jaw input in the FBS but does not alter input from the forepaw.
Conclusion: The source for the rapid expression of lower jaw input in the anterior FBS likely originates solely from the LJBSF, rather than from other cortical or subcortical sources.

Disclosures: L. Wang: None. **A.L. De Jongh Curry:** None. **J.W. Tsao:** None. **R. Waters:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.04/CC12

Topic: D.03. Somatosensation – Touch

Support: Eunice Kennedy Schriver National Institute of Child Health & Human Development, NIH Grant R01HD094588

Title: Removal of $GABA_B$ inhibition fails to unmask lower jaw input in the forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI)

Authors: *A. L. DE JONGH CURRY[1], L. WANG[2], J. W. TSAO[3], R. S. WATERS[2,1];
[1]Biomed. Engin., Univ. of Memphis, Memphis, TN; [2]Anat. & Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [3]NYU Langone, New York, NY

Abstract: Introduction: The forepaw barrel subfield (FBS) in rat primary somatosensory cortex (SI) receives exclusive input from the forepaw skin surface. However, immediately following forelimb deafferentation, new input from lower jaw is expressed in anterior FBS. The lower jaw barrel subfield (LJBSF) lies adjacent to the FBS in SI and sends sparse neuronal projections to the anterior FBS which are unexpressed in forelimb intact (normal) rats. We previously reported in normal rats that removal of $GABA_A$ receptor-mediated inhibition by injection of bicuculline methiodide leads to rapid expression of new lower jaw input in anterior FBS. The ionotropic $GABA_A$ receptor is responsible for fast inhibition. In this study, we report the contribution of the metabotropic $GABA_B$ receptor, which is responsible for slow inhibition, in rapid unmasking of lower jaw input in the FBS in normal rats.
Methods: In anesthetized normal rats, an electrode was inserted into the FBS and LJBSF to record receptive fields of neurons that were activated with mechanical and electrical stimulation applied to forepaw and lower jaw skin surfaces, respectively. Following mapping, selected sites in the FBS were chosen to study effects of $GABA_B$ antagonism on expression of lower jaw inputs in FBS. The single electrode was replaced with a dual iontophoresis pipette and carbon fiber electrode with tip separation of ~60 um. The iontophoresis pipette was filled with $GABA_B$ antagonist saclofen, which was ejected at sites in anterior and posterior FBS. Spontaneous

activity was recorded before, during, and after each instance of saclofen iontophoresis. Mechanical and electrical stimulation of lower jaw were used to test for presence of lower jaw input in FBS. Signal processing was done with IGOR-Pro. Electrolytic lesions were made at selected FBS sites to reconstruct electrode penetration locations following histological tissue processing (cytochrome oxidase staining was used to show barrel subfields).
Results: Iontophoresis of the $GABA_B$ antagonist in the FBS: a) failed to unmask lower jaw input in FBS, but selectively enlarged digit and pad receptive fields, and b) reversibly altered spontaneous background firing from regular firing to irregular spike bursting.
Conclusion: In contrast to removal of $GABA_A$ receptor-mediated inhibition, removal of $GABA_B$ receptor-mediated inhibition failed to unmask lower jaw input in FBS of normal rats. However, the selectivity of saclofen in unmasking digit/pad receptive fields but not lower jaw input remains to be determined. Nonetheless, $GABA_B$ inhibition is not a likely mechanism underlying rapid lower jaw-to-forepaw reorganization that follows forelimb deafferentation.

Disclosures: A.L. De Jongh Curry: None. **L. Wang:** None. **J.W. Tsao:** None. **R.S. Waters:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.05/CC13

Topic: D.03. Somatosensation – Touch

Title: Plasticity of feedforward circuits in superficial layers of sensory neocortex during learning

Authors: *J. CHRISTIAN[1], E. PARK[2], A. L. BARTH[2];
[2]Dept. of Biol. Sci., [1]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Sensory experience and learning drive synaptic changes in the neocortex, but the input- and target-specificity of these changes and how they evolve over time are not well understood. This information is critical for linking together in vitro mechanisms for synaptic plasticity with circuit changes observed in vivo. Here we evaluate plasticity of feedforward pathways in the mouse barrel cortex, focusing on the layer 4 (L4) to L2/3 excitatory synapse by expressing channelrhodopsin in Scnn1a-Cre transgenic mice. Using whole-cell patch clamp techniques, we recorded optically-evoked quantal EPSCs (qEPSCs) and found that qEPSCs from L4 inputs were similar between L2 and L3 pyramidal neurons under control conditions. After one day of sensory association training, qEPSC amplitude was unchanged in L3 but was significantly elevated in L2 pyramidal cells. These data suggest that L2 pyramidal neurons maybe selectively sensitive to conditions that drive learning. The strengthening observed in L2 was transient, renormalizing as animals learned the sensory association, and was not observed during pseudo-training, when stimuli and rewards were uncoupled. We also explored the effects of environmental enrichment, without explicit reward association, on these synapses. Taken together this data suggests that pyramidal neurons in superficial layers of sensory cortex,

separated by cortical depth, undergo different programs of synaptic plasticity during learning. Our experiments will further explore the timescale and mechanism for cortical feedforward synaptic plasticity.

Disclosures: J. Christian: None. **E. Park:** None. **A.L. Barth:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.06/CC14

Topic: D.03. Somatosensation – Touch

Support: NIH

Title: Subtype-specific plasticity in neocortical inhibitory neurons during learning

Authors: *M. B. MOSSO[1], M. B. KINSTLE[1], A. L. BARTH[2];
[1]Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA; [2]Carnegie Mellon U., Pittsburgh, PA

Abstract: Somatostatin (SST) expressing neurons are a diverse subclass of inhibitory cell with distinct morpho-electric phenotypes. SST cell activity and morphology has been shown to be altered by learning. Through viral-mediated transduction of PSD95.FingR-Citrine for SST cell-type specific labeling of the endogenous excitatory synaptic marker PSD95, we explore whether molecularly distinct SST cells may be regulated by experience. We trained animals using an automated, whisker-dependent sensory association task where mice learn to associate a gentle air puff with the presentation of a water reward. We trained a different set of animals to another task where the air puff was no longer predictive of the water reward. Confocal images of putative excitatory synapses across SST dendrites were collected to obtain volumetric stacks in L2/3 of fixed tissue from primary somatosensory cortex. Using immunohistochemistry to differentiate classes of SST neurons, we investigated whether the excitatory input on specific subtypes of SST cells was modulated by training condition. We found that a molecularly distinct population of SST cells showed a reduction in excitatory input during training while others were unperturbed. This effect was abolished when the stimulus was uncoupled from the reward during training. Artificial suppression of all SST molecular sub-types resulted in a broad reduction of inputs to SST cells. These data stimulate new hypotheses about circuits, which may be implemented differentially during learning and can be identified based on molecular subclass.

Disclosures: M.B. Mosso: None. **M.B. Kinstle:** None. **A.L. Barth:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.07/CC15

Topic: D.03. Somatosensation – Touch

Support: NIH

Title: Sparse and transient impact of reward-association training on stimulus-response coupling in the primary somatosensory cortex

Authors: *M. ZHU[1], S. J. KUHLMAN[1], A. L. BARTH[2];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Carnegie Mellon U., Pittsburgh, PA

Abstract: Synaptic potentiation has been linked to learning in sensory cortex, but a link between this potentiation and increased sensory-evoked neural activity is not clear. Here we used longitudinal in vivo calcium imaging in the barrel cortex of awake mice to test the hypothesis that increased excitatory synaptic strength during learning of a whisker-dependent, reward-association task would enhance stimulus-evoked firing. In order to isolate stimulus-evoked responses from dynamic, task-related activity, imaging was performed outside of the training context. Although multi-whisker stimuli have been shown to drive robust subthreshold activity in layer 2/3 (L2/3) pyramidal neurons, we observed sparse Ca transients in these neurons under control conditions prior to training. At the onset of sensory-association training, we identified a transient increase in stimulus-driven responses in a small subset of L2/3 pyramidal neurons that renormalized over a 10-day training period. Overall, sensory-association training maintained stimulus-evoked activity in barrel cortex. These results were in contrast to stimulus-evoked responses in L2/3 pyramidal neurons from mice subjected to pseudo-training, where the whisker stimuli and rewards were uncoupled. In pseudo-trained mice, sensory-evoked responses showed significant response suppression over a 10-day training period. These findings suggest that widespread synaptic potentiation across the L2/3 pyramidal cell population after sensory association training characterized in vitro is not necessarily linked to pronounced enhancements in stimulus-evoked activity in vivo. Furthermore, our data indicate that learning stabilizes sensory representations in the primary sensory cortex, in contrast to pseudo-training, where sensory-evoked responses become depressed.

Disclosures: M. Zhu: None. **S.J. Kuhlman:** None. **A.L. Barth:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.08/CC16

Topic: D.03. Somatosensation – Touch

Support: NIH

Title: Input- and target-specific thalamocortical plasticity in layer 1 during sensory learning

Authors: *A. RAY, J. A. CHRISTIAN, A. L. BARTH;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Changes in the strength of synaptic connections underlie the cellular basis of learning. Previously, we have demonstrated that genetically-encoded fluorescence-based reagents for pre- and post-synaptic labeling with a digital image analysis pipeline can identify pathway-specific synaptic changes in higher-order thalamocortical (POm) synapses onto layer 5 pyramidal neurons (L5 Pyr) during a whisker-dependent learning task. Excitatory synapses were identified using PSD95.FingR, and presynaptic POm axons were labeled with a tdTomato cell fill. Here we focused on changes in POm synapses onto genetically targeted L2/3 Pyr neurons in the mouse barrel cortex in the same task, using Drd3-Cre transgenic mice. Electrophysiological evidence for potentiation of this pathway has been reported (Audette et al., Neuron, 2019; 103(2):277-291), but the dendritic location of these changes remains unknown. We looked at POm synapses in L2/3 Pyr apical tufts located in L1 and their basal dendrites in L2, and compared them to all other excitatory synapses on these cells. During the first two days of training, we identified a gradual increase in the size of POm-associated synapses on the apical tufts, particularly in the outermost sublamina of L1. This change was concentrated in POm inputs, as PSD95.FingR puncta not aligned to POm boutons did not significantly change. Individual POm boutons contacted more PSD95.FingR puncta after training, suggesting further structural reorganization. The time-course of these synaptic changes were evaluated as the animals became expert in the task. These synaptic changes were also evaluated in animals that have undergone pseudotraining, where stimuli and rewards were uncoupled. These data suggest that apical tufts of L2/3 Pyr are particularly sensitive to learning-related plasticity in contrast to synapses on the tufts of L5 Pyr that decrease in size with learning (Ray et al., J Neurosci. 2023; 43(4):584-600). We propose that dendritic architecture and post-synaptic composition play a critical role in circuit reorganization. In addition, the transience of these changes in primary somatosensory cortex suggest that synaptic traces of sensory learning may be refined and ultimately reside elsewhere. Finally, our studies across different neocortical circuits also show the broad utility of our approach in studying plasticity in a high-throughput manner.

Disclosures: **A. Ray:** None. **J.A. Christian:** None. **A.L. Barth:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.09/CC17

Topic: D.03. Somatosensation – Touch

Support: AFOSR

Title: Suppression of neocortical SST neuron activity drives behavioral change during acquisition of a sensory association task

Authors: *R. A. SWINDELL[1], E. PARK[2], M. ZHU[1], A. L. BARTH[3];
[2]Biol. Sci. Dept., [3]Biol. Sci., [1]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: During learning in a whisker-dependent sensory association task (SAT), the inhibitory output of somatostatin (SST) neurons in superficial layers of somatosensory (barrel) cortex of mice is suppressed compared to animals where whisker stimuli and rewards are unpaired. Behavioral analysis in wild-type mice indicates that animals increase anticipatory licking to the stimulus prior to water delivery, during the first day of SAT training, indicating that this association can be rapidly acquired (Audette et al, 2019). Chemogenetic (HM4di) suppression of SST neuron activity without training was sufficient to reduce stimulus-evoked SST inhibition in vivo, and CNO administration in untrained mice initiated a persistent reduction of SST inhibition compared to mCherry control mice, revealed in acute brain slices. CNO treatment during behavior did not change the mean number of trials carried out and daily water consumption. We carried out a fine-scale behavioral analysis of trial initiation and licking behavior across the pretraining and SAT interval. At the onset of training, animals typically show an initial aversion to whisker stimulation that is rapidly lost as they habituate to the stimulus. Chemogenetic suppression of SST activity across the training period enhanced stimulus-evoked aversive behavior at the onset of training but was associated with accelerated learning of the stimulus-reward association compared to mCherry controls. These data indicate that chemogenetic regulation of SST activity in barrel cortex can influence both SST synaptic output and sensory-guided behavior during learning.

Disclosures: R.A. Swindell: None. **E. Park:** None. **M. Zhu:** None. **A.L. Barth:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.10/CC18

Topic: D.03. Somatosensation – Touch

Support: 1R01NS131549

Title: Dynamic representation of limbs in the gracile nucleus of mouse

Authors: *R. IWAMOTO[1,2], Z. HUBBARD[1], S. TAMURA[3], A. MATUNIS[4], E. STACY[6], Y. KAMBE[7], T. HIKIDA[8], T. IMAI[9], T. K. SATO[10], T. R. SATO[5];
[1]MUSC, Charleston, SC; [2]Osaka university, Osaka, Japan; [3]Developmental neurophysiology, Kyushu Univ., Fukuoka, Japan; [5]Dept. of Neurosci., [4]Med. Univ. of South Carolina, Charleston, SC; [6]Col. of Charleston Program In Neurosci., Charleston, SC; [7]Kagoshima Univ., Kagoshima, Japan; [8]Inst. for Protein Res., Osaka Univ., Suita-Shi, Japan; [9]Kyushu university, Fukuoka, Japan; [10]Kagoshima university, Kagoshima, Japan

Abstract: The gracile nucleus in the brainstem is located as the first relay point in the somatosensory pathways from the hindlimbs to the cerebral cortex. Despite its obvious significance, however, we do not have a complete picture of the precise representation of the hindlimbs in this area, and how such respresentation can be modified following lesions. To address these issues, in the current study, we developed novel approaches that allowed us to perform in vivo two-photon calcium imaging of neurons in the gracile nucleus while we apply precise stimulation of a small portion of the hindlimb (each finger, paw and knee) and the forelimb. While each neuron responds to multiple stimulation site, the center of the gravity moved systematically as we stimulate different portions of the hindlimb. Furthermore, we found that the neural responses to forelimb stimulation emerged after a few weeks following T3 spinal cord injury. Our data demonstrate the precise but dynamic representation of sensory stimuli in the brainstem sensory nucleus.

Disclosures: R. Iwamoto: None. **Z. Hubbard:** None. **S. Tamura:** None. **A. Matunis:** None. **E. Stacy:** None. **Y. Kambe:** None. **T. Hikida:** None. **T. Imai:** None. **T.K. Sato:** None. **T.R. Sato:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.11/CC19

Topic: D.03. Somatosensation – Touch

Support: Grant CMRPG3K2151-2
Grant CMRPG3M1651

Title: Neural code in barrel cortex following partial neurorrhaphy in infra-orbital nerve

Authors: *J.-J. TSENG[1], H.-P. CHEN[1,2], H.-T. LIN[3,2], C.-H. LIN[3,2,4], J.-J. HUANG[1,2,4], Y.-C. PEI[1,2,4,5];
[1]Physical Med. and Rehabil., [2]Ctr. of Vascularized Tissue Allograft, [3]Plastic & Reconstructive Surgery, Chang Gung Mem. Hospital, Linkou, Taoyuan City, Taiwan; [4]Sch. of Medicine, Chang Gung Univ., Taoyuan City, Taiwan; [5]Healthy Aging Res. Center, Chang Gung Univ., Taoyuan City, Taiwan

Abstract: Neuroplasticity has been considered as the major mechanism underlying the recovery of cortical topography following nerve reconstruction, but its underlying mechanism remains unclear. In this study, we designed an aberrant peripheral nerve reinnervation model to evaluate the change of direction tuning, whisker tuning, and topography reorganization in the barrel field of primary somatosensory cortex (S1BF). Rats received hemi-end-to-end shifted nerve reconstruction surgery, by which infra-orbital nerve was reconnected with a 50% offset and the other 50% was disconnected, which will induce a systematic shift of peripheral input. Neuronal activity first recovered in medial S1BF 1 month after surgery, and topography in SIBF shifted

from its original barrels by 2.5 mm, a finding that is compatible with systematic shift of peripheral input. We found that, over 6 months of recovery, neurons in lateral S1BF started to respond to whisker stimulation. The expected non-oriented expansion of receptive field toward the neighboring, originally silent S1BF was not observed. Instead, whole whisker-eliciting cortical area expanded to the silent barrels and was able to maintain its cortical topography with consistent receptive field area of each whisker and with well-organized row order. Surprisingly, around 50% of neurons showed an exaggerated directional tuning during the early phase of recovery, and the similar direction tuning property was observed at nascent cortical areas that lack well-organized topography of whisker tuning during the late recovery phase. We dubbed this phenomenon as "nascent tuning" and it was considered to result from scarcity of input and was gradually substituted by normal direction tuning. Our findings indicate that S1BF restores its original topography under distorted peripheral inputs after nerve reconstruction, a property that might be mediated by the expansion of receptive fields into the silent cortical area in a row-by-row-substitution manner. Besides, nascent tuning illustrated the distinctive neuronal signal processing in response to alteration of peripheral input and cortical topography, and it implied the potential mechanism of sensory disorder after nerve reconstruction.

Disclosures: **J. Tseng:** None. **H. Chen:** None. **H. Lin:** None. **C. Lin:** None. **J. Huang:** None. **Y. Pei:** 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.; CMRPG3K2151-2, CMRPG3M1651.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.12/CC20

Topic: D.03. Somatosensation – Touch

Support: R01AG072305
R01AG066768

Title: Spatial transcriptomics of layer 4 of the mouse barrel cortex reveals gradients of gene expression and differential transcriptional responses to deprived and spared inputs

Authors: *A. X. LUO[1], S. P. BROWN[2,3], L. A. GOFF[2,3,4];
[1]Dept. of Neurosci., Johns Hopkins Med. Institutions, Baltimore, MD; [2]Dept. of Neurosci., [3]Kavli Neurosci. Discovery Inst., [4]McKusick-Nathans Dept. of Genet. Med., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Experience-dependent plasticity (EDP), essential for adaptation to changes in the environment, has been extensively studied from the cellular and circuit perspectives. The topography of the mouse barrel cortex (BC) provides an advantageous system for assessing the

spatial organization of transcriptional responses to whisker deprivation and sparing, quantified via spatial transcriptomics (ST). Furthermore, studies of transcriptional responses to EDP have primarily focused on short-term (0-4hrs) responses. Here, we aimed to determine the transcriptional changes induced by long-term sensory deprivation, pertinent to the prolonged evolution of physiological changes in EDP. We used ST to determine how gene expression differs across the mouse primary barrel field in response to chessboard whisker deprivation. Tangential sections of layer 4 (L4) of BC were collected after 7 days of whisker deprivation. The sections were annotated with structural features of the barrel field (e.g. row, arc, septa) and sequenced using the 10x Genomics Visium platform. A region enriched for canonical L4 markers and primary somatosensory cortex markers encompassed the barrel field identified anatomically, validating that our transcriptional dataset captured the intended region and layer. Analyzing genes enriched in this region (relative to visual cortex and BC layers 2/3 and 5) identified additional genes significantly enriched in BC L4. We identified multiple depth-dependent and tangential transcriptional gradients within BC L4 using nonnegative matrix factorization (NMF). We tested for genes with significant differential expression between spared and deprived barrels. In addition, we identified NMF patterns that were differentially used between spared and deprived barrels, providing distinct transcriptional signatures of differential responses to whisker manipulation. These NMF patterns implicated processes associated with microglial function and regulation of synaptic function. Here, we harness the spatial organization of transcriptional responses to chessboard whisker deprivation in the mouse BC to identify patterns of gene expression enriched or depleted in spared and deprived barrels.

Disclosures: **A.X. Luo:** None. **S.P. Brown:** None. **L.A. Goff:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.13/DD1

Topic: D.03. Somatosensation – Touch

Support: NIH NM4R
NIH R25HD106896
NSF BRAIN Center award #2137255
NSF BRAIN Center award #2137272

Title: Intermittent Theta Burst Stimulation of Supplementary Motor Area for Balance Recovery in Chronic Stroke Survivors.

Authors: *K. KUKKAR[1], D. HUYNH[1], S. SHAH[1], S. SHEIKH[1], J. L. CONTRERAS-VIDAL[1], I. WEINBERG[2], P. J. PARIKH[1];
[1]Univ. of Houston, Houston, TX; [2]Weinberg Med. Physics, Rockville, MD

Abstract: Within 6-12 months after discharge from the hospital, approximately 73% of stroke survivors encounter a fall, resulting in significant complications and placing a financial burden on society. The ability to maintain balance is crucial for mobility and independence in daily activities, and its impairment is a leading cause of falls among stroke patients. Thus, it is essential to design effective interventions to improve balance control in stroke patients. Our previous work in healthy adults showed that a transient reversible disruption of supplementary motor area (SMA) using theta burst stimulation altered balance performance and disrupted activation within the brain balance network spanning frontal and parietal regions during a challenging balance task when compared with sham stimulation. These findings suggested that SMA is an important node in the fronto-parietal balance network and provided direct evidence for a causal role of SMA in the control of balance in healthy adults. Following stroke, it is not known how cortical reorganization affects the balance network and whether neuromodulation of SMA in stroke patients will improve balance function in chronic stroke patients. The brain network was assessed using electroencephalography (EEG) in eleven chronic stroke patients. Of eleven, four patients returned for additional neuromodulation sessions - treatment and sham sessions. For neuromodulation, we delivered intermittent theta burst stimulation (iTBS) over SMA in the affected hemisphere or sham stimulation in separate counterbalance sessions. We assessed changes in the balance performance and changes in the functional corticospinal integrity by computing coherence between brain's electrical activity measured with EEG and muscle signals from leg muscles. We found that iTBS compared to sham stimulation improved balance performance as shown in the standard center of pressure measures obtained during a challenging balance task. Following iTBS versus sham stimulation, we observed higher cortico-muscular coherence (CMC) in the delta frequency band during both low and medium balance task difficulty conditions. These findings suggest that a single session of iTBS over SMA has a potential to improve balance control by enhancing functional corticomuscular integrity in chronic stroke patients.

Disclosures: K. Kukkar: None. **D. Huynh:** None. **S. Shah:** None. **S. Sheikh:** None. **J.L. Contreras-Vidal:** None. **I. Weinberg:** None. **P.J. Parikh:** None.

Poster

PSTR478. Somatosensation: Plasticity and Reorganization

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR478.14/DD2

Topic: D.03. Somatosensation – Touch

Title: Cortical activation during virtual reality object grasping with neuro-haptic feedback and real-world object grasping with natural haptic feedback

Authors: *A. K. SHELL[1], J. ASBEE[2], A. PENA[2], J. ABBAS[1], R. JUNG[1];
[1]Biomed. Engin., [2]Inst. for Integrative and Innovative Research, Univ. of Arkansas, Fayetteville, AR

Abstract: Virtual reality (VR) has been used as a tool for sensorimotor rehabilitation of the upper limb. Touch feedback is important for rehabilitation; however, this sense is still largely missing in VR, which can be detrimental to overall sensorimotor recovery. There is a lack of understanding of how the inclusion of haptic feedback in VR influences cortical activity. In this study, we compared how the brain responds to neurostimulation-based sensory feedback (neuro-haptic feedback (neuroHF)) with its response to natural haptic feedback (natHF) from the hand. NeuroHF was delivered through non-invasive electrical stimulation of the median nerve at the wrist using ExtendedTouch (xTouch), our patented sensory neuromodulation approach. This emulated the tactile experience of grasping virtual objects. Functional near-infrared spectroscopy (fNIRS) was used to measure changes of hemoglobin in 3 cortical regions of interest (ROIs): the sensorimotor (SMC), somatosensory association (SMA) and prefrontal (PF) cortices during two object identification tasks. While blindfolded, participants (n = 5) grasped 6 virtual objects with neuroHF and 6 physical objects with natHF from the right hand in a randomized order for 36 and 18 total presentations, respectively. The object profiles were combinations of 2 sizes (small, large) and 3 compliances (soft, medium, hard). Paired samples t-tests were performed for each virtual/physical object profile pair by ROI. Preliminary results indicate that there was no statistical difference in cortical activation for four object profile pairs in all ROIs: Small-Soft, Small-Hard, Large-Medium, Large-Hard (p>0.05). However, differences in cortical activation were observed for two object profile pairs: Small-Medium (Contralateral SMC, Contralateral PF, SMA, p<0.01) and Large-Soft (Contralateral SMC, SMA, p<0.01). Results indicate that cortical patterns of activation between physical objects with natHF and virtual objects with neuroHF may be comparable for most combinations of size and compliance. These findings suggest that xTouch has the potential to provide an intuitive sense of touch within VR. This work lays the foundation for future studies investigating the potential benefits neuroHF affords a user in fully immersive VR environments, since targeting sensory pathways has the potential to promote neuroplastic changes like those observed in traditional rehabilitation settings. Knowledge of how neuroHF influences cortical neural activity can inform the design of VR-rehabilitation interventions that promote recovery, ultimately leading to enhanced translatability of VR-training to real-world functional improvements.

Disclosures: **A.K. Shell:** None. **J. Asbee:** None. **A. Pena:** None. **J. Abbas:** None. **R. Jung:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.01/DD3

Topic: D.03. Somatosensation – Touch

Support: ERC No 633428

Title: Modeling sensory perception with neurobiologically detailed artificial neural networks

Authors: *M. KEATON, R. FRUENGEL, M. OBERLAENDER;
Max Planck Inst. for Neurobio. of Behavior, Bonn, Germany

Abstract: Unraveling cellular and circuit mechanisms that underlie perception is extremely challenging, because even the simplest sensory stimulus activates hundreds of thousands of neurons distributed throughout the entire brain. Moreover, the data provided by the sensory systems, representing the state of the world, is noisy. Yet, the brain is able to classify this noisy input across the hierarchy of cortical processing stages, triggering flexible and nuanced behaviors - a hallmark of higher cognition. How the brain accomplishes such robust perception is unclear. Here we introduce a novel computational modeling approach that allows translating cellular and circuit mechanisms that represent neural substrates of perception into design principles for artificial neural networks (ANNs). For this purpose, we motivate the network architecture of ANNs with empirical anatomical data from both dense and sparse reconstructions of local and long-range connectivity in the thalamocortical whisker system of the rat. Moreover, we inform the activation functions of nodes in the ANNs with empirical physiological data to capture both the perisomatic and nonlinear dendritic physiology of cortical pyramidal neurons. We provide first evidence that our approach leads to an improved performance and ability of such ANNs to generalize across tasks, and less reliance on large training datasets. These results indicate that neurobiologically detailed ANNs could facilitate dissecting the neural basis of perception, and showcase how higher brain functions emerge from their neurobiological implementations.

Disclosures: M. Keaton: None. **R. Fruengel:** None. **M. Oberlaender:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.02/DD4

Topic: D.03. Somatosensation – Touch

Support: BMBF/FKZ 01GQ1002
ERC No 633428
NIH Grants R01 NS24328
NIH Grants P40 OD010996

Title: New Definition of Motor Areas in the Cerebral Cortex

Authors: *A. MAHARJAN[1], J. M. GUEST[2], J.-A. RATHELOT[3], P. L. STRICK[4], M. OBERLAENDER[1];
[1]Max Planck Inst. for Neurobio. of Behavior – caesar, Bonn, Germany; [2]In Silico Brain Sci., Ctr. of Advanced European Studies and Res., Bonn, Germany; [3]Inst. des Neurosciences de la TImone

(UMR 7289), Aix-Marseille Univ., Marseille, France; [4]Systems Neurosci. Inst., Univ. Pittsburgh Sch. Med., Pittsburgh, PA

Abstract: A primary function of the cerebral cortex is to control voluntary movement. How cortical circuits orchestrate movement remains however poorly understood. Dissecting the circuits for motor control is challenging because neurons in the cortex generally do not form direct monosynaptic connections with motoneurons (MNs) in the spinal cord or brainstem. Instead, pyramidal tract neurons in cortical layer 5 (L5PTs) connect to highly diverse sets of premotor neurons, which then connect to highly diverse sets of MNs, which then innervate several different muscles. Due to the enormous complexity of these disynaptic networks, activation of even neighboring L5PTs can evoke movements of very different body parts, such as facial or limb muscles, or combinations thereof. Identifying the L5PTs throughout the cerebral cortex that have disynaptic access to the MNs of a single muscle remains hence a major challenge. Here we address this challenge by utilizing wildtype rabies virus, which we inject into the facial muscle that moves a single whisker on the snout of the rat. We complement these experiments with injections into a single muscle that moves digits on the rats' forepaw. Due to the retrograde spread of rabies virus across multiple synapses, we reveal L5PTs throughout all cortical areas that are disynaptically connected to the MNs of these two muscles. We find that disynaptic connections from L5PTs to both muscles extend far beyond the primary motor cortex to the primary sensory cortices, higher-order motor and sensory cortices, and even to association areas, such as the insular cortex. Notably, the distributions of L5PTs within and across these cortical areas is highly specific for each muscle. Our findings set the stage to quantitatively dissect the organization of the circuits by which the cerebral cortex orchestrates movement.

Disclosures: A. Maharjan: None. **J.M. Guest:** None. **J. Rathelot:** None. **P.L. Strick:** None. **M. Oberlaender:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.03/DD5

Topic: D.03. Somatosensation – Touch

Support: NIH Grant F31NS120483
NIH Grant R01NS117536

Title: Sensorimotor interactions between primary vibrissal somatosensory and motor cortices

Authors: *R. PANCHOLI, A. SUN-YAN, M. LAUGHTON, S. PERON;
New York Univ., New York, NY

Abstract: Identifying and responding to stimuli depends on the precise integration of sensory and motor information. Understanding how sensory and motor cortices interact is thus central to

understanding how animals produce behavior. In the mouse whisker system, a subregion of primary vibrissal motor cortex (vM1) exhibits robust responses to object contact ('touch') and receives rich input from primary vibrissal somatosensory cortex (vS1). Vibrissal S1 and vM1 also exhibit responses to whisker movement ('whisking') and to licking. We use two-photon calcium imaging in mice performing an object detection task with two whiskers to compare sensorimotor representations in superficial layers of vS1 and vM1. We find that both areas contain robust responses to touch, with touch neurons in vM1 exhibiting broader receptive fields than touch neurons in vS1. Both areas also contain populations of whisking and licking neurons. We next use retrograde tracer injections in either vS1 or vM1 to assess neurons projecting from one area to the other. We find that touch neurons responsive to multiple whiskers are overrepresented in the projecting population relative to neurons responding to a single whisker, with relatively equal fractions of broadly tuned touch neurons projecting in both directions. Whisking activity is also transmitted bidirectionally, although the fraction of whisking neurons projecting from vM1 to vS1 exceeds the fraction of whisking neurons projecting from vS1 to vM1. Licking activity follows a similar pattern. Imaging of both areas over the course of several weeks shows that the activity of neurons tuned for a single whisker is relatively unstable whereas the activity of broadly tuned touch neurons, whisking neurons, and licking neurons is much more stable. Our work provides evidence that communication between sensory and motor cortex is bidirectional, implying that computations requiring multiple sensorimotor variables can be performed in both vS1 and vM1. Representations spanning both areas thus tend to depend on sparse but stable populations of neurons that may engage in interareal recurrence.

Disclosures: **R. Pancholi:** None. **A. Sun-Yan:** None. **M. Laughton:** None. **S. Peron:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.04/DD6

Topic: D.03. Somatosensation – Touch

Title: Coding of sensorimotor variables in dysgranular vibrissal somatosensory cortices

Authors: *A. AHMED[1], A. GARCIA BARRABEITG[2], M. LAUGHTON[3], A. SUN-YAN[2], S. PERON[4];
[1]New York Univ., New York, NY; [3]Ctr. for Neural Sci., [2]New York Univ., New York City, NY; [4]New York Univ. Ctr. For Neural Sci., New York, NY

Abstract: Understanding the circuit basis of somatosensory perception requires a detailed understanding of the distributed cortical representation of touch. In the mouse whisker system, somatosensory thalamus outputs to primary and secondary vibrissal somatosensory cortices (vS1 and vS2). Somatosensory thalamus also targets the dysgranular zone (Killackey et al. 1983), a strip of somatosensory cortex between the vibrissal and forepaw representations. Despite

extensive studies of vS1 and vS2, however, the dysgranular zone's response to whisker touch remains poorly understood. Vibrissal S1 sends outputs to three distinct areas within the dysgranular zone: the anteromedial (AM), centromedial (CM), and posteromedial (PM) dysgranular areas (Yamashita et al. 2018). In addition to vS1 and vS2, these three specific dysgranular areas may thus contribute to processing whisker touch. We recorded activity across layers 2, 3, and 4 of vS1, vS2, AM, CM, and PM using volumetric two-photon calcium imaging in transgenic mice expressing GCaMP6s in all cortical excitatory neurons. We trained mice to actively palpate a pole with their whiskers, reporting touch of the pole with licks to one of two lickports and the absence of touch with licks to the other. We trimmed the mice to either two or three whiskers (C2, C3, and sometimes C4), and we positioned the stimulus pole to yield many isolated touches by each whisker. We then compared touch, whisking, and licking activity across all five areas. Like vS2, dysgranular areas AM and CM had a larger fraction of touch neurons that were responsive to multiple whiskers compared to vS1. We also found a higher fraction of lick responsive neurons in CM, and a lower fraction of whisking neurons in AM and CM compared to vS1. These findings suggest that dysgranular areas may be crucial for the integration of touch information across multiple whiskers. Moreover, specific dysgranular areas likely serve to combine touch information with specific forms of motor information, such as licking and whisking.

Disclosures: **A. Ahmed:** None. **A. Garcia Barrabeitg:** None. **M. Laughton:** None. **A. Sun-Yan:** None. **S. Peron:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.05/DD7

Topic: D.03. Somatosensation – Touch

Title: Cortical contributions to context-dependent sensorimotor transformation

Authors: *P. GHADERI, S. CROCHET, C. PETERSEN;
Brain mind Inst., Lausanne, Switzerland

Abstract: Flexible integration of sensory stimuli in a context-dependent manner is a key cognitive process required to generate appropriate behavior. An intriguing question, then, is how the same sensory stimulus can be interpreted differently according to context in order to generate different behavioral responses. We designed a task in which mice were trained to lick for reward in response to a single whisker stimulus if it was preceded 1 s earlier by a brief Go-Tone, but not if it was preceded by a NoGo-Tone.Optogenetic inactivation of primary auditory cortex (A1), primary whisker somatosensory cortex (wS1), secondary whisker somatosensory cortex (wS2), secondary whisker motor cortex (wM2), and anterior lateral motor cortex (ALM) revealed prominent temporally-specific deficits in task performance for each area, whereas inactivation of

primary forepaw somatosensory cortex had no impact.We investigated neuronal correlates of context-dependent sensorimotor transformation using high-density extracellular Neuropixels recordings combined with high-speed video filming of facial movements. Neuronal activity in A1, wS1, wS2, wM2, and ALM differed comparing Go-context hit trials to NoGo-context correct rejection trials. We focused our analyses on two questions:i) How is context encoded in the delay period? Neurons in wM2 and ALM had prominent persistent activity during the delay period following the Go-tone presentation, even in trials without anticipatory facial movements. Using linear decoding of neuronal activity, we found that the accuracy of classifying context in the 200 ms before whisker stimulus was highest for wM2 and ALM. Consistent with an important role for this persistent activity, optogenetic inactivation of wM2 and ALM during the delay period profoundly reduced licking in the reward window.ii) How does context alter whisker sensory processing? Even the very first evoked responses of neurons in wS1 and wS2 were altered according to context, perhaps forming the first steps in gating the transformation of whisker sensation into licking motor output. Many cortical regions appear to contribute since optogenetic inactivation of A1, wS1, wS2, wM2, or ALM during the presentation of the whisker stimulus significantly decreased the probability of licking in the reward window. Future analyses will focus on examining interactions between cortical regions, which might underlie context-dependent goal-directed sensorimotor transformation.Our results indicate a critical role of frontal areas wM2 and ALM for encoding and maintenance of contextual information during the delay period and suggest early context-dependent sensory processing in wS1 and wS2.

Disclosures: **P. Ghaderi:** None. **S. Crochet:** None. **C. Petersen:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.06/DD8

Topic: D.03. Somatosensation – Touch

Support: Award no. 1T32NS126122-01

Title: Parallel processing of tactile information in S1 and superior colliculus

Authors: *A. NAM[1], K. HONG[2];
[1]Biol. Sci., [2]Neurosci. Inst. and Dept of Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Recent studies have demonstrated that inactivation of S1 leads to only partial and short-term behavioral impairments in whisker-mediated tactile detection. This suggests that primary somatosensory cortex (S1) activity is not absolutely essential for this behavior. In the absence of S1, the superior colliculus (SC) is thought to mediate tactile detection. The SC is an evolutionarily ancient midbrain structure that mediates rapid responses to the detection of salient stimuli. While thoroughly studied for its role in vision for saccade and orienting behaviors, SC is

a multisensory-motor hub. If SC is capable of mediating tactile detection, what does S1 contribute to the evolutionarily ancient SC? The intermediate layers of SC receive direct bottom-up tactile input from the brainstem as well as top-down input from S1. It remains unclear how S1 and SC dynamically coordinate sensory-guided behavior. To address this, we trained animals on a yes/no whisker-mediated tactile detection paradigm, while simultaneously recording S1 and SC. We find that during behavior, neural populations in S1 and SC each robustly encode deflection of single whisker stimuli. When we optogenetically silence S1, animals' performance is only partially impaired, consistent with previous studies. Surprisingly, S1 inactivation led to increased signal to noise ratio in SC- by decreasing SC's baseline activity while increasing the amplitude of stimulus-evoked firing rates. Thus, despite improved stimulus encoding in SC, S1 inactivation results in decreased detection abilities, suggesting that psychometric behavioral performance cannot be fully explained by SC activity alone. Our findings suggest that for an operant tactile detection task, while SC activity alone can account for a large extent of behavioral performance, S1 may serve to improve detection, especially for near-threshold stimuli. On-going work is aimed at manipulating SC activity to determine its contribution to detection behavior, as well as using an information theoretic approach to quantify redundant, unique, or synergistic information between S1 and SC.

Disclosures: **A. Nam:** None. **K. Hong:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.07/DD9

Topic: D.03. Somatosensation – Touch

Title: Neural activity in principal barrel column but not neighboring barrels is required for whisker guided active navigation

Authors: *A. G. ARMSTRONG, N. AL-KODMANY, K. HU, Y. VLASOV;
Neurosci. Program, Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: It has been reported the barrel cortex is dispensable for simple object detection tasks [1]. However, cortex and in particular interaction of multiple barrel columns might be required to produce more complex behavior, such as foraging and navigation. To study this, we have developed a tactile virtual-reality behavioral paradigm that mimics active whisker-guided navigation in the natural environment of rodents [2]. Mice with just a pair of C2 whiskers reliably and repeatably track the walls of the virtual environment after just 4 training sessions, while continuously navigating at high speeds (25cm/s) for up to 2 hours completely unrewarded, thus emphasizing the ethological relevance of the task. To determine the neural correlates of this behavior, Neuropixel silicon probes were inserted into the barrel cortex, with 438 cells measured in the principal C2 barrel column (n = 9) and 361 in nearest neighbor columns (n = 7). Neural

activity across all cortical layers in the C2 barrel column was strongly modulated during navigation, however this modulation was not seen in neighboring barrel columns. Significant modulation that directly precedes changes in run direction during navigation was only seen in the principal barrel and not the corresponding ipsilateral barrel. We hypothesize that this sensory activity in the principal barrel alone is used by downstream circuits to produce navigation behavior. Ultra small electrolytic lesions (150µm diameter) were generated highly localized in the center of layer 4 of the principal barrel column, the main thalamocortical input layer. Disrupting layer 4 significantly decreased navigation ability while having no effect on locomotion and whisking. Mean navigation success rate across animals dropped from 82% to 33% (n = 4) with no significant effect seen when lesioning layer 4 of neighboring barrels (n = 6) or in the ipsilateral C2 barrel. Contralateral principal barrel column activity, but not neighboring barrel activity, is therefore required for single whisker-guided active navigation. [1] K. Hong *et al. Nature*. **561**:542-546 (2018) [2] A. Armstrong *et al.* SfN 2022, P:208.05

Disclosures: A.G. Armstrong: None. **N. Al-Kodmany:** None. **K. Hu:** None. **Y. Vlasov:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.08/DD10

Topic: D.03. Somatosensation – Touch

Support: 3R01NS117536-04S1

Title: Behavioral role of individual mouse vibrissal somatosensory cortex barrels in discriminating between touch by distinct whiskers

Authors: *M. T. LAUGHTON, A. SUN-YAN, L. RYAN, S. PERON;
Ctr. for Neural Sci., New York Univ., New York City, NY

Abstract: In a range of behaviors, mice rely heavily on tactile sensory input from their facial whiskers to acquire information about their surroundings. Partitioned into a topographic map of well-defined columns also known as ‘barrels’ (radius: ~150 um), the mouse primary vibrissal somatosensory cortex (vS1) contains a highly stereotypical pattern of barrels that each receive input from a single whisker. Loss of individual barrels has a range of effects on behavior, including degrading touch intensity discrimination and gap crossing, but not object detection, by the relevant whisker. Mice typically sample their environment with multiple whiskers. We therefore sought to understand the role of individual barrels in multi-whisker behaviors. Mice with cranial windows over vS1 were trained on a two whisker discrimination task in which they reported which of two whiskers touched (of C1, C2 and C3) by licking one of two lickports, each of which was associated with a specific whisker. We then performed columnar scale laser mediated lesions of specific whisker barrels. Dual barrel lesions transiently impacted

performance of mice using two adjacent whiskers (C2 and C3), without disrupting vibrissal kinematics. Single barrel lesions also transiently impacted performance of mice using adjacent whiskers. We analyzed whether performance decline was whisker specific or the same across both whiskers. We next performed single barrel lesions in mice using a separated pair of whiskers (C1 and C3). Post-lesion, the resulting decline in behavior of mice using separated whiskers was smaller and the mice recovered performance more rapidly than when whiskers were adjacent. Thus, lesioning one or two barrels persistently reduced performance on a whisker touch discrimination task, with adjacent whisker discrimination being more sensitive to such lesions than separated whiskers.

Disclosures: **M.T. Laughton:** None. **A. Sun-Yan:** None. **L. Ryan:** None. **S. Peron:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.09/DD11

Topic: D.03. Somatosensation – Touch

Support: 5SC3GM122657-06

Title: Open-field testing on the exploratory behavior of barrelless mice

Authors: *S. A. F. LUTCHMAN[1,2], J. M. F. LUTCHMAN[1,2], G. CATALDO[1,2,3], J. C. BRUMBERG[1,2,3];
[1]Psychology, [2]Behavioral Neurosci. MA Program, Queens College, CUNY, Flushing, NY; [3]Biol. and Psychology PhD Programs, The Grad. Ctr. CUNY, New York, NY

Abstract: Rodents rely heavily on their whiskers to navigate and explore their surroundings in order to survive. In the somatosensory cortex sensory information from the stimulation of the whiskers are transmitted to the barrel region of the somatosensory cortex, which has a topographic arrangement, with each whisker corresponding to one barrel. To study if the cortical sensory information transmitted to the barrel cortex from the whiskers would affect mice performance; an open-field task was conducted. Barrelless (BRL) mice, an Adenylyl Cyclase 1 variation in which the thalamo-cortical afferents travel to the cortex but do not segregate into barrels were utilized. Previous research has demonstrated that BRL mice exhibited impairments on behavioral tasks. Due to the significant role that barrel patterning plays in navigation and active sensing we demonstrated that BRL mice expressed more willingness to explore an open-field arena over two consecutive days of behavioral testing. BRL mice also had increased locomotor activity and moved faster compared to wild type CD-1 mice across both days of behavioral testing. The next phase of this experiment is to test the mice on a texture-based discrimination task.

Disclosures: **S.A.F. Lutchman:** None. **J.M.F. Lutchman:** None. **G. Cataldo:** None. **J.C. Brumberg:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.10/DD12

Topic: D.03. Somatosensation – Touch

Support: NIH R01NS117536

Title: Cortical circuitry mediating inter-areal touch signal amplification

Authors: *L. RYAN, A. SUN-YAN, M. LAUGHTON, S. PERON;
Ctr. For Neural Sci., New York Univ., New York, NY

Abstract: Sensory cortical areas are often organized into topographic maps representing the sensory epithelium. Individual areas are richly interconnected, and, in many cases, they are coupled via reciprocal projections that respect the topography of the underlying map. Because topographically matched cortical patches process the same stimulus, their interaction is likely to be central to many neural computations, including object recognition, feature binding, and attention. Here, we ask how topographically matched subregions of primary and secondary vibrissal somatosensory cortices (vS1 and vS2) interact during whisker touch. In the mouse, whisker touch-responsive neurons are topographically organized in both vS1 and vS2, and both areas also receive thalamic touch input and are topographically interconnected. We focus on subregions of vS1 and vS2 that respond to touch from whiskers C2 and C3, ensuring topographically matched areas of both cortical regions. We first employ volumetric two-photon calcium imaging of these matched subregions to characterize touch neuron populations in both areas while a mouse is actively palpating an object with two whiskers. We find a sparse population of highly active, broadly tuned touch neurons responsive to both whiskers. These neurons are especially pronounced in superficial layer 2 in both areas. Next, we use retrograde labeling to determine which populations of touch neurons relay touch information to topographically matched targets across both areas. Despite their rarity, the broadly tuned population serves as the main conduit of touch-evoked activity between vS1 and vS2 and exhibit elevated synchrony. Finally, we selectively lesion patches of either vS1 or vS2 responsive to touch by whiskers C2 and C3 to assess how topographically matched sites mutually influence one another. Focal lesions of the whisker touch-responsive region in vS1 or vS2 degrade touch responses in the unlesioned area, with whisker-specific vS1 lesions degrading whisker-specific vS2 touch responses. Thus, a sparse and superficial population of broadly tuned touch neurons recurrently amplifies touch responses across vS1 and vS2.

Disclosures: **L. Ryan:** None. **A. Sun-Yan:** None. **M. Laughton:** None. **S. Peron:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.11/DD13

Topic: D.03. Somatosensation – Touch

Support: Whitehall Foundation
Purdue Institute for Integrative Neuroscience

Title: Bilateral integration in somatosensory cortex is controlled by behavioral context

Authors: *H. KERI[1,2], H. PARK[2], C. BI[2], C. YOO[3], S. R. PLUTA[2,4];
[1]Weldon Sch. of Biomed. Engin., [2]Dept. of Biol. Sci., [3]Dept. of Chem., [4]Purdue Inst. of Integrative Neurosci., Purdue Univ., West Lafayette, IN

Abstract: Many natural behaviors require the integration of tactile cues from both sides of the body. Traditional models argue that the integration of bilateral touch only occurs between homotopic regions of higher cortical areas. However, active touch requires high temporal resolution, implicating an important role for primary somatosensory cortex (S1). To test this hypothesis, we performed bilateral electrophysiology in mice trained to discriminate between two categories of bilateral cues using whisker-mediated active touch. During the task, mice performed bilaterally symmetrical whisking in a goal-directed manner. In S1 neurons, tactile information from the ipsilateral whiskers primarily facilitated the contralateral response. In accordance with task goals, bilateral facilitation prevailed for homotopic or heterotopic stimuli, while bilateral suppression dominated in untrained mice. Neural encoding of the ipsilateral stimulus was only accurate when mice made the correct behavioral choice. Temporally coordinated spiking and strong spike-field coupling between the hemispheres selectively emerged for the reward-associated stimuli. Thus, the flow of tactile information between the somatosensory cortices is controlled by goal-directed processing.

Disclosures: H. Keri: None. **H. Park:** None. **C. Bi:** None. **C. Yoo:** None. **S.R. Pluta:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.12/DD14

Topic: D.03. Somatosensation – Touch

Title: High frequency whisker vibrations encode texture information through temporally precise firing in the cortex

Authors: *Y. DING[1], Y. VLASOV[2];
[1]Univ. of Illinois, Urbana, IL; [2]Neurosci., Univ. of Illinois Urbana-Champaign Neurosci. Grad. Program, Urbana, IL

Abstract: Precise temporal coding has been observed in the vibrissae system with precisely time-aligned spiking with jitter less than a milliseconds following whisker stimulation. What aspects of such fast and complex spatiotemporal micromotions of a whisker are contributing to generation of these precisely timed spike trains? Recently, by measuring high frequency whisker micromotions induced by interaction with textured surfaces [1], we hypothesize that this temporally precise firing in cortical populations is encoding arrival times of vibrational shockwave travelling from the whisker tip to the base. To test this hypothesis in-vivo, we use a microphone with textured membrane to listen to micro-vibrations of the animal whisker while it is swept across. Extracellular electrophysiology is simultaneously recorded with linear 64 channel probe (Cambridge Neurotech) implanted into the principle whisker barrel column. High frequency components of the recorded acoustic signals that correspond to generation of vibrational eigenmodes are used as triggers to align spikes from single unit activity recorded across different cortical layers. We found a number of units with firing time strongly synchronized with onset of whisker micromotions detected from recorded acoustic signals, with majority of their spikes fired with 6ms latency. Closer inspection reveals that when triggered by higher order vibrational eigenmode (mode 8 at 7.5kHz), the maximum likelihood estimation (MLE) of spike latency is 5.48 milliseconds with a jitter of 0.8 milliseconds. This is in contrast to the MLE spike latency of 6.84 milliseconds when triggered by lower order vibrational eigenmode (mode 2 at 500Hz). This 1.36 millisecond difference in spike latency is comparable to the difference of arrival times at the whisker base between higher and lower order vibrational eigenmodes due to the 10X difference in their propagation speed [1]. This indicate that the shockwave generated at the whisker tip by collisions with external objects generate a time series of energy bursts that create a temporally unique “bar code” of a time-sequenced spike trains. The resulting ultra-high information capacity that is available for encoding (and discrimination) using such temporal code of time-aligned spikes exceeds by orders of magnitude the rate capacity of a traditional rate coding stick-slip model that does require a temporal integration of sequential collision events. [1] Y. Ding, Y. Vlasov, bioRxiv, DOI 10.1101/2022.06.15. 496141 (2022)

Disclosures: **Y. Ding:** None. **Y. Vlasov:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.13/DD15

Topic: D.03. Somatosensation – Touch

Support: Horizon 2020 ICT Grant DEEPER
NIH Grant U19 NS107464

Title: Multisensory contribution to texture discrimination in head-fixed mice

Authors: *I. ZANCHI[1,2,5,3], A. SEMPERE[2,3], M. CELOTTO[4,6], L. TAUSANI[2,3], D. VECCHIA[2,3], A. FORLI[2,3], J. BONATO[4,6], S. PANZERI[3,6,4], T. FELLIN[2,3];
[1]Italian Inst. of Technol., Genova, Italy; [2]Optical Approaches to Brain Function, [3]Neural Coding Lab., Inst. Italiano di Tecnologia, Genova, Italy; [4]Neural Computation Lab., Inst. Italiano di Tecnologia, Rovereto, Italy; [5]Univ. di Genova, Genova, Italy; [6]Dept. of Neural Information Processing, Ctr. for Mol. Neurobio. (ZMNH), Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Decision making tasks involving the somatosensory system, such as texture discrimination, have been extensively used in rodents to investigate the cellular and circuit mechanisms underlying the processing of sensory information and are believed to primarily rely on whisker-mediated inputs. However, when using whiskers to explore the environment, mice may combine tactile inputs with those from other sensory modalities (e.g., olfaction, vision). Here, we tested the hypothesis that sensory modalities other than somatosensation influence animal's behaviour in a Go/No-Go texture discrimination task in head-fixed mice. In expert animals, we found that trimming the whiskers prolonged reaction times, while this manipulation did not significantly affect the proportion of correct choices. We replicated these results when mice performed the task in darkness, suggesting that visual inputs were not responsible for the unaltered proportion of correct choices observed upon whisker trimming. In contrast, experimental manipulations interfering with the processing of olfactory signals significantly decreased the fraction of correct choices both in the presence and in the absence of whiskers. Using two-photon calcium imaging in the primary somatosensory cortex (S1) in combination with information theoretic analysis, we found encoding of task-related information in S1 both in the presence of whiskers and after whisker trimming. Finally, manipulations perturbing olfaction cancelled the encoding of task-related information in S1. Taken together, these results demonstrate that olfaction can be sufficient to sustain the ability to discriminate textures in head-fixed mice. Moreover, these findings suggest that S1 neurons may be a site for integration of multisensory (i.e., olfactory and somatosensory) information.

Disclosures: I. Zanchi: None. **A. Sempere:** None. **M. Celotto:** None. **L. Tausani:** None. **D. Vecchia:** None. **A. Forli:** None. **J. Bonato:** None. **S. Panzeri:** None. **T. Fellin:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.14/DD16

Topic: D.03. Somatosensation – Touch

Support: NRF-2019M3E5D2A01058328

Title: Synchronization of spike times across layers during texture perception in the mouse primary somatosensory cortex

Authors: *D. OH, J. KWAG;
Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Precisely synchronized activity among cortical neurons is crucial for encoding information about various stimulus elements from their environment. In the primary somatosensory cortex (S1), which is involved in whisker-related tactile perception. It is well established that different textures elicit distinct spike patterns in neurons across the laminar structure of S1. However, how different texture information is represented in synchronization across multiple neurons in each layer is poorly understood. To address this question, single-unit activities from S1 were recorded *in vivo* using a 32-channel silicon probe during 1-second presentations of two different textures, rough (P80) and smooth (P1200), to the whiskers of the head-fixed mouse. In response to each stimulus, the majority of neurons across layers of S1 displayed distinct spike patterns depending on textures. To investigate synchronization between units within and between layers, the similarity was analyzed between spike patterns of units during texture stimulation. Our preliminary results demonstrated that similarity among units, both within the same layer (intra-layer) and between different layers (inter-layer), was significantly higher during rough texture stimulation compared to smooth texture stimulation in most cases. Together, our preliminary results demonstrate that different textures may employ different synchronized activities of units across layers to encode texture information.

Disclosures: D. Oh: None. **J. Kwag:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.15/DD17

Topic: D.03. Somatosensation – Touch

Support: NIH Grant 1R01NS123681
NIH Grant 2 R01NS092367-08A1

Title: A novel high-speed 2-photon voltage imaging approach for exploring neural coding and dynamics in sensory cortex

Authors: *L. C. GOMEZ[1], G. ZHANG[2], S. LEE[2], D. JIANG[2], M. Z. LIN[3], N. JI[4], D. E. FELDMAN[5];
[1]Mol. and Cell. Biol., Univ. of California, Berkeley, Berkeley, CA; [2]Stanford Univ., Palo Alto,

CA; [3]Neurobio., Stanford, Stanford, CA; [4]Physics and MCB, California Clin. Trials, Berkeley, CA; [5]Mol. & Cell Biol. Dept., UC Berkeley, Berkeley, CA

Abstract: Recording activity from large neural populations simultaneously is critical for studying brain function. 2-photon (2P) calcium imaging has been the workhorse technique for acquiring population activity in deep scattering tissue, however it is a poor proxy for spiking activity and lacks the temporal resolution to capture fast (<20 ms) timing information. Genetically encoded voltage indicators (GEVIs) can directly overcome these limitations but have historically suffered from signal detection issues due to low photon output per imaging volume. Recent advances in GEVI engineering have increased fluorophore brightness and optimized kinetics to improve signal to noise ratio (SNR). An outstanding challenge for population voltage imaging has been the need for fast acquisition rates (>250 Hz) to track rapid spiking-related fluorescence changes. Standard methods to achieve fast sampling rates, like widefield microscopy, have limited imaging depth and contrast. For 2P imaging, spatiotemporal multiplexing based on free-space angular-chirp-enhanced delay (FACED) can achieve kHz frame rates. Here, we present a novel framework that combines an improved, positively tuned GEVI (ASAP4.6.2), with both conventional small-field and FACED large-field 2P imaging. We apply this approach to image whisker sensory responses in Layer (L) 2/3 pyramidal (PYR) cells of somatosensory cortex (S1) of awake mice. We injected AAV carrying Cre-dependent ASAP4 into S1 cortex of Drd3-Cre mice (L2/3 PYR cell-specific). We applied calibrated whisker deflections to awake, head-fixed mice while imaging voltage activity. ASAP4 had a bright baseline where spikes were evident as brief (2.5 ms FWHM) fluorescent transients with a peak response amplitude of 24.8 % $\Delta F/F_0$, showing minimal photobleaching (7.9 % F_0 decline) over 30 minutes of continuous imaging. Simultaneous voltage and calcium imaging, assessed by jRGECO1a co-expression, indicated a superior spike sensitivity by ASAP4. Robust sensory-evoked spiking was reported by ASAP4 with 36% of cells showing significant whisker responses (vs. ~30% as is common with calcium imaging). ASAP4 subthreshold responses showed whisker-evoked depolarization (from ~ 0-64 ms post-stimulus) and later hyperpolarization (65-135 ms), with 50 % of spikes evoked in <25-55 ms post-stimulus onset. Whisker receptive fields from spiking data resembled those from classical spike recordings. We are currently applying FACED 2P imaging to study network dynamics among S1 neurons tuned to different whiskers. These results show voltage imaging can be a powerful tool for population imaging in sensory cortex.

Disclosures: **L.C. Gomez:** None. **G. Zhang:** None. **S. Lee:** None. **D. Jiang:** None. **M.Z. Lin:** None. **N. Ji:** None. **D.E. Feldman:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.16/DD18

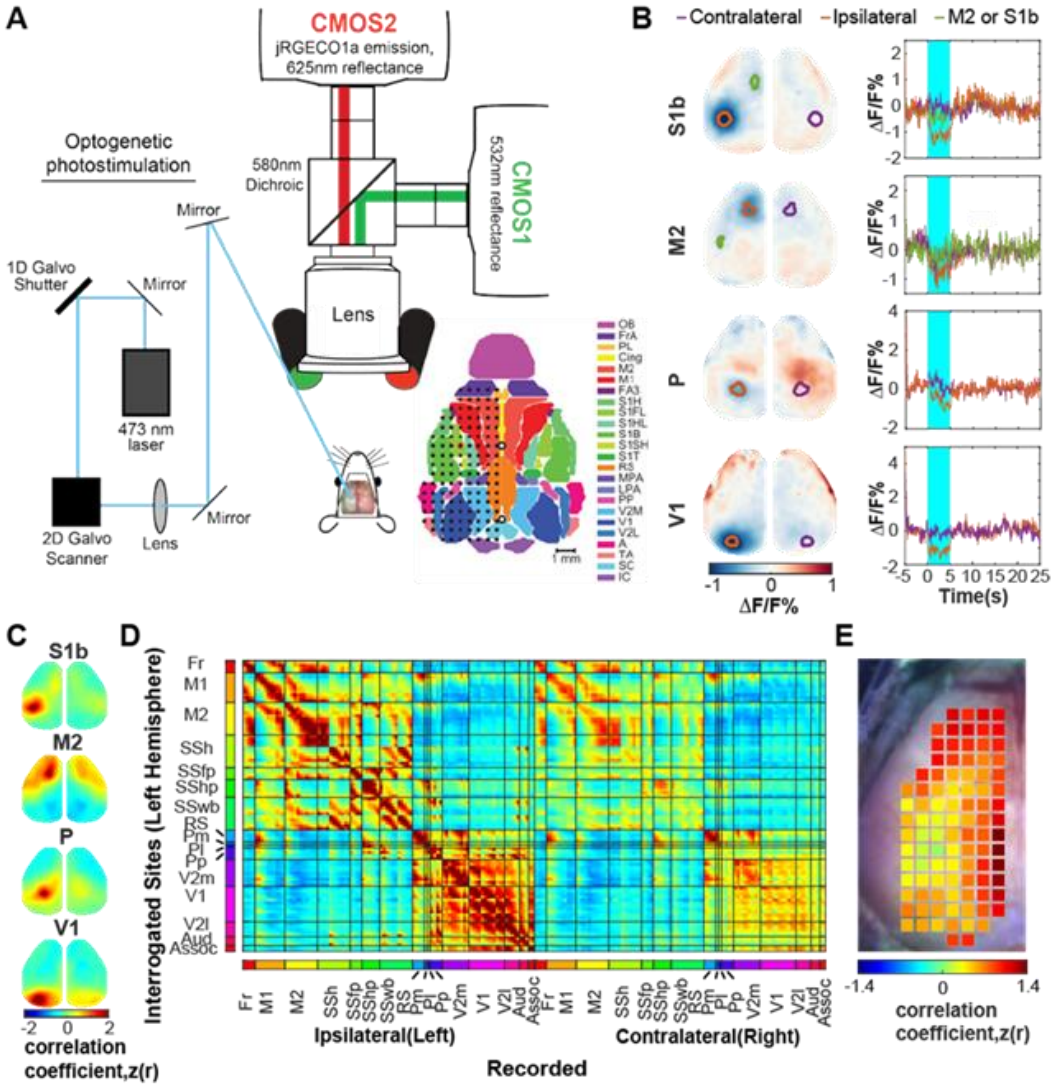
Topic: I.08. Methods to Modulate Neural Activity

Support: NIH - R01NS10287005
NIH - R01NS12632601
NIH - RF1AG07950301
NIH - P30CA09184221
Siteman Cancer Center Grant 5385
WUSTL Department of Radiology
McDonnell Center for Systems Neuroscience

Title: Mapping Local and Global Interactions between Parvalbumin Inhibitory Neurons and Excitatory Neurons over the Cortex in Awake Mice

Authors: *X. WANG[1], A. BICE[1], A. Q. BAUER[2];
[1]Washington Univ. in St. Louis, Saint Louis, MO; [2]Washington Univ. in St. Louis, Washington Univ. in St. Louis, St. Louis, MO

Abstract: Parvalbumin interneurons (PV-INs) are the largest subpopulation of GABAergic neurons, and play major roles in modulating plasticity. Coherent infraslow fluctuations in blood oxygenation are coupled in phase and amplitude to gamma brain rhythms. These patterns emerge from the rhythmic output of PV-INs to synchronize excitatory activity over long distances. Recent evidence suggests PV-INs exhibit long-range, transcallosal projections in select cortical regions. Whether transcallosal PV-INs feature prominently over the cortex, and the extent to which their local influence affects global excitatory activity is unknown. We created a novel imaging system and mouse line to allow for optogenetic targeting of PV-INs and mesoscopic imaging of excitatory activity via the red-shifted calcium indicator jRGECO1a (**Fig.1A**) to evaluate the interaction between PV-INs and excitatory cells over the cortex. Photostimuli were delivered over a grid in the left hemisphere of awake mice (**Fig.1A**). Photostimulation of PV-INs resulted in local reductions of jRGECO1a fluorescence, consistent with local inhibition of excitatory activity. Photostimulating primary somatosensory cortex(S1b) reduced S1b activity and ipsilateral motor (M2) activity (**Fig.1B**). Similarly, photostimulation of left M2 resulted in reciprocal, ipsilateral inhibition of left S1b, and right M2(**Fig. 1B**). Regional effective connectivity (EC) was determined by correlating the time course at the photostimulated site with all other time courses to map the global extent of local PV-based inhibition (**Fig.1C**). Systematically scanning photostimulti over the left hemisphere allowed for visualizing the "PV connectome" (**Fig.1D**) and site-wise homotopic EC (**Fig.1E**). We developed novel technology to examine the interaction between PV-INs and excitatory cells over the cortex. PV-based inhibition extended several mm locally and into more distant, ipsilateral regions. Further, PV-INs exhibit region-specific interhemispheric inhibitory influences. Future work will evaluate how PV-EC patterns relate to underlying anatomy.



Disclosures: **X. Wang:** None. **A. Bice:** None. **A.Q. Bauer:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.17/DD19

Topic: D.03. Somatosensation – Touch

Support: NIH Grant R01HD054453
NIH Grant R01 NS117597
Jessamine Hilliard Neurobiology Graduate Student Grant Program

Title: Adapting and facilitating responses of excitatory neuron populations in mouse somatosensory cortex are dynamic and shaped by experience across days.

Authors: *Z. DOBLER[1,2], T. CHARI[1,2], S. MULA[2], C. PORTERA-CAILLIAU[2,3];
[1]Univ. of California, Los Angeles Interdepartmental Ph.D. Program in Neurosci., Los Angeles, CA; [2]Neurol., [3]Neurobio., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: To construct a stable and coherent experience of the external world, sensory circuits must adapt their activity to the statistics of the surrounding environment and filter out irrelevant stimuli. This is achieved in part via stimulus-evoked sensory adaptation (SA), whereby neuronal activity is repeatedly adjusted in response to repetitive sensory stimuli. While SA has been extensively studied at the level of individual neurons on timescales of tens of milliseconds to a few seconds, little is known about SA at the population level, or whether SA dynamics are stable across hours or days. To address these knowledge gaps, we used in vivo 2-photon calcium imaging of layer (L) 2/3 or L4 excitatory neurons in the barrel field of the somatosensory cortex (S1BF) of awake adult Ai162 mice (GCaMP6s) x Slc17a7-Cre or Scnn1a-Cre, respectively. In addition to previously described adapting neurons that decreased their firing with repetitive stimulation, we found facilitating neurons that increased their activity, and still others that were neither adapting nor facilitating. Within each of these populations (adapting vs. facilitating) in either layer, individual responses to different whisker deflections were strikingly heterogeneous and stochastic from one whisker stimulus to the next. We also discovered that, for L2/3 neurons, adaptation to one stimulus does not generalize to different stimuli; when we delivered 10 whisker stimuli at one frequency followed by a second bout at an alternate frequency, we found that adapting L2/3 neurons (but not facilitating neurons) exhibited increased response peak amplitudes after switching to a higher frequency. We also investigated the stability of population SA dynamics by longitudinally imaging the same neurons over several days. Strikingly, most stimulus-responsive neurons did not maintain their SA response profiles (e.g., some adapting cells became facilitating and vice versa) and the ratio of adaptation to facilitation was dynamic across days. These results indicate that 1) Population-level SA is encoded heterogeneously in S1BF and does not universally generalize; 2) Adapting neurons are most sensitive to shifts in stimulus parameters; and 3) The balance between adaptation and facilitation at the population level is experience-dependent.

Disclosures: Z. Dobler: None. **T. Chari:** None. **S. Mula:** None. **C. Portera-Cailliau:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.18/DD20

Topic: D.03. Somatosensation – Touch

Support: NIH R37 NS092367
NIH R01 NS092367-08A1

Title: Factors that regulate tuning instability in the whisker map in L2/3 of mouse S1 cortex

Authors: *H. WANG[1], D. FELDMAN[2];
[1]Helen Wills Neurosci. Inst., Berkeley, CA; [2]Mol. & Cell Biol. Dept., UC Berkeley, Berkeley, CA

Abstract: Representational drift is a prominent phenomenon in cortices, not only in high-order association areas, but surprisingly also in the primary sensory cortex, where a sizeable fraction of pyramidal (PYR) cells change their tuning for basic sensory features over days and weeks, even in the absence of overt learning. Such tuning instability raises a challenge for stable perception and behavior, but may be linked to plasticity, memory consolidation, or hidden cognitive or behavioral modulation. In a recent study, we performed longitudinal Ca^{2+} imaging in L2/3 of whisker somatosensory cortex in mice with consistent performance on a whisker sensory task, and observed robust whisker tuning instability in ~40% of L2/3 PYR cells over a 5-18 day period (Wang et al., Nat. Comm. 2022). Tuning instability was highly concentrated in non-columnar whisker (non-CW) tuned neurons, and thus was structured in the whisker map. Here, we investigate possible sources of this tuning instability. Tuning instability was unchanged during chronic whisker paralysis, and thus does not require normal patterns of whisker experience outside the task. Tuning drift remained prominent when each imaging session was performed under isoflurane anesthesia, indicating the tuning changes were not driven by acute cognitive modulation of a stable default sensory circuit. When mice performed a task requiring discrimination of individual whiskers and selective reinforcement of some whiskers, the stability of CW-tuned neurons increased, but tuning of non-CW tuned neurons remained unstable. Thus, unstable tuning of non-CW neurons is not overtly modulated by whisker experience, whisker-reward association, or acute cognitive modulation of whisker sensory processing. We are currently investigating whether CW-tuned and non-CW tuned PYR neurons are different molecularly distinct cell types that may belong to distinct circuits with distinct plasticity properties.

Disclosures: H. Wang: None. **D. Feldman:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.19/DD21

Topic: D.03. Somatosensation – Touch

Support: ERC grant 2021

Title: What and where pathways in mouse whisker system

Authors: *R. OZ ROKACH, A. GILAD;
Med. Neurobio., Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The study of sensory integration in the brain cortex stands at the core of neuroscience research, contributing significantly to our understanding of the neural mechanisms underpinning cognition. In an attempt to further this understanding, we turned our focus to the whisker system of mice, drawing inspiration from the two-stream hypothesis, found in primates' visual system. This hypothesis postulates distinct 'what' and 'where' pathways responsible for object recognition and spatial positioning, respectively. In our study, we hypothesized the possible presence of analogous pathways in mice, diverging from the Barrel Cortex (BC), rather than the primary visual cortex (V1). Utilizing wide-field calcium imaging to monitor the brain activity in the dorsal cortex, we assigned mice to perform texture differentiation ('what') and location identification ('where') tasks using their whiskers. We found that in both tasks the mice used different behavioural strategies that varied across and within mice. We highlight different cortical areas in posterior and frontal parts of the cortex that are related to either task type or behavioural strategies. In general, we find a posterior or frontal activity (such as area P and M2 respectively) patterns that seem to alternate based on behavioural parameters. In addition, we find that other parameters such as motivation, order of task and prior knowledge, have an effect on cortex-wide neuronal dynamics. Taken together, these results emphasize distinct processing streams that are dependent both on external task parameters and internal processes.

Disclosures: **R. Oz Rokach:** None. **A. Gilad:** None.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.20/DD22

Topic: D.03. Somatosensation – Touch

Support: NIH R01NS119813
NIH R01AG075114
NIH R21MH125107
NIH K00AG084261

Title: Locus coeruleus modulation of population activity in the awake somatosensory cortex

Authors: *S. WELLMAN[1], C. L. SLATER[1,2], G. A. GONZALEZ[1], Q. WANG[1];
[1]Biomed. Engin., [2]Vagelos Col. of Physicians and Surgeons, Columbia Univ., New York, NY

Abstract: Perception and cognition are heavily influenced by neuromodulatory systems within the brain, including the locus coeruleus-norepinephrine (LC-NE) system. The LC is a small

noradrenergic brainstem nucleus with a well-established role in sensory processing and higher-order cognitive function due to its wide-spread projections throughout the brain. LC neurons exhibit distinct phasic and tonic firing patterns, yet their functional implications remain unclear. Furthermore, how different patterns of LC activity impact information coding at the level of individual neurons is poorly understood. Using a combination of two-photon calcium imaging and single-photon optogenetic stimulation, we investigated changes in spontaneous and sensory-evoked activity following LC modulation within layer 2/3 excitatory neurons in the primary somatosensory (S1) cortex of awake, head-fixed mice. For LC stimulation, we emulated phasic LC activity by delivering light at 0.5 s duration, 20 Hz frequency or tonic LC activity using 2 s duration at 2, 5, or 10 Hz frequency (10 ms pulse width at 2-3 mW of laser power). Preliminary results suggest that different patterns of LC stimulation elevate the firing rate of spontaneous activity within S1 neurons at different time scales. Specifically, the effect of phasic LC stimulation lasted much longer in duration compared to tonic LC stimulation while an overall increase in firing rate was intensity dependent. Similarly, correlation between neurons in S1 was increased following all stimulation patterns with phasic LC stimulation demonstrating the most prolonged effect. LC stimulation also increased both peak and time-to-peak of evoked calcium transients within individual cells following repeated deflection of the principal whisker (66 Hz for 1 s) in an intensity-dependent manner. Interestingly, our data revealed that 2 Hz stimulation induced the largest increase in the evoked calcium signals amongst the highest responding S1 neurons compared to 5 and 10 Hz stimulation. However, the increase in correlation between neurons appeared to be the largest following 10 Hz stimulation. Lastly, we demonstrate that LC activation enhances the mouse's behavioral performance during a whisker detection task, with perceptual improvement dependent on the interval between LC stimulation and tactile stimulus. Altogether, our preliminary data demonstrate that LC stimulation modulates both spontaneous and evoked responses of excitatory neurons within the S1 cortex in awake mice and the differential effects of phasic and tonic LC stimulation on neural activity occur at distinct timescales both within individual neurons and at the population level.

Disclosures: **S. Wellman:** None. **C.L. Slater:** None. **G.A. Gonzalez:** None. **Q. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Financial interest in Sharper Sense.

Poster

PSTR479. Barrel Cortex: Local and Long Distance Cortical Interactions in Somatosensation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR479.21/DD23

Topic: D.03. Somatosensation – Touch

Support: BRAIN Initiative NINDS U01NS103558

Title: Characterizing neuromodulator release in the somatosensory cortex during a whisker-guided detection task

Authors: *C. WANG[1], Y. LI[2], S. A. HIRES[1];
[1]Dept. of Biol. Sciences, Section of Neurobio., USC, LOS ANGELES, CA; [2]Peking Univ., Peking Univ., Beijing, China

Abstract: Neuromodulators are differentially but cooperatively involved in various cognitive functions such as attention, arousal, mood, and reward. While plenty of the past research has carefully dissected the physiological and behavioral effects of the neuromodulator-releasing neurons, few studies have been done to map the neuromodulator releases in the target brain regions due to a lack of techniques with high spatiotemporal resolutions. We use a toolkit of the newly developed GPCR Activation Based (GRAB) sensors (GRAB-gACh4h for acetylcholine, GRAB-AC-NE1h for norepinephrine, GRAB-5-HT3.0 for serotonin, GRAB-DA3m for dopamine) and two-photon microscopy to monitor neuromodulator releases in the somatosensory cortex (S1) when mice perform a whisker-guided pole detection task. Our preliminary results show that different neuromodulators have distinct temporal patterns and align to different behavioral events (first/last lick, reward lick, pole onset/offset, and whisking) in this task. We also performed the GRAB imaging while optogenetically activating the axon terminals in the S1 of different neuromodulator neurons. The results revealed the *in vivo* time courses of this array of GRAB sensors and that the photoactivation induces neuromodulator releases in a pulse number-dependent but light power-independent manner. In the case of GRAB-gACh4h expressed in the ChAT-Cre X Ai32 background, we saw 37.94% increase in GRAB signal after delivering 40 pulses of light, with a time constant of 25.1 seconds. This study helps us understand the *in vivo* dynamics of the state-of-the-art neuromodulator sensors and unravel the engagement of neuromodulators in sensorimotor integration and learning.

Disclosures: C. Wang: None. **Y. Li:** None. **S.A. Hires:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.01/DD24

Topic: D.04. The Chemical Senses

Support: 368482240/GRK2416

Title: Axonal projections of mouse main and accessory olfactory bulb principal neurons

Authors: *M. NESSELER, M. SPEHR;
Dept. of Chemosensation, RWTH Aachen Univ., Aachen, Germany

Abstract: Olfactory cues drive both innate and adaptive behaviors in mice. In the brain, these cues are processed via two complementary olfactory pathways, i.e., the main and the accessory olfactory systems. While sensory neurons of the main olfactory epithelium mainly detect airborne cues and send information to the main olfactory bulb, sensory neurons of the vomeronasal organ primarily transduce non-volatile semiochemicals and relay these signals to

the accessory olfactory bulb. Primary sensory processing in both the main and accessory olfactory bulb is mutually independent and information is thought to be relayed separately to downstream nuclei via mitral/tufted cell axonal projections. Today, a comparative analysis of main and accessory olfactory bulb principal neuron projections using modern axonal tracing techniques is lacking. Here, we implement stereotaxic microinjections of recombinant adeno-associated viruses in transgenic mouse driver lines to allow for system-specific transduction and detailed anatomical assessment. To this end, we combine the t-box transcription factor 21 Cre driver mouse line with Cre-dependent expression of viral genomes using the flip-excision genetic switch. Moreover, to ensure brain-wide axonal tracing, we established a tissue preparation yielding intact brain slices that are imaged using high-resolution confocal microscopy. Moreover, we clear tissue that is subsequently imaged using light-sheet fluorescence microscopy. With these tools, we comprehensively delineate axonal projections of both main and accessory olfactory bulb principal neurons and identify both unique and common integration areas in the mouse central nervous system. Altogether, we provide the anatomical foundation for future investigations into olfactory integration along the main and accessory olfactory pathways.

Disclosures: **M. Nesseler:** None. **M. Spehr:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.02/DD25

Topic: D.04. The Chemical Senses

Support: Gatsby Charitable Foundation GAT3361
Wellcome Trust 090843/F/09/Z

Title: Multisensory processing of social signals in the medial amygdala

Authors: ***M. D. EDWARDS**[1], S. BAILEY[1], D. REGESTER[1], J. YIN[1], R. SUEDA[1], S. XU[1], A. GUBANOVA[1], V. LAKNER[1], M. MURCHIE[1], C.-Y. LEE[2], B. BINTU[3], Y. ISOGAI[1]; [1]Sainsbury Wellcome Ctr. for Neural Circuits and Behaviour, Univ. Col. London, London, United Kingdom; [2]Max Planck Inst. of Animal Behaviour, Univ. of Konstanz, Konstanz, Germany; [3]Departments of Bioengineering and Cell. and Mol. Med., Univ. of California San Diego, San Diego, CA

Abstract: The recognition of social cues is a major determinant for the selection of social behaviors under specific contexts. How the brain gathers and integrates different streams of multisensory social information, which potentially arrive at different timing, remains unclear. The medial amygdala (MeA) is a critical hub in the innate social behaviour circuit. Previous studies have demonstrated that the MeA receives strong pheromonal inputs, and that activating specific cell populations within the MeA can drive specific innate social behaviors including mating, aggression, infanticide, and parenting. Despite this, how the MeA integrates incremental

social information to form an abstract social representation critical for the expression of specific behaviors remains elusive. Furthermore, a detailed understanding of the functional MeA neuronal cell types, their connectivity with other brain areas and their involvement in this integration is unknown. Here, we addressed these problems using *in vivo* physiology and transcriptomic profiling of MeA neurons. We developed a novel paradigm using Neuropixels probes that allowed us to chart the selectivity of single MeA neurons to different types of social stimuli, including monomolecular pheromones, pheromonal blends, volatile scents, and social touch. We found that MeA neurons respond with at least two different types of kinetics and that the dynamics of neural response depend on stimulus modality and are modulated by prior social experience. Moreover, we performed in situ transcriptomics of the MeA neurons and found 26 subtypes of neurons and identified a unique cell type responsible for regulating the balance of multimodal chemosensory inputs. Taken together, these results suggest the convergence of multi-pheromonal and multisensory inputs within MeA neurons with experience-dependent modulation and establish the MeA as a major hub of multisensory integration of social cues.

Disclosures: **M.D. Edwards:** None. **S. Bailey:** None. **D. Regester:** None. **J. Yin:** None. **R. Sueda:** None. **S. Xu:** None. **A. Gubanova:** None. **V. Lakner:** None. **M. Murchie:** None. **C. Lee:** None. **B. Bintu:** None. **Y. Isogai:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.03/DD26

Topic: D.04. The Chemical Senses

Support: PMRF, MoE, India

Title: Neural codes of variation in olfactory behavior

Authors: *S. GUPTA, A. K. GUPTA, N. GUPTA;
Biol. Sci. and Bioengineering, Indian Inst. of Technol., Kanpur, India

Abstract: No two organisms of a species are alike. Even in an isogenic population of Drosophila melanogaster, variation occurs in the nervous system, say in neuronal connections and firing patterns across individuals. We want to understand these loci of variation in distinct neuronal layers that govern the perception of an olfactory stimulus, its valence, and decision-making. We also want to tease out the mechanisms which compensate for these variations and bring about a stereotyped olfactory behavior in flies. The ability to record neurons of an animal simultaneously as it performs the behavior is vital for understanding the underlying mechanisms. We have designed an arena for Drosophila with which one can simulate a planar olfactory environment of any complexity as the flies move freely in the environment, while simultaneously recording calcium signals from neurons of interest. We are using this setup for finding the causes of behavioral stereotypy. In line with the current research, we record from the Mushroom Body

Output Neurons that govern the valence associated with the odor, and from the Dopaminergic Neurons that bias the olfactory output in accordance with reward prediction and motivation.

Disclosures: S. Gupta: None. **A.K. Gupta:** None. **N. Gupta:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.04/DD27

Topic: D.04. The Chemical Senses

Support: R21 DK118503
ZIA DA000642

Title: Appetite-regulating hormones modulate odor perception and odor-evoked responses in hypothalamus and olfactory cortices

Authors: *Y. ZHAO[1], S. BHUTANI[2], T. KAHNT[1];
[1]NIDA Intramural Res. Program, NIH, NIDA IRP, Baltimore, MD; [2]San Diego State Univ., San Diego, CA

Abstract: Across species, olfactory cues are fundamental for guiding food-seeking, and food intake, in turn, affects olfactory function. Previous work indicates that this relationship may be driven by appetite-regulating hormones like ghrelin, insulin, and leptin, but the neural mechanisms through which they exert this effect have yet to be determined in humans. Specifically, it remains unclear where in the brain metabolic hormones affect neural responses to odors. The olfactory bulb sends odor information through three main striae of the olfactory tract to the cortex, terminating in the piriform cortex (PirC), amygdala (AMY), olfactory tubercule (OT), and anterior olfactory nucleus (AON). All of these areas also connect to the hypothalamus. Here, we test the hypothesis that olfactory processing in these areas is influenced by changes in metabolic hormone levels. To address this question, we examined odor-evoked fMRI responses and plasma levels of ghrelin, leptin, and insulin after a meal. We derived a composite measure of orexigenic and anorexigenic hormones using principal component analysis (PCA). We correlated the first PCA factor (PCA1) with odor intensity ratings, and fMRI responses in the hypothalamus and the cortical endpoints of olfactory tract striae. PCA1 captured variance related to both anorexigenic and orexigenic hormones, with a major positive contribution from insulin, a similar but negative contribution from ghrelin, and a near-zero contribution from leptin. Importantly, PCA1 was inversely correlated with odor intensity ratings and with fMRI responses to odorized vs. clean air in the hypothalamus, OT, and AON. Interestingly, no significant correlations were found in PirC or AMY. Whole-brain voxel-wise correlation analysis with PCA1 revealed peak correlations near the diagonal band of Broca (DBB) and the parahippocampal gyrus (PHG). Our findings suggest that high blood plasma concentrations of insulin decrease perceived odor intensity and odor-evoked activity in the cortical targets of the medial and intermediate striae of

the olfactory tract, as well as the hypothalamus. The present study expands our understanding of insulin's role in olfactory processing and implicates OT, AON, and hypothalamus as anatomical sites in which metabolic hormones affect neural responses to odors.

Disclosures: Y. Zhao: None. **S. Bhutani:** None. **T. Kahnt:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.05/DD28

Topic: D.04. The Chemical Senses

Support: NIDCD R01DC018075

Title: Neural responses to odors are decodable by identity, pleasantness, and edibility

Authors: *S. CORMIEA[1], N. DIKECLIGIL[1], J. STEIN[2], I. CHEN[3], K. DAVIS[1], J. GOTTFRIED[1];
[1]Neurol., [2]Radiology, [3]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Odors are not static stimuli; they unfold over the duration of a sniff (and beyond). Previous research in human olfaction has sought to understand the salient perceptual features that differentiate odors as well as the time-course over which such features arise in behavior and brain activity. Here, we devised an odor feature rating task to measure how people perceive and identify odors. Resultant odor ratings provide a framework to decipher neural responses to odors in a time-resolved manner. On each trial, participants were presented with one of eight real-world odors (e.g., cheese, dirt, lemon, shampoo) and asked to evaluate it. Participants rated odors on one of three feature dimensions, which were presented randomly on a trial-by-trial basis: (i) pleasantness, (ii) edibility, or (iii) identity. Behavioral results reveal that participants reliably differentiate between pleasant and unpleasant odors as well as between edible and inedible odors. When asked to endorse the true label of an odor versus an incorrect foil label, participants overwhelmingly chose the true label. Our analyses also take advantage of the high temporal resolution of intracranial EEG to investigate how odor-evoked brain activity evolves during perception, memory, and evaluation of odor stimuli. Participants in the present study performed behavioral tasks while their brain activity was simultaneously recorded via surgically implanted electrodes, which were placed as part of a treatment plan for intractable epilepsy. After preprocessing of neural signals, electrodes located in piriform (primary olfactory cortex) were selected for further analysis. Electrodes were localized using a combination of pre-implantation anatomical MRI scans and post-implantation CT images. Consistent with previous findings, we saw a pronounced increase in theta band oscillatory activity following odor stimulus onset (in piriform, but not control regions). In order to differentiate neural patterns for different stimuli, a set of support vector machine classifiers were trained to decode odors on the basis of identity, valence, or edibility. Behavioral results have already shown that odor features such as valence,

edibility, and identity are separable from stimulus ratings alone. Future analyses will reveal the precise timing of such relationships between stimuli as they arise and unfold over time.

Disclosures: **S. Cormiea:** None. **N. Dikecligil:** None. **J. Stein:** None. **I. Chen:** None. **K. Davis:** None. **J. Gottfried:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.06/EE1

Topic: D.04. The Chemical Senses

Support: BRAIN 1R01NS111673
TR01 5R01DC017876

Title: High-throughput cell type identification and mapping of single neuron projections via DNA barcoding to analyze the wiring logic of the olfactory system

Authors: *C. SOITU[1], D. HERNANDEZ TREJO[2], E. BULZOMI[2], Y.-C. WU[2], A. KOULAKOV[3], A. ZADOR[3], F. ALBEANU[4];
[1]Cold Spring Harbor Lab., Brooklyn, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Cold Spring Harbor Lab., Cold Spg Hbr, NY; [4]Cold Spring Harbor Labs., Cold Spring Harbor, NY

Abstract: Across sensory modalities, neuronal connectivity of cortical areas generates ordered spatial maps that reflect behaviorally relevant stimulus features, such as spatial location, visual object orientation, and sound frequency. In contrast, the prevailing view of the olfactory cortex, based on reconstructions of dozens of neurons, is that it lacks structured connectivity, as suggested by reports of broad and distributed connections both from the olfactory bulb (OB) to the piriform cortex (PCx) and within the cortex. These studies have inspired computational models of circuit function that rely on random connectivity. Recently, we found that the architecture of the piriform cortex is structured, and thus need not rely on algorithms that assume random connectivity. Specifically, we uncovered a novel, but simple, principle of olfactory circuit organization and information processing: a triad. For example, a mitral cell in the bulb that targets the anterior portion of the piriform likely also projects to the anterior olfactory nucleus (AON); and pyramidal neurons in the targeted (anterior) piriform locus complete the triad by also projecting to the AON. Moreover, the same matched input-output triadic organization is replicated at different positions within the piriform cortex, along its anterior-posterior (A-P) axis, for other functionally distinct targets, such as the cortical amygdala and lateral entorhinal cortex. This organization enables parallel computations, spatially segregated along functionally distinct streams and cross-referencing, since olfactory information reaches a given target brain region via both direct and indirect pathways. We are currently investigating the topography of projections in three dimensions (A-P, D-V, M-L axes), with cell-type and

single-cell resolution, focusing on the bulb-anterior piriform-AON triad. Specifically, we are exploiting the high throughput of Barcoded Anatomy Resolved by sequencing (BARseq) to read out simultaneously gene expression, cell body position and brain-wide projections of thousands of individual OB, PCx and AON output neurons within individual brains. We further aim to relate the projections of mitral and tufted cells and downstream cortical pyramidal neurons to the molecular identity of odorant receptors (ORs) in the input glomeruli as a starting point to uncover the algorithms for processing olfactory information.

Disclosures: C. Soitu: None. **D. Hernandez Trejo:** None. **E. Bulzomi:** None. **Y. Wu:** None. **A. Koulakov:** None. **A. Zador:** None. **F. Albeanu:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.07/EE2

Topic: D.04. The Chemical Senses

Support: NIMH Grant F31MH126622-01A1

Title: Disruption of Odor-Reward Coding in CA2 in the Df(16)A$^{+/-}$ Mouse Model of the 22q11.2 Microdeletion

Authors: *S. BIGLER[1], S. HASSAN[2], S. A. SIEGELBAUM[3];
[1]Columbia Univ., NEW YORK, NY; [2]Zuckerman Institute, Columbia Univ., New York, NY;
[3]Dept of Neurosci., Columbia Univ. Postdoctoral Dept. of Neurosci., New York, NY

Abstract: Disruption of Odor-Reward Coding in CA2 in the *Df(16)A*$^{+/-}$ Mouse Model of the 22q11.2 Microdeletion
Shivani Bigler, Sami Hassan, Steven A. Siegelbaum
Neuropsychiatric disorders are often associated with abnormal social behaviors, including decreased social memory—the ability to recognize and remember past experiences with another individual. Prior findings demonstrate that *Df(16)A*$^{+/-}$ mice, a genetic model of the 22q11.2 microdeletion which is strongly linked to schizophrenia, have a deficit in social memory due to dysfunction of the CA2 region of the hippocampus, an area critical for social memory. As CA2 neuron activity discriminates social odors (urine from different mice) and helps mediate social odor-reward associations, we asked whether the social memory deficits in *Df(16)A*$^{+/-}$ mice result from an altered ability of CA2 to discriminate social odors and/or from an impairment in the ability to associate a social stimulus with a given experience (i.e. an associated reward). We used in vivo two-photon calcium imaging in CA2 as water-restricted, head-fixed *Df(16)A*$^{+/-}$ mice and wild-type (WT) littermates were trained to lick at a water port to receive a water reward in response to one social and one nonsocial odor (GO odors, A and C) and to withhold licking to another pair of unrewarded social and nonsocial odors (NOGO odors, B and D). The *Df(16)A*$^{+/-}$ mice showed a profound deficit in learning both social and nonsocial odor-reward associations.

However, CA2 population-level analyses, based on decoding of odor identity using a linear SVM classifier, showed that CA2 activity in both WT and *Df(16)A*$^{+/-}$ mice decoded social and non-social odor identity with a similarly high accuracy prior to learning. We next determined whether CA2 contained a generalized representation of social versus nonsocial odors by training a classifier on one pair of social/nonsocial odors (e.g. odors A versus C) and testing on the withheld pair of odors (e.g. odors B versus D). This cross-condition generalized performance (CCGP) for generalized decoding social versus nonsocial odors had a similarly high accuracy for both WT and *Df(16)A*$^{+/-}$ mice. In contrast, CCGP for decoding rewarded versus nonrewarded odors (e.g. training on odor A versus B and testing on odor C versus D) was much weaker for *Df(16)A*$^{+/-}$ compared to WT mice. Linear regression analysis showed a positive correlation between behavioral performance and CCGP accuracy for both the *Df(16)A*$^{+/-}$ mice and WT mice. Thus, whereas CA2 of *Df(16)A*$^{+/-}$ mice can accurately decode social and nonsocial odor identity, these neurons fail to form a generalized or abstract representation of reward valence, thereby impairing odor-reward learning.

Disclosures: S. Bigler: None. **S. Hassan:** None. **S.A. Siegelbaum:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.08/EE3

Topic: D.04. The Chemical Senses

Support: NIDCD Grant 1F31DC020671-01A1

Title: Psychedelics impact active sampling and odor perception in freely-moving mice

Authors: *A. WELCH[1], S. C. STERRETT[4], K. JONES[2], M. SMEAR[3];
[1]Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [2]Univ. of Oregon, University of Oregon, OR; [3]Univ. of Oregon, Univ. of Oregon, Eugene, OR; [4]Univ. of Washington, Seattle, WA

Abstract: Olfactory hallucinations occur in many disorders, including Parkinson's disease, epilepsy, schizophrenia, and migraines, but the mechanisms underlying these hallucinations are unknown. Mechanistic studies of hallucination in animal models are fundamentally limited, since animals do not verbalize what they perceive. However, in lieu of a verbal report, internal states can be inferred from an animal's externally observable behavior. Using computational tools, our lab has shown that a mouse's perceptual states can be inferred from close analysis of strategic sniffing behavior. We have found that injection of the psychedelic DOI alters the rhythmic structure of sniffing behavior and the accuracy of odor report. In ongoing work, we are investigating how DOI impacts population dynamics in the olfactory bulb. This work will provide fresh insights into the link between active sampling, olfaction, and psychedelics.

Disclosures: A. Welch: None. **S.C. Sterrett:** None. **K. Jones:** None. **M. Smear:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.09/EE4

Topic: D.04. The Chemical Senses

Title: Distorted representations in olfactory bulb output underlie generalization errors in target odor recognition in novel olfactory environments in Shank3B mice

Authors: G. H. OTAZU;
Biomed. Sci., NYIT-COM, Old Westbury, NY

Abstract: Individuals with autism often experience significant stress when confronted with novel stimuli, but the underlying neural mechanisms behind this aspect of "insistence on sameness" remain poorly understood. In this study, we used a behavioral task in mice that enables quantitative analysis of target odor recognition within novel odor backgrounds (Li et al., 2023). Using this task, we previously demonstrated behavioral deficits in odor recognition in novel backgrounds in the Cntnap2$^{-/-}$ (Peñagarikano et al., 2011), as well as in Shank3B$^{+/-}$ (Peça et al., 2011) mouse models of autism, despite their similar performance to wild-type (WT) mice in familiar backgrounds (Li et al., 2023, Ryndych et al., under revision). To investigate the role of mitral and tufted cells' glomerular representations in odor recognition within novel environments in mouse models of autism, we crossed GP5.11 mice, which express GCaMP6f in mitral and tufted cells in the olfactory bulb, with Shank3B mice. We performed widefield calcium imaging to record olfactory bulb output responses during behavior after thinning the skull. Water-deprived Shank3B+/- mice were trained to identify odors in the presence of background odors using a go/no-go behavior paradigm, in which they were required to lick a water spout for the go target odor and refrain from licking for the no-go stimuli (2 mice, 4 sessions). Shank3B mice exhibited good performance with familiar background odors (80.8%, 478 trials), but their performance dropped to near-chance levels for novel background odors (59.7%, 176 trials).Odor glomerular responses were found to be stable and synchronized with the respiratory signal. We trained a linear classifier using glomerular responses recorded during correct trials with familiar background odors. For the go stimuli, glomerular responses were quantified as the average signal in a 500 ms window preceding the lick response, while for the no-go stimuli, glomerular responses were quantified during an equivalent time period of 500 ms preceding the median value of the lick responses. The linear classifier generalized well for the glomerular representations with novel background odors and accurately discriminated the go stimuli from the no-go stimuli (66.2±7.2%, mean±st.d., 4 sessions) for trials that were correctly discriminated by the Shank3B mice. However, the performance of the linear classifier dropped for trials in which the Shank3B mice made errors (38.4±9.1%). These findings indicate that the activity of mitral and tufted cells reflects the performance of Shank3B mice, and trials with altered glomerular responses are associated with lower performance in this mouse model of autism.

Disclosures: G.H. Otazu: None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.10/EE5

Topic: D.04. The Chemical Senses

Title: A Study of Aromatherapy and Sleep

Authors: *M. L. LEDFORD[1], D. A. MACQUEEN, III[2];
[1]Psychology, Univ. of North Carolina Wilmington, Wilmington, NC; [2]Psychology, Univ. of North Carolina, Wilimington, Wilmington, NC

Abstract: Disrupted sleep negatively impacts quality of life. It can also serve as a risk factor for mental illnesses, exacerbate psychiatric symptoms, and limit treatment effectiveness. Aromatherapy has the potential to alleviate symptoms of mental illness including sleep improvement and could be easily implemented in clinical practice at low-cost. Empirical evidence is needed to support claims that aromatherapy can aid in sleep improvement. Prior research suggests that essential oils have various effects. For example, lavender has demonstrated sedating and calming effects, whereas peppermint has evidenced an increase alertness and arousal. In this experiment, participants initially completed an intake where self-report measures were collected. They were then sent home with a Fitbit Charge 2 and Essence aromatherapy ring. Participants completed two trials (active odor and placebo) in which their sleep patterns were measured. Each participant received either lavender or peppermint as their active odor. It was hypothesized that lavender would have sedating effects, improving sleep, while peppermint essential oil would have no effect or mildly disrupt sleep. 59 participants completed the study, 43 being female (72.88%), 15 being male (25.42%), and 1 did not specify (1.69%). Ages ranged from 18 to 27, with an average age of 19.29 years old (M= 19.29, SD=1.81). Results from a 2x2x2 mixed ANOVA with variables including active odor, odor type, and gender indicated that the control odor had significantly different minutes asleep, F= 5.65 (1, 59), p< 0.02, and sleep efficiency outcomes, F= 5.59 (1, 59), p < 0.02. Participants spent more time asleep in the control condition as compared to the active odor condition. Similarly, participants had higher sleep efficiency scores in the control condition (M= 0.90, SD= 0.06), rather than the active odor condition (M= 0.83, SD= 0.18). When decomposing a significant interaction between the active odor and odor type on sleep efficiency, it was found that peppermint had significantly different sleep efficiency outcomes than the lavender odor type, t(57)= 2.05, p= 0.045. Specifically, peppermint had lower sleep efficiency (M= -0.09, SD= 0.02), as compared to the lavender condition (M= -0.05, SD= 0.01). Results suggest that some essential oils, such as peppermint, have the potential to disrupt sleep. Peppermint likely has a different mechanism of action by acting on the trigeminal nerve activating an alert response. Further research is needed to determine if lavender could aid in sleep through continuous behavioral reinforcements as a sleep cue, rather than a one-time sleep trial as shown in this experiment.

Disclosures: M.L. Ledford: None. **D.A. MacQueen:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.11/EE6

Topic: D.04. The Chemical Senses

Support: NIH Grant R01DC019405-01A1

Title: Re-examining the role of temporal processing in human olfactory perception

Authors: *B. M. LINNE, J. A. GOTTFRIED;
Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: A “primacy” model has recently been proposed to explain the role of temporal processing in olfaction. This model purports olfactory bulb glomeruli activated within a defined early temporal window play an outsized role in shaping its qualitative percept. The existence of this defined early temporal window and its impactful role on odor identification has been supported in rodent models. Yet in the human olfactory system, the relevance and implications of a temporal primacy model have received little attention. Theoretically, if neural activations inside this early temporal window were a crucial determinant of odor quality, then shifting activations by odor mixture subcomponents in and outside of this window should perceptibly impact odor mixture quality. One recent study employing this logic concluded that, contrary to rodents, humans did not seem able to leverage this temporal structure alone to meaningfully discriminate odors. This work, however, utilized low participant numbers and relatively insensitive discrimination metrics and so these findings were re-examined in the present work. Presently, odors composed of two components temporally staggered within a single sniff were constructed and participants were tasked with discriminating this odor from another in which the components were identical but temporally inverted. This was achieved via two delivery formats—a “short pulse” method (SP) in which subcomponents were each presented as 200 ms pulses, and a “long hold” method (LH) in which subcomponents were extended for the duration of the 1.5s sniff. Within each format, ability to discriminate temporally inversed odorants (TIOs) was then probed using TIOs composed of three distinct monomolecular odor pairs, a robust number of subjects (n = 30) and evaluations, and a signal detection framework with confidence assessment to account for unstable response criteria. Contrary to previous findings, our results demonstrate that on both individual and group levels, humans are capable of meaningfully discriminating temporally inverted odor pairs. Group level discrimination between all TIOs regardless of presentation format is imperfect but evidently greater than chance (discrimination probabilities = 0.63-0.68; all p’s $\leq$ 0.015). On the individual level, TIOs were discriminated (p’s $\leq$ 0.05) by 16.7%, 50%, and 33.3% (SP) and 50%, 53.3% and 40% (LH) of individuals prior to group-level analysis. These findings open the door for more fine-grained investigations into the role of temporal coding and validity of the primacy model in human olfaction, some of which will be addressed in forthcoming fMRI work.

Disclosures: **B.M. Linne:** None. **J.A. Gottfried:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.12/EE7

Topic: D.04. The Chemical Senses

Support: CIHR Grant 507489 (Junchul Kim)
NSERC Discover Grant 506730 (Junchul Kim)
NSERC CGS M (Andrew Cheon)

Title: Temporal dynamics of the anterior olfactory nucleus in odor-context memory

Authors: J. BANNING[1], ***A. CHEON**[1], J. KIM[2,1];
[1]Dept. of Cell & Systems Biol., [2]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: The Anterior Olfactory Nucleus (AON) serves a fundamental role in early olfactory processing. It modulates odor-guided behaviours by processing both bottom-up inputs from primary olfactory structures and top-down inputs from higher-order limbic structures. We previously demonstrated that the AON functions as an engram repository for odor memory, and that hippocampal projections to the AON form an experimentally tractable neural circuit model of episodic odor memory. However, the temporal dynamics of intrinsic AON activity during odor memory encoding and retrieval remain unknown. In this study, we coupled in vivo fiber photometry with an olfactory go/no-go paradigm to analyze AON activity in Thy1-GCaMP6s mice during the development and expression of odor-context memory. We found that AON activity dynamics shift significantly when odor-context associations are necessary to receive a reward. Specifically, the AON is pre-emptively activated prior to odor sampling when performing a two-context olfactory go/no-go task, but not during a single-context go/no-go task where contextual information is unnecessary for reward (one-way ANOVA, $n = 9$, $F(1.654, 13.23) = 10.29$, $p = 0.0029$). This supports our hypothesis that the AON functions as an odor-context memory repository. We also discovered a robust suppression of AON activity during the reward consumption phase of the go/no-go task. To investigate this novel effect, we conducted a series of experiments designed to isolate the effects of licking, reward consumption, and reward anticipation, revealing that the suppression corresponded to reward anticipation rather than reward consumption itself. Next, we adopted an all-optical approach to investigate whether the pre-emptive AON activity represents a hippocampus-dependent signal to the AON that propagates contextual information required for successful odor-guided behaviour. In an open-field assay, we used concurrent fiber photometry and optogenetic techniques to stimulate ChrimsonR-expressing hippocampal terminals at the AON while recording AON activity via the same optic fiber implant. Overall, this study provides novel insights into the central role of the AON in processing odor-context memory. Our research on the temporal dynamics of AON activity and the hippocampal-AON circuit enhances our understanding of how the brain

processes sensory elements of episodic memory, which has significant implications for the field of memory research.

Disclosures: **J. Banning:** None. **A. Cheon:** None. **J. Kim:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.13/EE8

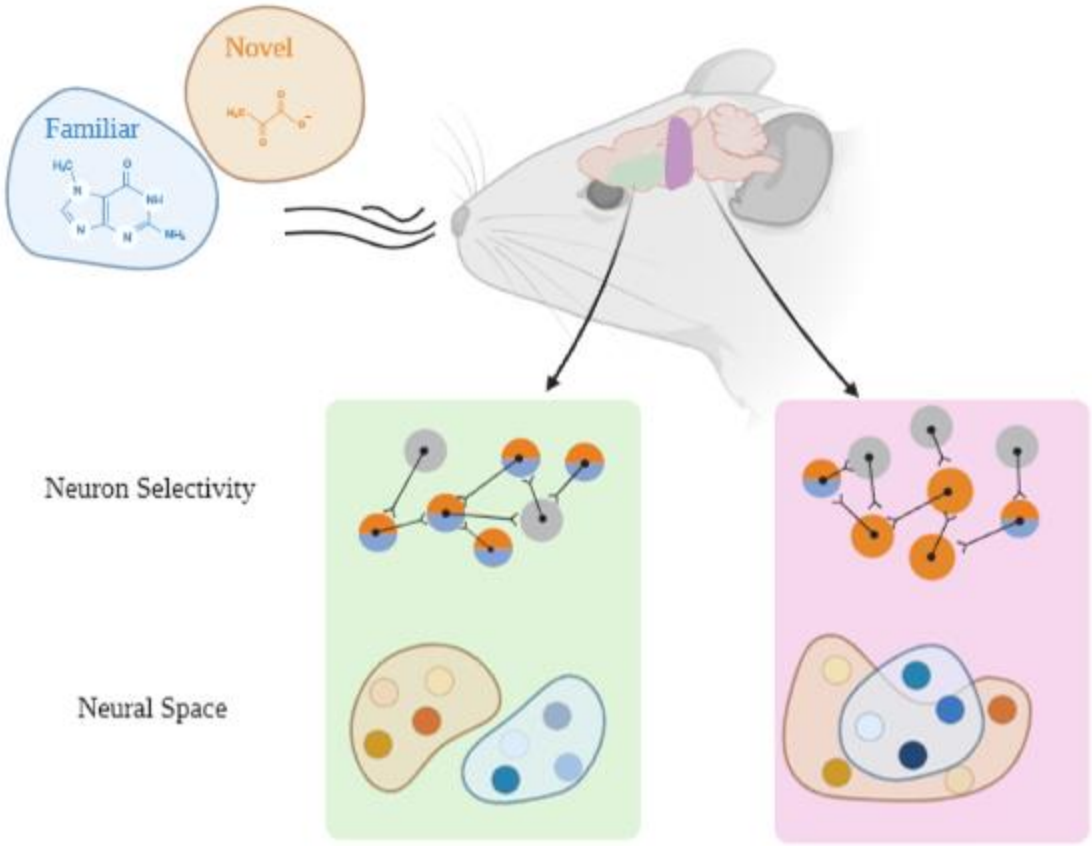
Topic: D.04. The Chemical Senses

Support: FWO Grant Fundamental Research 1135122N

Title: Experience shapes the transformation of olfactory representations along the cortico-hippocampal pathway

Authors: *E. SCHILTZ[1,2,3], M. BROUX[2], S. HAESLER[2];
[1]Biomed. Sci., Katholieke Univ. Leuven, Leuven, Belgium; [2]Neuro-Electronics Res. Flanders (NERF), Herverlee, Belgium; [3]Fonds Wetenschappelijk Onderzoek, Bruxelles, Belgium

Abstract:



New chemicals in the air signal a change in the environment, triggering interest and arousal in mammals. While neural representations of odour identity in the brain have been widely studied, how these representations are transformed across multiple olfactory brain areas and how they are affected by sensory experience is less well understood. In this study, we recorded 1404 neurons in mice along the cortico-hippocampal pathway, including the Anterior Olfactory Nucleus (AON), Piriform Cortex (PIR), Lateral Entorhinal Cortex, CA1 and Subiculum, in a passive novelty detection paradigm. We observe that while the proportion of cells responding to odours slightly decreases along the pathway, the selectivity of cells increases. This eventually leads to a sparsening of olfactory representations in the medial temporal lobe (MTL) structures. Using a decoding approach with support vector machines (SVM), we demonstrate that AON reaches the highest level of accuracy in the representation of odour identities, while all subsequent regions have lower values. Interestingly, in PIR, the decoding capacity of the SVM was not identical for novel and familiar odours, as a small fraction of the population was distinctly well tuned to familiar odour identities. We further find that experience differentially affects odorant representations along the pathway. In the anterior regions, most cells respond to both familiar and novel odours, and the level of experience is encoded through the firing rate modulation of the whole population. This leads to a clear separation between novel and familiar odours representations in the neural space. In the MTL on the contrary, a fraction of the population show an increase selectivity for novel odours, but it hardly affects the representation of odour identities in the neural space. As such, we concluded that the processing of olfactory stimuli along the pathway leads to an uncorrelation in the representation of identity and experience through a

sparsening mechanism. This mechanism could sustain an efficient relaying of behaviorally relevant information across the brain.

Disclosures: E. schiltz: None. **M. Broux:** None. **S. Haesler:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.14/EE9

Topic: D.04. The Chemical Senses

Support: SERB Grant SB/SJF/2021-22/04-C
Council of Scientific and Industrial Research, Government of India

Title: Understanding mechanisms contributing to stereotyped behaviors despite variability in neural circuits across individuals

Authors: *A. K. GUPTA, S. GARG, A. M. MITTAL, N. GUPTA;
Dept. of Biol. Sci. and Bioengineering, Indian Inst. of Technol., Kanpur, India

Abstract: Animals rely on several genetically hardwired (innate) behaviors for their survival. For example, in insects, innate behaviors linked to foraging, searching for a mate, oviposition, and avoiding harmful stimuli are all crucial for survival and reproduction and are, therefore, stereotyped across individuals. Olfactory navigation plays a central role in many of these behaviors. Genetic and environmental factors result in variability at every level in the olfactory circuit in fruit flies: for example, olfactory receptor expression levels in the sensory neurons and the numbers of the receptor neurons, projection neurons (PNs), and local neurons (LNs), and their connectivities differ across individuals. Similarly, at the next layer, there is randomness in the connectivity between PNs and Kenyon Cells (KCs). Although one might expect the variabilities at all these levels to accumulate and result in highly inconsistent behaviors, individual flies show reasonably stereotyped behaviors. We have built a new assay for measuring the olfactory behavior of individual fruit flies in response to attractive and aversive odors. Using this setup, we have quantified the amount of stereotypy in their innate olfactory behaviors. Two-photon calcium imaging confirmed that the amount of stereotypy present in the total KC responses across individuals is lower than that observed in the behavior, suggesting a compensatory mechanism for reducing the variability. Existing literature points toward neuromodulators as possible candidates for controlling variability in various behaviors. In ongoing experiments, we are exploring how dopamine signaling affects stereotypy in the innate olfactory behaviors of flies.

Disclosures: A.K. Gupta: None. **S. Garg:** None. **A.M. Mittal:** None. **N. Gupta:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.15/EE10

Topic: D.04. The Chemical Senses

Title: Exploring olfactory-spatial association in the lateral entorhinal cortex of freely-moving mice

Authors: *O. MCKISSICK, C. DONOHO, T. PHAM, K. BAKER, J. RITT, A. FLEISCHMANN;
Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: The ability to use past sensory experiences to effectively navigate the world is crucial for survival. The lateral entorhinal cortex (LEC), which exhibits strong connectivity with the hippocampus and receives inputs from various cortical areas, plays a key role in integrating sensory and spatial information.
We are investigating the LEC's involvement in integrating olfactory and spatial information. We employ mini-endoscope recording techniques while mice learn and recall information in a novel behavioral task. Initially, mice navigate to a cue port located at the "North" end of a triangle arena, where they encounter one of two odor cues. The cue predicts the location of reward, which can be at either "East" or "West" ports. Once mice have learned these associations, we introduce them (by moving a transparent barrier that splits the arena in half) to the opposite side of the arena, where they now obtain cues from the "South" port, while still navigating towards the same East and West reward ports. This design, fixing reward locations while altering the origin at which cues are received, isolates the processes of stimulus identification, navigational planning, and reward acquisition temporally and spatially. Additionally, we can explore the differences in neural activity under allocentric (Go-East, Go-West) and egocentric (Go-Left, Go-Right) task demands.
Preliminary results indicate that mice can successfully learn and perform the egocentric version of this task, and generalize across arena configurations. Ongoing work aims to incorporate mini-endoscope fluorescence imaging to establish connections between behavioral events and LEC activity. I hypothesize that our neural recordings will reveal spatially tuned and odor specific cells as well as conjunctive odor-spatial cells. These experiments collectively investigate the role of the LEC in associative memory formation related to sensory and spatial information.

Disclosures: O. McKissick: None. **C. Donoho:** None. **T. Pham:** None. **K. Baker:** None. **J. Ritt:** None. **A. Fleischmann:** None.

Poster

PSTR480. Olfaction: Central Mechanisms and Behavior II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR480.16/EE11

Topic: D.04. The Chemical Senses

Support: KAKENHI, Grant number 20K03482

Title: Scents of remembrance: Unveiling the power of odors in eliciting vivid autobiographical memories and visual and frontal neuronal correlates in individuals

Authors: *Y. MASAOKA[1], M. YOSHIDA[3], A. YOSHIKAWA[2], N. KOIWA[4], M. HONMA[1], M. IZUMIZAKI[1];
[1]Physiol., Showa Univ. Sch. of Med., Tokyo, Japan; [2]Sch. of Nursing and Rehabil. Sci., Showa Univ. Sch. of Med., Kanagawa, Japan; [3]Dept. of Ophthalmology, Jikei Med. Univ., Tokyo, Japan; [4]Dept. of Hlth. and Sci., Univ. of Human Arts and Sci., Saitama, Japan

Abstract: Specific odors can trigger autobiographical memories (AM-odor) accompanied by vivid visual scenes, heightened emotional arousal, and a sense of comfort. Compared to other sensory cues such as auditory and visual stimuli, the recall of memories prompted by specific odors can evoke powerful visual imagery. Our hypothesis was that AM-odor could enhance individual cognitive abilities through activations in the visual cortex. In this study, we utilized functional magnetic resonance imaging to explore the relationship between blood oxygen levels in olfactory regions and cortical areas during AM-odor-induced subjective memory retrieval and emotional experiences in both young and older adults. A total of 44 subjects, ranging from 32 to 84 years old, participated in the study. The research protocol was approved by the Ethical Committees of Showa University School of Medicine, and all participants provided written informed consent. We found that left fusiform gyrus activation increased in correlation with memory retrieval, comfort levels, intensity of recollection, and vividness of memories during AM-odor experiences. Notably, older subjects exhibited enhanced connectivity between the left fusiform gyrus and the left posterior orbitofrontal cortex. The fusiform gyrus is known to be associated with mental imagery, particularly vivid and precise images. AM-odor can evoke vivid visual imagery of past experiences, potentially influencing motivation and self-efficacy. This effect is especially prominent in older subjects, as it correlates with the activation of the orbitofrontal cortex. Considering the reported improvements in quality of life, cognition, and social communication through reminiscence therapy in psychotherapy, we propose that AM-odor could serve as an effective prompt for such therapy.

Disclosures: Y. Masaoka: None. **M. Yoshida:** None. **A. Yoshikawa:** None. **N. Koiwa:** None. **M. Honma:** None. **M. Izumizaki:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.01/EE12

Topic: D.05. Auditory & Vestibular Systems

Title: Deciphering the genetic interactions between Pou4f3, Gfi1 and Rbm24 in maintaining cochlear hair cell survival

Authors: *G. WANG[1,3], Y. GU[1,3], Z. LIU[1,3,4], *G. WANG[2];
[1]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China; [2]Inst. of Neuroscience, Chinese Acad. of Sci., ShangHai, China; [3]Univ. of Chinese Acad. of Sci., Beijing, China; [4]Shanghai Ctr. for Brain Sci. and Brain-Inspired Intelligence Technol., ShangHai, China

Abstract: Mammals have a limited number of hair cells (HCs) in their cochleae that detect sound, and once these cells are damaged, they cannot regenerate. This makes it crucial to investigate the molecular mechanisms that govern the survival of these HCs in order to prevent hearing problems. When the Pou4f3 or Gfi1 genes are absent in these cells, they rapidly degenerate, whereas HCs lacking the Rbm24 gene degenerate at a later stage. However, we still have limited understanding of the transcriptional processes involving Pou4f3, Gfi1, and Rbm24. In our study, we discovered that the expression of Rbm24 is completely suppressed in Pou4f3-deficient HCs, while its expression remains unchanged in Gfi1-deficient HCs. Additionally, the absence of Rbm24 does not affect the expressions of Pou4f3 and Gfi1 in HCs. Using in vivo transgenic reporter assays in mice, we identified three enhancers of Rbm24 that interact with Pou4f3. Finally, we investigated whether restoring Rbm24 could slow down the degeneration of Pou4f3-deficient HCs. Our genetic experiments conducted in vivo showed that ectopic Rbm24 alone is not sufficient to prevent the degeneration of Pou4f3-deficient HCs. In conclusion, our study provides valuable new insights into the regulation of HC survival at the molecular and genetic levels.

Disclosures: **G. Wang:** None. **Y. Gu:** None. **Z. Liu:** None. **G. Wang:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.02/EE13

Topic: D.05. Auditory & Vestibular Systems

Support: Hearing Health Foundation 2018 ERG to A.C.V.
UK CCTS pilot grant to A.C.V. through NIH (NCRR and NCATS) UL1TR001998

Title: Trpa1 channels are essential for the maintenance of cochlear outer hair cell innervation

Authors: D. LLANES-CORONEL, A. M. KRUSE, D. CALDERÓN-BRICEÑO, ***A. VELEZ-ORTEGA**;
Physiol., Univ. of Kentucky, Lexington, KY

Abstract: TRPA1 channels are activated by tissue damage and they trigger pain-like responses in nociceptive neurons. In the inner ear, we recently showed that TRPA1 channels regulate

hearing sensitivity after noise exposure (Velez-Ortega, Nat Commun, 2023). Although mice lacking TRPA1 channels (*Trpa1*$^{-/-}$) have normal hearing thresholds, we recently uncovered abnormal wave amplitudes in auditory brainstem responses (ABR) of *Trpa1*$^{-/-}$ mice as they age. These differences in ABR waveforms could not be explained by differences in inner hair cell ribbon counts. However, we did observe age-related abnormalities in fibers presumed to be type II spiral ganglion neurons (SGNs). In *Trpa1*$^{-/-}$ mice, neurofilament labeling in the first postnatal week showed type II SGNs with largely normal innervation that became significantly disordered by six weeks of age. At five months of age, some *Trpa1*$^{-/-}$ mice even showed regions devoid of type II SGNs. These unmyelinated afferent fibers innervate the outer hair cells, respond to cochlear tissue damage, activate neurons in the cochlear nucleus following high sound intensity stimulation, and may trigger the medial olivocochlear (MOC) efferent negative feedback. Thus, type II SGN are likely to be the damage-sensing pathway in the cochlea. In conclusion, our results show that TRPA1 channel activity is required to maintain the proper innervation of cochlear outer hair cells by type II SGNs, which could potentially make *Trpa1*$^{-/-}$ mice more susceptible to noise-induced hearing loss as they age.

Disclosures: D. Llanes-Coronel: None. **A.M. Kruse:** None. **D. Calderón-Briceño:** None. **A. Velez-Ortega:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.03/EE14

Topic: D.05. Auditory & Vestibular Systems

Support: 1R01DC020209-01A1

Title: Understanding galectin-3: mechanisms for age-related cochlear protection and modulation by female hormones

Authors: *B. HU[1], M. YE[2], C. ZHANG[3];
[1]Univ. of Buffalo, Buffalo, NY; [2]Univ. at Buffalo, Buffalo, NY; [3]Univ. of the Pacific, San Francisco, CA

Abstract: Galectin-3 (Gal-3) is a member of the β-galactoside-binding protein family and is expressed in various tissues and cell types, including immune cells, epithelial cells, endothelial cells, and fibroblasts. Its functions involve the modulation of inflammation, immune responses, and cell-matrix interactions. Our previous study demonstrated that the absence of galectin-3 function in mice exacerbated age-related hearing loss, with females showing a greater decline in auditory function. In this current study, we aimed to gain a better understanding of the underlying mechanisms by which galectin-3 protects cochlear homeostasis. We first characterized galectin-3 expression at various ages in mouse cochleae. We observed that galectin-3 expression was absent in the early stages of cochlear development but emerged around

postnatal day 8 (PND-8) at the basal end of the cochlea. Over time, its expression gradually expanded toward the apex. By PND-20, the galectin-3 expression pattern resembled that of adult ears. Specifically, within the organ of Corti, galectin-3 expression was confined to Hensen's cells. As the mice aged, we noted a loss of sensory cells occurring in both the apical and basal regions of the cochlea. Interestingly, the expression of galectin-3 increased concomitantly, with more Hensen's cells acquiring galectin-3 expression. Additionally, galectin-3 expression was observed in Deiters cells surrounding areas with missing hair cells and in a portion of cochlear macrophages. To further explore the underlying mechanisms, we examined the expression of Estrogen receptor alpha (Erα) in the cochlea. Our findings revealed a high level of Erα expression in the nuclei of Hensen's cells, suggesting that estrogen-mediated regulation of Hensen's cells may contribute to galectin-3 function. To provide additional evidence, we conducted ovariectomy or sham surgery in female mice lacking galectin-3 expression. Auditory brainstem response tests conducted six months after the surgeries showed that mice undergoing ovariectomy developed fewer threshold shifts compared to those that received only the sham surgery. This observation suggests that female hormones play a role in the development of auditory dysfunction caused by galectin-3 deficiency. In summary, our study reveals that the impact of galectin-3 on sensory cell homeostasis is mediated through its role in supporting cells and immune cells. Furthermore, our findings highlight the modulatory role of female hormones in the effects observed with galectin-3 deficiency.

Disclosures: **B. Hu:** None. **M. Ye:** None. **C. Zhang:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.04/EE16

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD/NIH R01DC013817
DoD RH200052

Title: Estrogen Receptor-2 Agonists for Protection Against Noise-Induced Hidden Hearing Loss in Female Mice

Authors: *R. AMANIPOUR[1], B. SHUSTER[1], B. MILON[1], R. HERTZANO[2,1];
[1]NIH, Natl. Inst. on Deafness and Other Communication Disorders, Bethesda, MD; [2]Dept. of Otorhinolaryngology, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Hearing loss affects more than 20% of the world's population, and it is the fourth cause of disability globally. One of the most common forms of hearing loss in adults is noise-induced hearing loss (NIHL). NIHL due to excessive exposure to loud noise can result in temporary or permanent threshold shift (TTS or PTS, respectively). A noise exposure that only causes a TTS can nevertheless result in permanent damage to, or uncoupling of, ribbon synapses

between inner hair cells (IHCs) and spiral ganglion neurons (SGNs). This form of hearing loss, known as noise-induced hidden hearing loss (NIHHL) or cochlear synaptopathy, impairs the encoding of sounds (like speech) in the presence of background noise. Work from our and other laboratories shows that female mice are less susceptible to NIHL compared with males, and that this protection is due to endogenous 17β-estradiol. Additionally, it has been demonstrated that this protective effect is partially mediated through estrogen receptor β (ESR2). In this study we investigate whether augmentation of ESR2 signaling via systemic administration of DPN (diarylpropionitrile, an ESR2 specific agonist) or 17β-estradiol (E2) can protect gonadally intact female mice against NIHHL.Methods: Female B6CBAF1/J mice were obtained at 7 weeks of age. At 8 weeks of age, mice were implanted with slow release (21-days) subcutaneous pellets containing DPN, E2, or a placebo. Baseline auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) thresholds were recorded in all mice at 9 weeks of age. At 10 weeks of age, mice were exposed to noise (8-16 kHz, 93- or 97-dB SPL, for 2-hours). ABRs and DPOAEs were re-evaluated at 1-day, 1-week, and 6-weeks after noise exposure. At each of these time-points, mice were euthanized, and cochlear tissue was collected for quantification of outer hair cells and IHC synapses. Result: Comparison of ABR and DPOAE threshold shifts between treated and placebo animals revealed lower threshold shifts for the E2- and DPN-treated mice at all timepoints following exposure to 93- and 97-dB SPL noise. Comparison of ABR wave-I amplitudes revealed a smaller reduction in amplitudes in E2- and DPN-treated mice after 93- and 97-dB SPL noise exposure. Furthermore, histologically, treatment with E2 and DPN resulted in significantly higher survival of IHC synapses 6-weeks after noise exposure at 24 kHz frequency.Conclusion: These results show that augmentation of ESR2-mediated signaling ameliorates NIHHL in intact female mice. This study indicates that ESR2 specific agonists, which do not induce feminizing effects, are promising candidates for future clinical trials for hearing preservation in females.

Disclosures: **R. Amanipour:** None. **B. Shuster:** None. **B. Milon:** None. **R. Hertzano:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.05/EE17

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC012957 to EG
NIH Grant R01 DC019380 to SGS
NIH Grant F31 DC014910 to ZY
NIH Grant P30 DC005211 to Center for Hearing and Balance at Johns Hopkins

Title: Non-quantal transmission in the vestibular periphery is sufficient for encoding fast head movements

Authors: D. ZHOU, W. SCHOO, Z. YU, T. KODAMA, S. DU LAC, E. GLOWATZKI, *S. SADEGHI;
Johns Hopkins Univ., Baltimore, MD

Abstract: Vestibular sensors located in the inner ear serve a key role in providing information about head movements, which will be used for maintaining stability of gaze, head and body. Unique afferent terminals, the calyx, ensheath the basolateral walls of one or more type I hair cells (HCs). In addition to the quantal vesicular glutamatergic release, the type I HC - calyx synapse uses a non-quantal (NQ) transmission, independent of and faster than the quantal transmission. However, the function of this NQ transmission is not clear. We investigated whether NQ transmission alone could encode fast head movements. We first used mice that lack vesicular glutamate transporter-3 (vglut3 KO). These animals are known to be deaf, have no startle reflex, and show no auditory brainstem responses (ABR). In the vestibular periphery, we used in vitro patch clamp recording from calyx terminals (P14 - P21) and found no glutamatergic synaptic events in calyx terminals even when HCs were depolarized. However, in vivo vestibular nerve responses in vglut3 KO mice (P30 - P60) were normal as measured by vestibular sensory evoked potentials (VsEP). This suggests that the most phasic afferents had normal responses to fast linear head movements. These mice also showed normal vestibulo-ocular reflex (VOR) responses during fast head movements (1 - 8 Hz). Because of the chronic nature of the condition in KO animals, the responses could have been affected by compensatory changes in the peripheral and central pathways. To rule out this possibility, as an acute model for testing the function of NQ transmission, we used bilateral intratympanic injections of the AMPA receptor antagonist NBQX in wild type mice. Penetration of the drug into the inner ear was confirmed by lack of ABR responses that was observed about 20 - 60 min after injections, with effects lasting up to 3 hours. Both VsEP and VOR responses were normal after intratympanic injection of NBQX, confirming the results from KO animals. Together, the above results show that the glutamate-independent NQ signals could effectively encode fast head movements (normal VsEP) and drive vestibular responses (normal VOR).

Disclosures: D. Zhou: None. **W. Schoo:** None. **Z. Yu:** None. **T. Kodama:** None. **S. du Lac:** None. **E. Glowatzki:** None. **S. Sadeghi:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.06/EE18

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01 DC019380 to SGS

Title: Large conductance calcium-activated potassium (BK) channels regulate response of vestibular nerve afferents to fast head movements

Authors: W. SCHOO, *D. BALLINAS, S. SADEGHI;
Otolaryngology - Head and Neck Surgery, Johns Hopkins Univ., Baltimore, MD

Abstract: Vestibular nerve afferents that have the most phasic response properties receive inputs from central regions of the neuroepithelia in the inner ear, mainly through calyx nerve terminals that cover the basolateral walls of type I hair cells. These afferents respond to fast head movements and show highly reproducible spike patterns in response to complex head movements. We have previously shown that efferent modulation of the activity of KCNQ, BK, and SK potassium channels affects the response of calyces to a step current injection, resulting in a change in the number of spikes, first spike time, and response threshold. Here, we further investigated the effect of activity or lack of expression of BK channels on response properties of calyx terminals and phasic afferents in mice. We show that intralabyrinthine or intratympanic application of baclofen, a GABA-B agonist that acts through inhibition of BK/SK channels in calyces, resulted in an increase in afferent responses to fast head movements as measured by vestibular sensory evoked potentials (VsEP). Intralabyrinthine application of a GABA-B antagonist resulted in the opposite effect. Furthermore, using *in vitro* patch clamp recording from calyx terminals we found that that inhibition of BK/SK activity resulted in an increase in phase locked responses of calyx terminals to sinusoidal current injections. Finally, VsEP responses in BK knockout mice (n = 3) were decreased by about 50% compared to their wild type littermates and our previous VsEP recordings from C57BL/6 mice. These findings suggest an important role for BK channels in coding of fast head movements and fast synchronous firing by afferents.

Disclosures: W. Schoo: None. **D. Ballinas:** None. **S. Sadeghi:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.07/EE19

Topic: D.05. Auditory & Vestibular Systems

Support: NIH grant R01 DC019369

Title: Identification of Vestibular Ganglion Pathology in an Animal Model for Syndromic, Congenital Vestibular Disorders

Authors: K. L. PHILLIPS[1], V. JAIN[1], Z. SHAW[1], N. BELL[1], B. HENTZ[3], E. B. BOGIN[4], J. C. HIRSCH[4], A. POPRATILOFF[2], *K. D. PEUSNER[5];
[1]Biol., [2]George Washington Univ., Washington, DC; [4]Neurol. & Rehabil. Med., [3]George Washington Univ. Sch. of Med., Washington, DC; [5]Neurol. & Rehabil. Med., Sch. of Med., Washington, DC

Abstract: Children with syndromic, congenital vestibular disorders (CVDs) form an abnormal inner ear early in development, resulting postnatally in severe challenges in maintaining posture, balance, walking, eye-hand coordination, eye tracking, reading, and language acquisition. The

most common pathology observed is a sac-like inner ear with truncated or missing semicircular canals. The semicircular canals normally emerge during the first trimester of gestation. It is not known how malformation of the semicircular canals early in development affects the emerging vestibular neural circuitry. Accordingly, this lab designed and implemented an animal model that forms a sac-like inner ear early in development which resembles that found in CVD children. In two-day old chick embryos (E2), surgical Anterior-posterior Rotation 180°of the Otocyst creates a sac-like inner ear on one side, called the ARO chick. After hatching, ARO chicks experience balance and walking problems. Since vestibular-ganglion (VG) neuron number is reported to be reduced in children with CVDs, our first step was to determine whether VG neurons survive in E13 ARO chicks. VG cells were counted by two approaches: (1) a classical approach using transverse, serial, Nissl-stained tissue sections (20 µm thick) visualized with light microscopy and analyzed with QuPath software, and (2) a novel approach using biocytin Alexa Fluor labeling of VG cell bodies, fixed and cleared in whole-mount preparations that were imaged using confocal microscopy and analyzed with Imaris software. With both Nissl-staining and biocytin-labeling, VG neuron number on the rotated side of ARO chicks was slightly but consistently reduced compared to those on the intact side and in normal chicks. Furthermore, VG neuron cell bodies on the rotated side of ARO chicks failed to form the orderly, contiguous anterior and posterior ganglionic masses found on the intact side and in normal chicks. Thus, the VG acquires nearly normal neuron numbers two-thirds of the way through prenatal development, despite connecting to an abnormal sac-like inner ear. However, absence of normal VG topography in ARO chick embryos may presage pathological changes in VG connectivity postnatally. Ongoing studies of the hatchling ARO chick will determine the fate of VG neurons postnatally.

Disclosures: **K.L. Phillips:** None. **V. Jain:** None. **Z. Shaw:** None. **N. Bell:** None. **B. Hentz:** None. **E.B. Bogin:** None. **J.C. Hirsch:** None. **A. Popratiloff:** None. **K.D. Peusner:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.08/EE20

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC017147
NIH Grant DC018785
NIH Grant DC005965

Title: Autophagy proteins are essential for aminoglycoside-induced hearing loss

Authors: J. LI[1], U. MULLER[3], ***B. ZHAO**[2];
[1]Indiana Univ. school of medicine, Indianapolis, IN; [2]Indiana Univ. school of medicine, INDIANAPOLIS, IN; [3]Johns Hopkins, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Aminoglycosides (AGs) are widely used to treat severe infections. However, systemically administered AGs preferentially kill cochlear hair cells, resulting in irreversible hearing loss. Recently, we found that AGs bind to RIPOR2 and trigger its rapid translocation in cochlear hair cells. Reducing RIPOR2 expression entirely prevents AG-induced hair cell death and subsequent hearing loss in mice. Next using yeast two-hybrid screening, we found that RIPOR2 interacts with GABARAP, a key macroautophagy/autophagy pathway protein. Following AG treatment, RIPOR2 colocalizes with GABARAP and regulates the activation of autophagy. Remarkably, reducing the expression of GABARAP, or another key autophagy protein MAP1LC3B/LC3B, entirely prevents AG-induced hair cell death and subsequent hearing loss in mice. Furthermore, we found that AGs activate the autophagy pathway specific to mitochondria. Reducing the expression of PINK1 or PRKN/parkin, two key mitophagy proteins, protects hair cells against AG toxicity. Thus, our findings demonstrated that RIPOR2-mediated autophagic dysfunction is essential for AG-induced hearing loss and provided potential therapeutic strategies for preventing AG toxicity.

Disclosures: **J. Li:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J. L. is named an inventor on patent applications related to this work. **U. Muller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U. M. is named an inventor on patent applications related to this work. **B. Zhao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); J. L., U. M. and B. Z. are named inventors on patent applications related to this work..

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.09/EE21

Topic: D.05. Auditory & Vestibular Systems

Support: DC017532

Title: Waveform similarities and differences of eye-movement-related-eardrum-oscillations (EMREOs) in subjects with normal hearing

Authors: *C. KING[1], S. N. LOVICH (SCHLEBUSCH)[2], D. L. MURPHY[2], R. LANDRUM[3], D. KAYLIE[2], C. A. SHERA[4], J. GROH[2];
[1]Neurobio., [2]Duke Univ., Durham, NC; [3]Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA; [4]USC, Los Angeles, CA

Abstract: We recently discovered a unique type of low-frequency otoacoustic emission (OAE), time-locked to the onset and offset of saccadic eye movements and occurring in the absence of external sound (Gruters et al., 2018). These eye movement-related eardrum oscillations

(EMREOs) contain parametric information about horizontal and vertical eye displacement and position, revealing that information about the position of the visual spatial map (i.e. retina) with respect to the head is available to the auditory system at the level of the periphery (Lovich et al, 2023). EMREOs are likely to be produced by some combination of middle ear muscles and outer hair cells, and thus abnormalities in this signal may ultimately have clinical relevance for diagnosing efferent causes of hearing dysfunction. However, before this promise can be realized, normative data from participants with normal hearing are needed. By identifying attributes of EMREOs that are similar across normal participants, we can set the stage for future comparisons with EMREOs in individuals with abnormalities that affect various motor components of the ear. We find that in subjects with normal hearing thresholds and normal middle ear function, all ears exhibit measurable EMREOs characterized by a phase reversal for contralaterally versus ipsilaterally-directed horizontal saccades. There is a large peak in the signal occurring soon after saccade onset, and an additional large peak time-locked to saccade offset. We find that waveforms are less variable to horizontally versus vertically-directed saccades, and we report evidence that saccade duration is encoded in the waveform. Components of EMREOs that are most consistent across subjects, such as the phase reversal for contraversive vs ipsiversive saccades, are the ones that are most likely to play an essential role in their function. In contrast, response differences between subjects are likely to reflect normal variation in individuals' auditory system anatomy and physiology, similar to traditional measures of auditory function such as auditory-evoked OAEs, tympanometry and auditory-evoked potentials. In future analysis, focusing on the most consistent EMREO characteristics, identified here in a normal population, provides the best strategy for pinpointing differences in abnormal systems.

Disclosures: C. King: None. **S.N. Lovich (Schlebusch):** None. **D.L. Murphy:** None. **R. Landrum:** None. **D. Kaylie:** None. **C.A. Shera:** None. **J. Groh:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.10/EE22

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD intramural program 1ZIADC000021

Title: Gene expression analysis of the developing vestibular ganglion of the inner ear

Authors: P. RAIKWAR, *D. WU;
NIH/NIDCD, Bethesda, MD

Abstract: The organization of the vestibular ganglion (VG), which constitutes afferent neurons that innervate the five vestibular sensory organs and project to two central targets, is poorly understood. Recent findings in transcriptomics have suggested there are genes that distinguish between two complementary subpopulations of the VG named VGN-1 and VGN-2, which

largely corresponded to the respective superior and inferior VG (Sun et al., 2022). For example, *Sall3* was thought to be expressed in VGN-1 and *Gata3* in VGN-2. We investigated the expression patterns of these genes during VG development to address whether they can provide insight into how the VG is organized. Using wild-type CD-1 mice, we conducted *in situ* hybridization experiments between embryonic day (E) 11.5 and postnatal day (P) 1. We compared the gene expression patterns of *Sall3* and *Gata3* to known temporal markers of neuronal maturity, including *Neurod1* for vestibular neuroblasts and *NF68* for mature neurons. cDNAs for anti-sense probe generation were validated with sequencing prior to experiments. Gene expression patterns were analyzed qualitatively using Amira-Avizo Software 2020.2. At E11.5, *Sall3* and *Gata3* appear to have complementary expression patterns. *Sall3* expression concentrates in a *NF68*-positive zone of maturing neurons. *Gata3*-positive cells flank this cluster, largely overlapping with *Neurod1*-positive migrating VG-fated neuroblasts. Instead of identifying VG subtypes, these expression patterns suggest *Gata3* and *Sall3* have a developmental relationship. We thus examined their expression at more mature stages of VG development. At E16.5, *Sall3* and *Gata3* expression patterns again appear complementary but do not segregate between the superior and inferior VG. Namely, *Sall3* is expressed in the most superior and inferior portions of the VG. *Gata3* transcripts are clustered in anterior, posterior, and lateral foci between these *Sall3*-positive domains. This dynamic complementary relationship is maintained at P1, except *Gata3* expression is reduced spatially compared to E16.5. Our study concludes that while *Sall3* and *Gata3* show regional expression in the VG, they are not markers that distinguish between the superior and inferior VG. Furthermore, the spatial relationship between VGN-1 and VGN-2 is more complex than that suggested by transcriptomic data, potentially reflecting the maturation of VG-fated cells during VG development. Further investigations are underway to explore the relationship between *Gata3* and *Sall3* at earlier stages and to identify candidates that distinguish between the superior and inferior VG.

Disclosures: **P. Raikwar:** None. **D. Wu:** None.

Poster

PSTR481. Hair Cells and the Periphery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR481.11/EE23

Topic: D.05. Auditory & Vestibular Systems

Support: ANR-20-CE37-0016 INVEST
ANR-20-NEUR-0005
PCI2020-120681-2

Title: Subchronic alteration of vestibular hair cells in mice: implications for multisensory gaze stabilization

Authors: *L. SCHENBERG[1,2], A. PALOU[3,4,5], F. SIMON[1,2], D. FRICKER[1,2], M. TAGLIABUE[1,2], J. LLORENS[3,4,5], M. BERANECK[1,2];

[1]CNRS UMR 8002, Paris, France; [2]Univ. Paris Cité, Paris, France; [3]Inst. de Neurociènces Univ. de Barcelona, Barcelona, Spain; [4]Departament de Ciències Fisiològiques, Univ. de Barcelona, 08907 l'Hospitalet de Llobregat, Spain; [5]Inst. d'Investigació Biomèdica de Bellvitge (IDIBELL), 08907 l'Hospitalet de Llobregat, Spain

Abstract: The functional complementarity of the vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) allows for optimal combined gaze stabilization responses (CGR) in light. While sensory substitution has been reported following complete vestibular loss, the capacity of the central vestibular system to compensate for partial peripheral vestibular loss remains to be determined. Here, we first demonstrate the efficacy of a 6-week subchronic ototoxic protocol in inducing transient and partial vestibular loss which equally affects the canal- and otolith-dependent VORs. Immunostaining of hair cells in the vestibular sensory epithelia revealed that organ-specific alteration of type I, but not type II, hair cells correlates with functional impairments. The decrease in VOR performance is paralleled with an increase in the gain of the OKR occurring in a specific range of frequencies where VOR normally dominates gaze stabilization, compatible with a sensory substitution process. Comparison of unimodal OKR or VOR versus bimodal CGR revealed that visuo-vestibular interactions remain reduced despite a significant recovery in the VOR. Modeling and sweep-based analysis revealed that the differential capacity to optimally combine OKR and VOR correlates with the reproducibility of the VOR responses. Overall, these results shed light on the multisensory reweighting occurring in pathologies with fluctuating peripheral vestibular malfunction.

Disclosures: L. Schenberg: None. **A. Palou:** None. **F. Simon:** None. **D. Fricker:** None. **M. Tagliabue:** None. **J. Llorens:** None. **M. Beraneck:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.01/EE24

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC019341

Title: Nucleus vestibularis ovalis of the Tokay gecko (Gekko gecko): relay nucleus between the saccule and the torus semicircularis

Authors: *D. HAN, C. E. CARR;
Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: Otolithic endorgans such as the saccule are considered to have dual auditory and vestibular functions in fish and amphibians, but the saccule's auditory role is thought to be lost in amniotes. Using tract tracing methods, we have found evidence supporting a novel saccular pathway related to audition in the Tokay gecko (*Gekko gecko*). Injections of biotinylated dextran amines (BDA) in endorgans of the inner ear revealed that nucleus vestibularis ovalis (VeO),

located lateral to nucleus magnocellularis and dorsal to the descending vestibular nucleus, exclusively receives input from the saccule. Injections of Neurobiotin in the torus semicircularis resulted in retrogradely labelled cells in VeO. Injections of BDA in VeO labelled terminals in the contralateral superior olivary nucleus and lemniscal fibers that projected to the torus semicircularis. An anatomical connection between the saccule and auditory midbrain, as well as a dedicated first order relay nucleus, parallels the anuran condition and supports the addition of the saccule as a second auditory endorgan in the Tokay gecko. In Nissl material we found that VeO in other lepidosaurs, including snakes and *Sphenodon*. It is likely that the saccular pathway in the Tokay gecko is widespread among lepidosaurs and plays a functional role in allowing the animal to sense low frequency vibrations.

Disclosures: D. Han: None. **C.E. Carr:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.02/EE25

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC019341

Title: Comparative quantitative analysis of cochlear nuclei in archosaurs and lepidosaurs

Authors: *R. FUQUEN[1], D. HAN[2], W. KAMAL[3], C. CARR[2];
[1]Univ. of Maryland, Col. Park, Arnold, MD; [2]Univ. of Maryland, College Park, MD; [3]Univ. Maryland, College Park, MD

Abstract: While the brainstem auditory nuclei of birds have been well-studied, it is unclear how they might compare with those of non-avian reptiles. In archosaurs, the two ears typically act as weakly connected pressure receivers, with sound direction computed from binaural interactions in the brain. Turtles, which are a sister group to archosaurs, do not have connected middle ears. In most extant lizards, the eardrums are strongly coupled and interact acoustically to produce a strongly directional response from the ear itself. Snakes and lizards are phylogenetically closely-related suborders, but snakes have secondarily lost their eardrums. Since the size of the brainstem auditory nuclei may reflect the requirements for computation of sound source direction, we compared the sizes of nucleus magnocellularis (NM), a first-order cochlear nuclei for the ascending binaural pathway in diapsids, including three archosaurs (the barn owl, the chicken and the American alligator), two turtles (the red-ear slider and the common snapping turtle), and three lepidosaurs (the tokay gecko, the green iguana and the Western ratsnake). For all species, we quantified the total number of cells in NM, and then normalized each to the adult brain weight. In larger archosaurs, cell numbers were determined using stereological methods, while nucleolar counts were used to determine neuronal numbers, with correction factor for split nucleoli, in smaller animals. NM is largest in the barn owl, which is an auditory specialist

adapted for sound localization, and smallest for the Western ratsnake, which is insensitive to airborne sound. The size of NM was similar for the tokay gecko, a vocal auditory specialist with highly directional ears, and the red-ear slider, an amphibious reptile sensitive to low frequencies. Overall, NM was largest in the archosaurs, which compute sound source location in the brain, and smallest in snakes and lepidosaurs.

Disclosures: **R. Fuquen:** None. **D. Han:** None. **W. Kamal:** None. **C. Carr:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.03/EE26

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC020109

Title: Stimulus selection of salient stimuli in the sound localization pathway of barn owls

Authors: ***A. BAE**[1], R. FERGER[2], J. L. PENA[3];
[1]Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Dominick P. Purpura Dept. of Neurosci., [3]Neurosci., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY

Abstract: As sound localization specialists, barn owls provide a unique opportunity to examine the neural computations underlying spatial coding. In particular, their well-described midbrain stimulus selection network, a circuit containing a map of auditory space dedicated to localizing sounds, can be leveraged to investigate how this circuit prioritizes salient sounds in environments with competing sounds. Earlier *in vivo* recordings in the owl's optic tectum (OT) have shown that neuronal responses and gamma oscillations are spatially tuned to both visual and auditory information, and may play a role in stimulus selection. However, these previous recordings have relied on single electrodes in single regions, and open questions remain regarding how network responses and brain oscillations facilitate information flow across regions for stimulus selection. Towards this end, we recorded spike responses and local field potentials in OT and one of its downstream forebrain regions simultaneously in awake head fixed owls while presenting competing auditory stimuli at different speaker positions from a free field speaker array. Relative salience of the competing stimuli was controlled by altering intensity differences between the two stimuli. Additionally, competing stimuli consisted of two unfrozen broadband noise or two amplitude modulated broadband noise to examine the contribution of the envelope to stimulus selection. Preliminary findings show that spike responses from areas of the map representing less salient stimuli decreases as the intensity of the more salient competing stimulus increases. This was observed at all competing stimulus locations and stimulus conditions, consistent with previous findings that the midbrain stimulus selection network exhibits global inhibition to suppress activity across the topographic space map for non-salient locations for competing visual

or bimodal (visual + auditory) stimuli. Our findings also demonstrate that global inhibition occurs across hemispheric spatial regions. In addition, spike patterning influenced by brain oscillations across midbrain and forebrain regions was analyzed.

Disclosures: **A. Bae:** None. **R. Ferger:** None. **J.L. Pena:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.04/EE27

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant NS104911
NIH Grant DC007690

Title: Stimulus competition and adaptation to free field stimuli in the barn owl's inferior colliculus

Authors: *R. FERGER[1], A. BAE[2], J. L. PENA[3];
[1]Neurosci., Albert Einstein Col. of Med., Bronx, NY; [2]Dominick P. Purpura Dept. of Neurosci., [3]Neurosci., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY

Abstract: The barn owl has been a model organism for sound localization for decades. It's outstanding abilities, the distinct use of interaural time difference (ITD) and interaural level difference (ILD) for localizing sound sources in azimuth and elevation, respectively, as well as the topographical organization of the owl's midbrain have elucidated many fundamental principles of sound localization. The external nucleus of the inferior colliculus (ICX) contains the part of the sound localization pathway, where multiple frequency channels merge and neurons respond to distinct combinations of ITD and ILD in dichotic stimuli (delivered via earphones), or equivalently to sound source locations in free field (more distant speakers). The ICX projects to the optic tectum (OT), the avian homologue of the superior colliculus, where neurons respond to auditory and visual stimuli. The OT is part of a global inhibition network which effectively suppresses responses to the less salient of two stimuli presented at different locations. This was shown for visual-visual stimulus pairs as well as responses to auditory stimuli in presence of competing visual outside of a neurons receptive field. In this study, we compare the amount and effect of global inhibition and adaptation between ICX and its direct down-stream projection target OT. Previously, stimulus competition has been shown in ICX and was explained by circuit-independent mechanism like binaural de-correlation. In a parallel study, we investigate responses in OT, and here we build upon this to elucidate the relative contribution of peripheral mechanisms and global inhibition on auditory-auditory competition.

Disclosures: **R. Ferger:** None. **A. Bae:** None. **J.L. Pena:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.05/EE28

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC018580
NIH Grant EY032230
NIH/NICHD award T32HD108079
UCSC IBSC

Title: Auditory cortex modulates information processing in the mouse superior colliculus

Authors: *B. MULLEN[1], S. SCARLETT[3], J. YAMADA[3], Y. SI[3], A. LITKE[1], D. A. FELDHEIM[2];
[2]MCD Biol., [1]Univ. of California, Santa Cruz, Santa Cruz, CA; [3]UC Santa Cruz, Santa Cruz, CA

Abstract: Localization of sound in space is critical for the survival of a wide range of species and relies on multiple brain structures and circuits. The superior colliculus (SC) is a midbrain area that plays a critical role in processing auditory information to assess saliency and promote action. We have previously performed extensive physiological analysis of auditory responsive neurons in the SC of awake behaving mice and showed that auditory neurons are located in the dSC and have spatially restricted RFs that form a smooth topographic map of azimuthal space that is aligned with the visual map in the superficial SC. We also found that auditory responses in the SC have early (5-20 ms) and late (20-150 ms) time components. Unlike the early component, the late component can be highly non-monotonic to sound level and overlaps in time with visual responses, suggesting that early and late responses arise from different sources. Here we describe experiments that test the hypothesis that the late time component of the auditory SC response comes from the auditory cortex. We investigate the organizational patterns of the subpopulation of layer 5 neurons that project from the auditory cortex to the SC through viral tracing experiments. Further, we show that application of muscimol and optogenetic silencing of the auditory cortex influences the processing of auditory information in the SC. Taken together, we show that the auditory cortex projects to the SC to shape auditory responsive neurons in the SC.

Disclosures: B. Mullen: None. **S. Scarlett:** None. **J. Yamada:** None. **Y. Si:** None. **A. Litke:** None. **D.A. Feldheim:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.06/FF1

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC016363

Title: Signatures of Eye-Movement Related Eardrum Oscillations Detected in the Inferior Colliculus

Authors: *J. L. HERCHE[1], M. N. SCHMEHL[1], D. A. BULKIN[2], G. TOSTAEVA[3], J. GRIEGO[1], J. M. GROH[1];
[1]Neurobio., Duke Univ., Durham, NC; [2]Mathworks, Natick, MA; [3]Northwell Hlth., Manhasset, NY

Abstract: Audiovisual integration involves coordinate translation between the auditory system's head-centered and the visual system's eye-centered reference frames. In primates, saccades cause frequent shifts between the visual and auditory scenes. Robust cross-referencing is critical to audiovisual processing. Eye movements generate a phenomenon in the auditory periphery called Eye-Movement Related Eardrum Oscillations (EMREOs) (Gruters et al., PNAS 2018). The EMREO encodes precise, parametric information about saccade direction and amplitude (Lovich et al., Biorxiv 2022) in both humans and monkeys (Gruters et al., 2018; Lovich et al., Phil Trans B in press), but its origin and impact on central auditory processing remains unknown. Here, we search for signals similar to the EMREO in the central auditory system. Specifically, we analyzed local field potential recordings throughout one rhesus macaque's inferior colliculus (IC). The IC helps localize sounds and modulates its response to them based on eye position. It has substantial connections to oculomotor areas and auditory effector structures (middle ear muscles and cochlear outer hair cells) hypothesized to generate EMREOs. Analysis of >45,000 free saccades across 760 recording locations showed an event-related potential response (latency about 20 ms after saccade onset) as well as a continuing oscillation, in the frequency range of the EMREO (30-40 Hz), discernable as early as >100 ms before saccade onset. Consistent with past EMREO findings, multiple regression analysis of the IC data shows most robust encoding of a saccade's horizontal displacement. Future work should clarify the chronicity of EMREO with the IC's saccadic signature and variability across subjects.

Disclosures: J.L. Herche: None. **M.N. Schmehl:** None. **D.A. Bulkin:** None. **G. Tostaeva:** None. **J. Griego:** None. **J.M. Groh:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.07/FF2

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC003180
NIH Grant DC005808
Kavli Foundation Grant GD2651

Title: Cortical map of auditory space

Authors: *C. CHEN[1], X. SONG[2], Y. GUO[3], X. WANG[4];
[1]Biomed. engineering, [2]Johns Hopkins Univ. Dept. of Biomed. Engin., Johns Hopkins Med. Institutions, Baltimore, MD; [3]Johns Hopkins Univ., Axoft, Baltimore, MD; [4]Johns Hopkins Univ. Sch. Med., Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Despite four decades of research, the nature of the neural representation of sound location in the auditory cortex remains unclear. Previous studies have failed to identify any maps or patches of spatial representation in the mammalian auditory cortex (Middlebrooks and Pettigrew, 1981, J Neurosci; Middlebrooks, 2021, J Neurosci). A prevailing hypothesis of cortical spatial processing is the distributed population coding, supported by the evidence that neurons respond broadly to sound locations on the contralateral hemifield (Ortiz-Rios et al., 2017, Neuron; van der Heijden et al., 2019, Nat Rev Neurosci). However, electrophysiology and fMRI methods have limited spatial resolution to evaluate the cortical representation of sound locations.

In the present study, we took advantage of the flat brain of the marmoset, a highly vocal New World monkey, and used wide-field calcium imaging methods to investigate the neural representations of sound location in the auditory cortex and neighboring multisensory region (medial superior temporal, MST) in awake condition.

Most cortical areas preferred contralateral sound locations, but regions tuned to the front and ipsilateral locations formed five to ten patches. We found those patches in both primary and nonprimary, rostral, and caudal auditory cortex. Next, we investigated whether spatial tuning of patches depends on interaural time and level differences (ITD and ILD) cues. We found patches that prefer low frequency were ITD cue dependent. In contrast, patches that prefer high frequency were cue independent. Patches identified with spatial and binaural stimuli were relatively stable across sound levels. Furthermore, a neighboring multisensory MST has weak sound-driven responses and was not topographically organized by sound frequency. Surprisingly, MST was organized topographically by sound locations that range from far-contralateral to front. Finally, a horizontal visual stimulus will evoke a strong response only in the MST but not in the auditory cortex. We also identified a retinotopic map in the MST. Notably, multisensory auditory and visual-spatial maps in the MST largely overlapped.

In summary, we found that auditory space is represented in the cortex by patch and map.

Disclosures: C. Chen: None. **X. Song:** None. **Y. Guo:** None. **X. Wang:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.08/FF3

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC017924
NIH Grant T01DC018401

Title: Auditory brainstem response abnormalities in aging and autistic animal models

Authors: *B.-Z. LI[1,2], S. POLEG[1], T. C. LEI[2,1], A. KLUG[1];
[1]Dept. of Physiol. and Biophysics, Univ. of Colorado Anschutz Med. Campus, Aurora, CO;
[2]Dept. of Electrical Engin., Univ. of Colorado Denver, Denver, CO

Abstract: Binaural spatial cues for sound localization are initially processed in the auditory brainstem, where neurons in the superior olivary complex compare encoded auditory inputs from both ears, extracting interaural time and level differences for afferent regions. Despite extensive investigation into the sound localization process over the past decades, the potential impact of pathological or age-related neuronal alterations on spatial hearing is understudied. This study addresses this question by recording the auditory brainstem response (ABR) from aging gerbils and Fmr1-KO mice, a model of autism with Fragile X Syndrome. Both aging and Fmr1-KO animals exhibited aberrant ABRs in comparison to young adults and wild-type controls. Notably, the aging gerbils displayed a significantly attenuated or absent peak III, while the Fmr1-KO mice showed prolonged peak III to peak V and reduced binaural interaction components. To further analyze potential mechanisms underlying spatial hearing deficits, a computational model of the mammalian auditory brainstem was implemented and fitted with recorded ABRs. Simulation results suggest that abnormal ABRs in elderly gerbils may be attributable to increased variation in axon myelination and reduced inhibitory input strengths. Meanwhile, the abnormality in Fmr1-KO mice could be caused by myelination deficits and hyperexcitability. These findings demonstrate how the sound localization process could be directly influenced and degraded by underlying disease or age-related alterations in neural circuits. This insight may inspire the development of therapeutic applications aimed at restoring compromised spatial hearing abilities.

Disclosures: B. Li: None. **S. Poleg:** None. **T.C. Lei:** None. **A. Klug:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.09/FF4

Topic: D.05. Auditory & Vestibular Systems

Support: ONR/ ILIR

Title: Effects of exposure to occupational noise and jet fuel inhalation on hearing-related injury in a rodent model

Authors: *S. ROMER[1], E. W. DIRR[2,3], V. ETHRIDGE[2,3], M. SONNER[2,3], N. GARGAS[2,3], N. HANANDEH[2,3], E. A. PHILLIPS[2,3], C. GUT[2,3], B. SHARITS[2,3], P. D. SALO[2];

[1]Envrn. Hlth. Effects Lab., Naval Med. Res. Unit Dayton/Leidos, Dayton, OH; [2]Naval Med. Res. Unit Dayton, Dayton, OH; [3]Leidos, Reston, VA

Abstract: Hearing-related injuries are the most common Military service-related disability. In addition to high noise levels, inhalation exposure of ototoxic JP-5 jet fuel while aboard Naval aircraft carriers is common. The effects of the combination of these exposures on hearing function have not been well characterized. The objective of this study was to evaluate the impact of noise exposures and inhalation of jet fuel aerosols and vapor on the development of hearing-related injury in a rodent model. Adult Long-Evans rats (n=10 per exposure group) were exposed to 6 hours per day of 8 kHz octave band noise, (~89 decibels (dB) 8 hour equivalent, meant to represent an occupational noise exposure), 1000 mg/m^3 of a JP-5 jet fuel aerosol/vapor mixture for 6 hours a day, or a combination of these exposures. Rats were exposed for five days per week for four weeks total. Auditory function was assessed the week following exposures. The function of the peripheral and brainstem segments of the ascending auditory neural pathway were assessed by measuring the auditory brainstem response (ABR). ABR is a non-invasive evoked potential that is recorded through the placement of electrodes over the auditory cortex that generally produces a response of 5 to 7 waves. Each wave corresponds to different signal generators in the auditory pathway providing a unique tool to localize specific exposure effects. Co-exposure to occupational noise and JP-5 showed a synergistic effect without significantly impacting ABR hearing thresholds. Despite this, the noise + JP-5 co-exposures produced decrements in neural auditory processing. Specifically, we did not find significant changes in waves 1 and 2 suggesting the cochlear nerve is not impacted by the JP-5 and occupational noise co-exposure. However, we found a significant decrease in the amplitude of ABR waves 3 and 4 suggesting exposure related effects in the cochlear nucleus, medial nucleus of the trapezoid body, and/or superior olivary complex of the auditory brainstem. We also found a significant decrease in wave 2-4 latencies consistent with auditory brainstem effects. Altogether these data indicate that the brainstem rather than cochlear nerve is the primary target effected by exposure to JP-5 and occupational noise exposure.

Disclosures: S. Romer: None. **E.W. Dirr:** None. **V. Ethridge:** None. **M. Sonner:** None. **N. Gargas:** None. **N. Hanandeh:** None. **E.A. Phillips:** None. **C. Gut:** None. **B. Sharits:** None. **P.D. Salo:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.10/FF5

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC019341

Title: Experience dependent plasticity in the barn owl ITD circuit

Authors: *C. E. CARR[1], N. ABOUELSEOUD[1], A. K. FORSBERG[1], W. KAMAL[1], M. F. KUBKE[2], C. KÖPPL[3], R. KEMPTER[4], P. T. KUOKKANEN[5];
[1]Univ. of Maryland, College Park, MD; [2]Otahuhu Col., Auckland, New Zealand; [3]Univ. Oldenburg, Oldenburg, Germany; [4]Humboldt-Universität Zu Berlin, Berlin, Germany; [5]Inst. for Theoretical Biology, Dept. of Biol., Humboldt-Universitaet zu Berlin, Berlin, Germany

Abstract: Barn owls experience increasing interaural time differences (ITDs) during development, because their head width more than doubles in the month after hatching. We hypothesized that their ITD detection circuit might be modified by experience. To test this, we raised owls with unilateral ear inserts that delayed and attenuated the acoustic signal, then used the binaural neurophonic to measure the ITD representation in the brainstem nucleus laminaris (NL) when the birds were adult. The ITD circuit is composed of delay line inputs from the nucleus magnocellularis (NM) to the coincidence detectors in NL, and we predicted that plastic changes would lead to shorter delays in the axons from the manipulated ear, and complementary shifts in ITD representation on the two sides. In owls that received ear inserts starting around P14, the maps of ITD shifted in the predicted direction, but only on the ipsilateral side, and only in those tonotopic regions that had not experienced auditory stimulation prior to insertion. The contralateral map did not change. We examined the consequences of reducing afferent auditory activity on this circuit, and measured NM cells and GABAergic innervation after plug rearing. The GABAergic input originates from the superior olive and matures in a low to high best frequency gradient in both NM and NL in the first month post-hatch. In ear plug raised birds, the NM cells and axons ipsilateral to the earplug were significantly smaller than contralateral cells, but only those in the high best frequency tonotopic regions that had not experienced auditory stimulation prior to insertion. Thus, altered auditory input during development is associated with long-lasting changes in the circuit for computation of ITD.

Disclosures: C.E. Carr: None. **N. Abouelseoud:** None. **A.K. Forsberg:** None. **W. Kamal:** None. **M.F. Kubke:** None. **C. Köppl:** None. **R. Kempter:** None. **P.T. Kuokkanen:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.11/FF6

Topic: D.05. Auditory & Vestibular Systems

Support: Hong Kong GRF grant 11100219
Hong Kong GRF grant 11101020
Martin Lee Foundation / Macquarie University

Title: Sensitivity of Inferior Colliculus Neurons to Interaural Time and Level Differences in Adult Neonatally Deafened Rats

Authors: M. ZEESHAN[1], F. PENG[1], B. CASTELLARO[1], S. FANG[1], N. ROSSKOTHEN-KUHL[2], ***J. W. H. SCHNUPP**[1];
[1]City Univ. of Hong Kong, Hong Kong, Hong Kong; [2]Dept. of Otorhinolaryngology, Neurobiological Res. Laboratory, Univ. of Freiburg, Freiburg, Germany

Abstract: Bilateral cochlear implants (biCIs) are increasingly used to treat severe hearing loss. However, human biCI users usually exhibit relatively poor binaural cue sensitivity, with interaural time difference (ITD) sensitivity in prelingually deaf patients being particularly poor. To better understand these shortcomings in prosthetic binaural hearing, it would be helpful to know what the "innate" ITD and ILD sensitivity of the neonatally deafened (ND), mature mammalian auditory pathway is like, but this cannot easily be investigated in humans. We therefore recorded neural responses in the inferior colliculus (IC) of rats deafened by i.p. kanamycin injection. When the deaf rats reached maturity (>p60) they were urethane anesthetized and implanted with biCIs. IC multiunit responses to pulse train stimuli at rates of 1, 100, and 900 pps with combinations of ITD $\in \pm\{0, 0.04, 0.08, 0.12\}$ ms and ILD $\in \pm\{0, 1, 4\}$ dB were recorded extracellularly, and analyzed for ITD or ILD. At pulse rates of 1, 100, and 900 pps, 85.6%, 99.7% and 97.2% respectively of multiunits were significantly ITD sensitive (Kruskal-Wallis tests), 88.5%, 96.4% and 88% were ILD sensitive, and 76.8%, 96.1% and 85.5% were sensitive to both. Sensitivity to small electrical stimulus ITDs and ILDs was therefore very widespread in the IC of adult, hearing-inexperienced, acutely CI-stimulated ND rats. While most multiunits showed significant sensitivity to both cues, examining the proportions of variance explained by ITD or ILD respectively revealed that multiunits in the naive IC nevertheless form two distinct clusters that are either predominantly ITD sensitive or predominantly ILD sensitive.

Disclosures: M. Zeeshan: None. **F. Peng:** None. **B. Castellaro:** None. **S. Fang:** None. **N. Rosskothen-Kuhl:** None. **J.W.H. Schnupp:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.12/FF7

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant 5R01DC012938

Title: Functional Maturation of Binaural Hearing in the Murine Auditory Brainstem

Authors: *A. DAGOSTIN[1], M. SERGISON[2], D. J. TOLLIN[2], H. VON GERSDORFF[1];
[1]OHSU, Portland, OR; [2]Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: The auditory brainstem develops rapidly from birth until hearing onset in rodents (around postnatal day 12). Although the system undergoes further maturation until adulthood, this process evolves more slowly and has not been fully studied to date. Using auditory brainstem recordings (ABR), we measured the activity of the auditory brainstem nuclei of young

juvenile (postnatal day 30; P30) and adult-like 3-6 months old (3MO) mice. Acoustic stimuli were monaural and binaural broadband clicks and tone pips at 8, 16 and 24 kHz and interaural time differences (ITDs) were varied from ±1500 μs in 250 μs steps. To avoid potential early age-related hearing loss (ARHL) effects in C57 mice, we used the CBA strain, which is not susceptible to the cadherin 23 mutation which leads to early ARHL of high frequency sounds. ABR wave amplitudes were not different between groups, indicating similar neuronal population activity. However, latencies were shorter in 3MO animals for waves II, III and IV. This suggests that the signal conduction velocity and/or synaptic strength is improving with maturation. Our results suggest that myelination and synaptic maturation is still occurring in the first 3 months of development. ABR wave IV is believed to be associated with the binaural nuclei of the brainstem including the Lateral Superior Olive (LSO). The LSO receives inhibitory inputs from the contralateral ear and excitatory ones from the ipsilateral ear, and this E/I balance plays a paramount role in binaural signal processing. We further studied the LSO binaural processing by computing the binaural interaction component (BIC). The BIC signal is obtained by subtracting the binaural-evoked ABR with a given ITD from the sum of the left and right ear monaural ABRs. The BIC waveform contains a large negative potential (DN1) that has a latency corresponding to LSO neurons activity. BIC DN1 had similar amplitudes in P30 and 3MO but with a significantly smaller latency in the latter. The BIC DN1 amplitude vs ITD plots (believed to reflect processing of ITDs in the LSO nuclei) were fitted with single gaussians, with the curves for the older animals being twice as narrow than those for the young ones (P30: σ=405 μs; 3MO: σ=186μs). This result alongside the shorter latency seen in older animals might be expected to translate into an improved capability of LSO neurons to extract ITD cues to sound source location in the horizontal plane in adult animals. We conclude that although the auditory brainstem circuit is believed to be fully formed in P30 mice, they undergo further maturation of the synapses and circuits into adulthood. This process provides additional sensory fine tuning for a more precise sound localization.

Disclosures: **A. Dagostin:** None. **M. Sergison:** None. **D.J. Tollin:** None. **H. von Gersdorff:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.13/Web Only

Topic: D.05. Auditory & Vestibular Systems

Support: NRF-2022R1A2C1004862
NRF-2019R1A2B5B01070129
Hallym University Research Fund

Title: Neuroplasticity for sound localization in single-sided deafness occurs in the early auditory cortices, and the higher auditory areas stand for functional compensation

Authors: *H.-J. LEE[1], J. KIM[3], G. KIM[2], L. SHIM[2];
[1]Otolaryngology, [2]Lab. of Brain and Cognitive Sci. for Convergence Med., Hallym Univ. Col. of Med., Anyang-si, Korea, Republic of; [3]Otolaryngology, Hallym Univ. Sacred Heart Hosp., Anyang-si, Korea, Republic of

Abstract: Studies on cortical plasticity in single-sided deafness (SSD) have consistently reported increased activity in the auditory cortex ipsilateral to the hearing ear. In neuroimaging studies with passive listening, functional consequences of the ipsilateral strengthening of auditory cortical response have been related to auditory localization performance and a later outcome with aural rehabilitation. Yet, evidence in the population with SSD is still limited on the neuroplasticity directly related to binaural processing, such as auditory localization, and is unclear whether the plasticity is specific to the ear side of deafness. Two groups of single-sided deafness with different ear sides deafness (17 left and 18 right-sided SSD) and 13 normal-hearing (NH) controls performed a functional MRI experiment with an auditory localization task. The NH group additionally conducted the same experiment with each ear acutely plugged. The cortical network for auditory localization was explored and compared across groups, and correlation analyses were conducted to reveal areas of activity associated with the duration of SSD and localization performance. Response laterality in the cytoarchitectonically defined auditory cortex to each sound source was analyzed in the right and left SSD groups, respectively, and compared to controls in a binaural condition as well as right- and left-monaural conditions. The extended duration of single-sided hearing modulates auditory cortical response in the right primary auditory cortex. The direction of change differs by the side of the deaf ear but resulting the same consequence of decreasing asymmetry in this early auditory area. Analyses of laterality reveal that functional asymmetry in the PAC decreases as the duration of SSD when localizing sound from the impaired ear side. Areas in the posterior STG, in the dorsal auditory pathway in the contralateral hemisphere to the intact ear, are related to better localization performance. Activity in the cingulo-opercular attention network increased in the Lt SSD and was related to better localization performance in the Rt SSD. Auditory cortical response for spatial tasks is differently modulated by the ear side of deafness. Engagement of cortical attentional resources contributes to auditory spatial behavior in the SSD, which is indispensable when hearing is impaired in the left ear.

Disclosures: H. Lee: None. **J. Kim:** None. **G. Kim:** None. **L. Shim:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.14/FF8

Topic: D.05. Auditory & Vestibular Systems

Title: Eeg activity and motor response differences between left and right-handed on spatial ability:an exploratory study on sound loclization task

Authors: *M. CASTRO GONZÁLEZ[1], Y. DEL RÍO PORTILLA[2];
[1]Univ. Nacional Autónoma De México, México City, Mexico; [2]Sleep Lab., Univ. Nacional Autónoma de México, México City, Mexico

Abstract: Differences in brain organization and brain processing between right and left-handed have been described, nevertheless is continue to be an issue of fact. There is also known that sound processing is different for both hemispheres in relation to spatial abilities related to sound localization. The aim of this study was to analyze brain processing and behavioral differences during a sound localization task. We used 120 stimuli (musical note A, 2s each). Stimuli were presented in a classical random block design, for each group (homogeneous left-handed and right-handed/ 80-100% of hand preference; and left and right-handed / 60-75% of hand prefference). After each run, subjects (n=20 male) respond on a keyboard according to where they heard the stimuli (right, middle or left side) and at the same time to gaze on the direction they heard the stimuli (right or left side and not eye movement if it is at the middle). For eye movements recording, we placed electrodes according EOG. Preliminary results between 20 males (10 homogeneous right-handed and 10 homogeneous left-handed) showed significant differences between eye movements EOG and motor response. For left side, left-handed have higher relation for eye and motor response to left side in comparison with right-handed $F = 1.36$ $t = 0.03$. Furthermore, right-handed have higher relation between eye movements and motor response for right side in comparison with left-handed $F = 1.38$ $t = 0.02$. Contralateral results between eye movements and motor response founded on average for interaction between eye movements (left and right) and all stimuli $F = 14.26$ $p = 0.01$. There were no significant results for Reaction Time (RT) between groups $F = 1.02$ $t = 0.15$. For EEG analysis, we found higher INTERr correlation during the sound localization task for beta1 r=.0001 and gamma r=.0001 for left-handedand and INTRAr higher correlation for right-handed for temporal-anterior regions on right hemisphere for alpha2 r=0.002 and beta2 r= 0.003 and higher for left-handed on temporal-posterior regions for gamma band on left hemisphere. Contralateral responses on eye movements and motor response, may be related to TR between saccades and tapping. For EEG activity, we expected that correlation increase as we have more participants and also may be differences between laterality groups.

Disclosures: M. Castro González: None. **Y. Del Río Portilla:** None.

Poster

PSTR482. Auditory Processing-Sound Localization and Binaural Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR482.15/FF9

Topic: D.05. Auditory & Vestibular Systems

Support: NSERC Grant RGPIN-2022-04402

Title: Auditory space perception is modulated by induced vestibular asymmetry: preliminary results.

Authors: *A. CÉDRAS[1,3,2], C. ORSINI[2,3], F. CHAMPOUX[2,4], M. MAHEU[2,3];
[1]Univ. of Montreal, Longueuil, QC, Canada; [2]Sch. of Speech Language Pathology and Audiol., Univ. of Montreal, Montreal, QC, Canada; [3]CRIR-IURDPM, Pavillon Laurier, CIUSSS du Centre-Sud-de-L'Île-de-Montréal, Montreal, QC, Canada; [4]Ctr. de recherche de l'Institut Universitaire de Gériatrie de Montréal, Montreal, QC, Canada

Abstract: It has been proposed that the vestibular system is necessary to integrate spatial information from other senses. For example, it has been established that vestibular dysfunction leads to distortions in tactile and temporal-spatial representations. However, less is known about the vestibular system's influence on auditory spatial representations. Studying the impact of vestibular dysfunction on the ability to localize sound sources will help us understand the mechanisms underlying auditory-vestibular interactions. Thus, this study aims to evaluate the influence of galvanic vestibular stimulation (GVS) on the capacity of localizing sound sources. A total of 14 young healthy participants were recruited. A sound localization task under earphones with 11 positions in the azimuth plane was created (-90°; -45°; -30°; -20°; -10°; 0°; 10°; 20°; 30°; 45°; 90°). Participants were asked to verbally identify the exact position of the sound source under 3 vestibular conditions.: 1) Without GVS 2) GVS with anode at the right mastoid 3) GVS with anode at the left mastoid. In each condition, all 11 positions were presented 6 times (6 blocks) in random order. We analyzed the error rate (average error rate across all 11 positions) for each condition using repeated measures ANOVA 3 conditions (baseline, anode left, anode right). Post-hoc t-test using Bonferroni correction factor was performed to reveal differences between conditions. post-hoc Preliminary results showed a significantly higher error rate on average when GVS is applied either at the left ($p = 0.002$) or the right mastoid ($p = 0.005$) compared to no vestibular stimulation. In addition, the anode-left condition appears to produce a significantly higher error rate on average as opposed to the anode-right condition ($p = 0.03$). These results shed light on the possible mechanism linking the vestibular system with auditory spatial representations.

Disclosures: **A. Cédras:** None. **C. Orsini:** None. **F. Champoux:** None. **M. Maheu:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.01/FF10

Topic: E.02. Cerebellum

Support: NIDA Grant R01DA044761
NIMH Grant R01MH115604

Title: Monosynaptic extra-pontine cerebro-cerebellar pathways

Authors: *J. GUARQUE-CHABRERA[1,2], M. OÑATE[1], M. MIQUEL[1,2], K. KHODAKHAH[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]Univ. Jaume I, Castellón, Spain

Abstract: The cerebellum has been classically implicated with high computational demands for motor coordination. However, increasing evidence based on its connectivity with other brain regions revealed its involvement in non-motor tasks such as fear memory, cognitive flexibility, goal-directed behavior, rewarded behavior, and emotional processing. Moreover, several findings link cerebellar dysfunction to neuropsychiatric conditions such as autism, obsessive-compulsive disorder, depression, schizophrenia, and substance use disorders. Direct and indirect ascending cerebellar pathways modulate activity in the medial prefrontal cortex, limbic regions, basal ganglia, midbrain, and nucleus accumbens. Descending pathways provide the information to which the cerebellum has access, and the pontine nuclei being the largest of the pre-cerebellar nuclei, have practically received all the attention when considering cerebro-cerebellar inputs. Therefore, very little to no information is available concerning extra-pontine cerebro-cerebellar pathways and what information these routes might convey to the cerebellum, reflecting a different functional organization than the canonically delivered via the pontine nuclei.Here, we injected each deep cerebellar nuclei (medial, interposed, or lateral) of wild-type and EGFP-reporter mice (RCE:loxP) with classic (Fluorogold) and viral (AAVrg-Cre) tracing agents to map the monosynaptic extra-pontine cerebro-cerebellar projections. Interestingly, we observed differential input cell distribution of the monosynaptic projections to each deep cerebellar nuclei from diverse cerebral nuclei.

Disclosures: J. Guarque-Chabrera: None. **M. Oñate:** None. **M. Miquel:** None. **K. Khodakhah:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.02/FF11

Topic: B.07. Network Interactions

Support: R01MH128888
1 RF1 MH128861-01

Title: Topography of corticopontine projection in mouse

Authors: *Q. ZHAO, B. ZINGG, J. SUN, M. RUDD, L. GOU, C. CAO, K. MORADI, N. FOSTER, H. HINTIRYAN, H. DONG;
UCLA, Los Angeles, CA

Abstract: The topographic organization of brain circuits underlies their functional properties. Wiring patterns in rat have been extensively studied in the cortico-pontine-cerebellar system, one of the largest projection systems in the brain. The pontine nuclei, between the cerebral cortex and the cerebellum, are typically attributed a complex clustered organization, related to a map transformation from orderly maps in the cerebral cortex to disseminated representations in the granular layer of the cerebellar cortex. In this study, we will construct a map of all cortico-

pontine-cerebellar projections and delineate the distinctive input and output of cortico-pontine-cerebellar subnetworks. Following single or multiple tracer injections into different cerebral cortex, pontine nuclei, and cerebellar regions, distributions of anterogradely or retrogradely labeled pathways in this network will be mapped. This study will provide a brain-wide connectivity map for understanding how this loop is working and implicated in behaviors.

Disclosures: Q. Zhao: None. **B. Zingg:** None. **J. Sun:** None. **M. Rudd:** None. **L. Gou:** None. **C. Cao:** None. **K. Moradi:** None. **N. Foster:** None. **H. Hintiryan:** None. **H. Dong:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.03/FF12

Topic: E.02. Cerebellum

Support: Lingang Laboratory Grant No. LG-QS-202201-02 to C.L.

Title: An anatomical and connectivity atlas of themarmoset cerebellum

Authors: *X. ZHU[1,2,3], H. YAN[2], Y. ZHAN[2], C. WEI[2], Y.-G. YAO[1,3], C. LIU[2,3,4];
[1]Kunming Inst. of Zoology, Chinese Acad. of Sci., Kunming, China; [2]CAS Ctr. for Excellence in Brain Sci. and Intelligence Technol., Shanghai, China; [3]Univ. of Chinese Acad. of Sci., Beijing, China; [4]Shanghai Ctr. for Brain Sci. and Brain-Inspired Intelligence Technol., Shanghai, China

Abstract: The cerebellum, despite its homogeneous cytoarchitectures, encompasses distinct regions that are associated with various motor control and cognitive processes. A thorough examination of the structural and functional attributes of these distinct cerebellar regions is crucial for achieving a comprehensive understanding of the cerebellum's role. The common marmoset, a small non-human primate, offers considerable benefits for exploring cerebello-cerebral circuits. In this study, we used multimodal MRI techniques to develop a comprehensive atlas of the marmoset cerebellum. Leveraging ultra-high-resolution ex-vivo MRI, we offered precise anatomical atlases, surfaces, and a flat map of the cerebellar cortex, facilitating a detailed characterization of anatomical features across regions. Through awake resting-state fMRI, we uncovered unique patterns of intra-cerebellar gradient and cerebello-cerebral gradient, the latter of which mirrors the pattern seen in humans. These disparities may originate from the species-specific cerebral influences on the cerebellum, with the human cerebellum demonstrating stronger intrinsic functional connections with the cerebral cortex than marmosets. We quantified the functional connections between the cerebellar cortex and 15 functional networks, revealing that cerebellar regions with higher cerebello-cerebral gradient values maintain stronger connections with more functional networks, underscoring the vital role of these regions in cerebellar-brain connectivity. Utilizing diffusion MRI tractography, we generated a structural connectivity map between cerebellar nuclei and various cerebellar cortex regions. We found that

the majority of the dentate nucleus connects to the region with higher cerebello-cerebral gradient values, displaying the most complex connectivity patterns, in contrast to the other nuclei. Further, the principal gradient presents a clear continuous change from the dentate nucleus (highest extreme) to the fastigial nucleus (lowest extreme), mirroring patterns found in the structural-connectivity mapping of the cerebellar nuclei. Overall, the atlas elucidates the anatomical details of the marmoset cerebellum, reveals distinct gradient patterns of intra-cerebellar and cerebello-cerebral functional connectivity, and maps the topological relationship of cerebellar nuclei in cerebello-cerebral circuits. As version 5 of the Marmoset Brain Mapping project, this atlas is publicly available at https://marmosetbrainmapping.org/MBMv5.html.

Disclosures: **X. Zhu:** None. **H. Yan:** None. **Y. Zhan:** None. **C. Wei:** None. **Y. Yao:** None. **C. Liu:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.04/FF13

Topic: E.02. Cerebellum

Support: NIH Grant R01NS032405
NIH Grant R35NS097284
NIH Grant K99NS110978
NIH Grant F32NS101889

Title: Comprehensive description of all Purkinje cell projections to the brainstem

Authors: *Z. YAO[1], C. CHEN[1], S. WU[2], W. REGEHR[2];
[1]Penn State Col. of Med., Hershey, PA; [2]Harvard Med. Sch., Harvard Med. Sch., Boston, MA

Abstract: Purkinje cells (PCs) are inhibitory cells of the cerebellar cortex and project to the deep cerebellar nuclei, which in turns carry cerebellar signals to the rest of the brain. PCs' direct projection in the brainstem is much less studied, providing alternate pathways for the cerebellar cortex to directly influence other circuits. Here we evaluated the extent and strength of PC projections outside the cerebellum. In order to comprehensively compare PC synapses in all the brainstem nuclei, we expressed synaptophysin-tdtomato under the control of *pcp-2(L7)* gene so that fluorescence proteins are restricted to presynaptic boutons in all PCs. We determined the percent of PC synapses in each brainstem nuclei by comparing PC signal to all vestibular GABA transporter signals marked by immunohistochemical staining. Each brainstem regions were delineated by genetic and histological landmarks according to the Allen Mouse Common Coordinate Framework. To test the strength of these PC synapses, we optogenetically activated PC synapses and measured evoked currents using in vitro patch clamp. As expected, PCs make the most abundant connections in the four vestibular nuclei and make up a bigger portion of these regions' total inhibitory inputs. Regional variations exist between and within these large

nuclei. Moderate inputs in the parabrachial nucleus, prepositus nucleus, and cuneate suggest non-canonical pathways for the cerebellar cortex to influence more diverse behaviors. Few connections were found in the pontine central gray regions, including the locus coeruleus, Barrington's, and tegmental nuclei, suggesting a lack of significant connection between PCs and these nuclei.

Disclosures: Z. Yao: None. **C. Chen:** None. **S. Wu:** None. **W. Regehr:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.05/FF14

Topic: E.02. Cerebellum

Support: 5R01MH115604-03
1R01DA044761-01A1

Title: Cerebellum targets the dopamine centers in the midbrain through distinct direct pathways

Authors: *M. OÑATE, J. VERA, L. KHATAMI, K. KHODAKHAH;
Albert Einstein Col. of Med., Bronx, NY

Abstract: There is increasing evidence that the cerebellum is involved in a wide range of functions from motor control to cognition and its dysfunction is associated with a range of pathologies ranging from motor diseases such as dystonia and Parkinson's to behavioral and mental disorders such as addictive behavior, schizophrenia, and autism spectrum disorder. Previous work of our laboratory has shown that the cerebellum (Cb) sends monosynaptic excitatory projections to the main midbrain dopaminergic nuclei, the ventral tegmental area (VTA), and the substantia nigra pars compacta (SNc). Specific activation of these projections results in dopamine release in the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), the main targets of VTA or in the dorsal striatum (DS), the main target of SNc. Consistent with these findings, behavioral experiments in mice suggest that dopamine modulation by the Cb-VTA projection activates reward circuits and promotes social preference, while the Cb-SNc projections may promote movement vigor. Yet, little is known on whether these projections arise as collaterals of the same cerebellar output axons or if distinct cerebellar projections target each dopaminergic pathway. Using different intersectional anatomical tracing experiments we find that although cerebellar projections to the VTA and SNc arise from similar territories in the three deep cerebellar nuclei (DCNs), they emerge primarily from different populations of neurons. Furthermore, functional connectivity experiments indicate that these pathways have differential activity. To explore the specificity of the Cb-VTA projections, we combine anterograde and retrograde viral tracers and find that all three DCNs send disynaptic projections (via the VTA) throughout all of mPFC and NAc. Analysis of subcircuits in the NAc show that different regions of the NAc are targeted by different VTA neuron targets, which in

turn, originate from predominantly different groups of neurons in the DCNs. Thus, cerebellar projections to the VTA dopamine pathway appear to have specific disynaptic targets in the NAc. Similar experiments describing the cerebellar projections to the mPFC and DS via VTA and SNc, respectively, support specific cerebellar targets through different disynaptic circuits. Thus, cerebellar projections appear to provide specific and distinct modulation of the different dopamine pathways targeting the basal ganglia and the cortex.

Disclosures: **M. Oñate:** None. **J. Vera:** None. **L. Khatami:** None. **K. Khodakhah:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.06/FF15

Topic: E.02. Cerebellum

Support: NIH Grant MH060605
NIH Grant MH115604
NIH Grant DA044761
NSF Grant IOS-2002863

Title: Modeling potential functions of the cerebellar projections to the substantia nigra dopamine neurons

Authors: *F. NADIM[1], J. YOSHIDA[3], K. KHODAKHAH[4], M. ESKANDAR[2], H. ROTSTEIN[5];
[2]Biol. Sci., [1]New Jersey Inst. of Technol., Newark, NJ; [3]Dominick P. Purpura Dept. of Neurosci., Yale Univ., New Haven, CT; [4]Albert Einstein Col. of Med. Postdoc Dominick P. Purpura Dept. of Neurosci., Bronx, NY; [5]NJIT, Jersey City, NJ

Abstract: Both the basal ganglia and the cerebellum (Cb) are involved in movement control and learning. The functions of both systems are mostly understood in terms of their respective reciprocal interactions with the cortex. The Cb ensures that movements are performed smoothly and efficiently, whereas the basal ganglia are important in proper movement initiation and the control of movement speed (vigor), functions that depend crucially on dopamine modulation. dopamine is released by the midbrain nucleus substantia nigra pars compacta (SNc) in the striatum, the primary input nucleus of the basal ganglia and its actions are known to be important for movement initiation and speed as well as reward-based learning. The Cb is also known to be essential for motor learning, but recent studies have shown the cerebellum may also participate in reward-based functions. We recently characterized functional monosynaptic projections (Cb-SNc) from the Cb to the SNc. Recordings in mice show that the Cb-SNc pathway is active during movement, suggesting a possible role for these projections in movement modulation. Consistent with this prediction, stimulation of this pathway increases locomotion vigor. Interestingly, in a simple Pavlovian task, the Cb-SNc activity is highly responsive to reward (water) consumption

and to reward value (sweet vs regular water), indicating a possible role for this pathway in modulating reward-based functions in the basal ganglia. The goal of this modeling and computational project is to disambiguate the potential contributions of the Cb-SNc pathway to reward-based function vs movement vigor. We examine the trial-by-trial simultaneous fiber photometry signals recorded from Cb-SNc projections, SNc dopamine neurons and the SNc dopamine axons in the dorsolateral striatum and deconvolve the components (kernels) of these signals that correlate with sensory cue, movement and reward. We use these signals to build simple rate models of the activity of cerebellar neurons that project to the SNc and the SNc dopamine neurons, including either feedback or feedforward inhibition. Finally, we examine how these signals change on a session-to-session basis by estimating the model parameter changes using reinforcement learning models and algorithms.

Disclosures: **F. Nadim:** None. **J. Yoshida:** None. **K. Khodakhah:** None. **M. Eskandar:** None. **H. Rotstein:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.07/FF16

Topic: E.02. Cerebellum

Support: NIH Grant MH115604
NIH Grant DA044761

Title: Direct cerebellar projections to the substantia nigra can affect movement vigor

Authors: *J. VERA[1], P. DIAZ[1], M. ONATE[1], F. NADIM[2], K. KHODAKHAH[1];
[1]Albert Einstein Col. of Med., Bronx, NY; [2]New Jersey Inst. of Technol., Newark, NJ

Abstract: Even the simplest voluntary movement involves coordinated activities of the motor cortex, the basal ganglia, and the cerebellum. It is believed that the cerebellum communicates with the cortex to ensure smooth and efficient execution of movement, whereas the basal ganglia control the initiation, termination, and speed of movement. The involvement of the basal ganglia in these functions is supported by their defect due to the loss of the substantia nigra pars compacta (SNc) dopamine neurons in Parkinson's disease, and by the dysregulation of striatal dopamine observed in patients with dystonia. These dopamine neurons target the striatum and modulate cortico-striatal circuits involved in movement. We recently showed that there is a monosynaptic glutamatergic projection from the cerebellum to the SNc (Cb-SNc) that is active during movement and is capable of driving the SNc dopamine neurons. Considering the importance of the cerebellum in producing efficient movements, a possible function of Cb-SNc may be to modulate movement vigor, the speed at which the movement is performed. To examine this possibility, we used optogenetic stimulation to the Cb-SNc projection in head-restrained mice walking on a treadmill, while recording dLight dopamine signals in the

dorsolateral striatum using fiber photometry. We found that increasing levels of Cb-SNc optogenetic activation proportionally increased striatal dopamine levels. Optogenetic stimulation of Cb-SNc also increased the speed and probability of walking. In our recordings, dopamine transients were not predictive of the onset of locomotion, indicating that it is unlikely that the cerebellar projections contribute to movement initiation. However, there was a tight linear correlation between locomotion speed and the levels of striatal dopamine, supporting the involvement of these projections in vigor. These results strongly suggest that the Cb-SNc projections contribute to dopamine release in the striatum, and that the cerebellum and the SNc may work together to modulate movement vigor.

Disclosures: J. Vera: None. **P. Diaz:** None. **M. Onate:** None. **F. Nadim:** None. **K. Khodakhah:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.08/FF17

Topic: E.02. Cerebellum

Support: NIH NIDCD R01-DC018061
Society of Neurologic Surgeons Neurosurgeon Scientist Training Program Grant

Title: Cerebellar and basal ganglia interactions in nonhuman primates during reaching

Authors: *K. R. LEHNER[1], K. E. CULLEN[2];
[1]Neurosurg., Johns Hopkins Hosp., Baltimore, MD; [2]Johns Hopkins Univ., The Johns Hopkins Univ., Baltimore, MD

Abstract: The cerebellum contributes to accurate motor control through computation of sensory predictive errors, and cerebellar dysfunction causes well-defined movement abnormalities. The dysfunction of the basal ganglia in movement disorders and its role in reward is also well described. Increasing evidence suggests that basal ganglia and cerebellar function are intimately linked, and multiple neurologic and neuropsychiatric disorders ranging from Parkinson disease (PD) and dystonia to Tourette syndrome (TS) and obsessive compulsive disorder (OCD) result from broader dysfunction of a network including the basal ganglia and cerebellum. Additionally, deep brain stimulation (DBS) of the subthalamic nucleus (STN) results in modulation of cerebellar function, and DBS of the dentate nucleus (DN) is being investigated for treatment of dystonia and rehabilitation after stroke. There are well-described disynaptic pathways linking the subthalamic nucleus (STN) of the basal ganglia to the cerebellum via pontine nuclei; similarly, the dentate nucleus (DN) of the cerebellum is linked to the striatum via disynaptic pathways with thalamic relays which likely contribute to these observations, however, the function of these pathways remains unclear.

To date, few studies have explored the characteristics of these pathways in non-human primates (NHPs) with simultaneous neural recordings. We report the results of simultaneous high-density neural recordings of the STN and DN in a NHP during a simple reaching task. The subject was trained to perform a simple touchscreen-based reaching task where a correct choice was associated with a reward. The target was moved in varying proportions during the reach, generating a sensory prediction error, and we observed differential firing patterns of task-responsive neurons during trials with a high proportion of moving targets. We also performed focal microstimulation of the DN with simultaneous high-density recording of striatal and DN neurons, finding modulation of striatal neurons similar to that seen in rodent studies. Similarly, we report the results of focal microstimulation of the STN with simultaneous high density neural recordings of the cerebellar cortex and DN. These results provide important mechanistic insights into the influences of the basal ganglia and cerebellum on each other during reaching as well as insight into the mechanisms of action of STN and DN DBS.

Disclosures: K.R. Lehner: None. **K.E. Cullen:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.09/FF18

Topic: E.02. Cerebellum

Support: R01MH093727
R01NS112917

Title: Control of self-timed movement initiation via sequential activation of neurons in prefrontal cortex and cerebellum

Authors: *M. V. MONAKHOV, J. F. MEDINA;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Making the right movement at the right time requires precise coordination of neural activity in multiple brain areas. For self-timed motor responses that need to be precisely triggered without the help of external sensory cues, an important step in this process involves the generation of an internal ‘go’ signal, which must be quickly relayed to brain centers responsible for movement initiation. It has been challenging to disentangle the neural sources of the internal ‘go’ and movement initiation signals because the two signals are separated by short delays and may partially overlap in time and space. Here, we trained mice to make a self-timed motor response in an omitted oddball task, and subsequently used brief (35 ms) photoinhibition of neurons in two brain regions to define their relative contributions at different times during the ‘go’—init period. Our results provide support for a fuse-detonator model in which the self-timed movement is triggered via sequential activation of ‘go’ neurons in the dorsomedial prefrontal cortex (dmPFC) followed by ‘init’ neurons in the dentate nucleus of the cerebellum (DN). This

type of serial signal processing may allow the brain to initiate movements quickly and with high temporal precision in the absence of external sensory cues.

Disclosures: M.V. Monakhov: None. **J.F. Medina:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.10/FF19

Topic: E.02. Cerebellum

Support: Simons Collaboration on the Global Brain
McKnight Foundation
NIH RF1 NS132025
NIH R01 NS113110
NIH R01 NS131229
NIH R01 NS112312

Title: A cerebello-thalamo-cortical pathway drives ramp-to-threshold activity in frontal cortex

Authors: *R. GAO, J. ZHU, L. LIU, H. KAKU, N. LI;
Baylor Col. of Med., Houston, TX

Abstract: Before volitional actions, neurons in frontal cortex exhibit ramping activity that reaches a consistent threshold before movement onset. This ramp-to-threshold dynamic has been observed across various behavioral tasks, brain regions, and species, but its neural circuit substrate remains unknown. We used Neuropixels probes to map ramping activity in frontal cortex and thalamus preceding volitional licking in mice. In a delayed response task, mice measured the position of a pole using their whiskers (anterior or posterior) during a sample epoch and reported their decision after a 'Go' cue by licking one of two lick ports (left or right). A delay epoch of fixed duration (1.3 s) separated the sample epoch and the 'Go' cue. We used two Neuropixels probes to simultaneously record activity in left frontal cortex and left thalamus. The probe tracks and recorded units were reconstructed in Allen Mouse Brain Common Coordinate Framework (Liu et al., eNeuro 2021). In both frontal cortex and thalamus, a subset of neurons exhibited consistent increase or decrease in activity during the delay epoch that reached a peak before the 'Go' cue. In frontal cortex, ramping activity was enriched in anterior lateral motor cortex (ALM). In thalamus, ramping activity was enriched in cerebellar-recipient thalamus, including parts of the ventral-medial nucleus (VM), ventral-anterior-lateral nucleus (VAL), paracentral nucleus (PCN), and central lateral nucleus (CL). The same thalamic regions were also anatomically coupled with ALM, forming a cerebello-thalamo-cortical pathway. To examine cerebellar contribution to ramping activity, we photostimulated the right cerebellar cortex in L7-cre X Ai32 mice that expressed ChR2 in Purkinje cells, thereby inhibiting cerebellar nuclei. Photostimulation of the cerebellar regions that reciprocally connect to ALM, crus 1/2 and

lobule 7 (Zhu, Hasanbegović et al., 2023), collapsed ramping activity in the thalamus. After a transient photostimulation in the first 500 ms of the delay epoch, subsequent ramping activity did not recover. Transient cerebellar photoinhibition thus 'paused' ramping activity. Behaviorally, photoinhibition increased licking reaction time (RT, 195ms vs 168 ms, photoinhibition vs control trials, $p<0.05$, paired t-test, n=8). In contrast, photostimulation of the cerebellar regions that do not reciprocally connect with ALM (lobule 4/5 and lateral simplex) did not affect ramping activity in the thalamus. Together, these results suggest that the ramp-to-threshold dynamic preceding volitional action is supported by a cerebello-thalamo-cortical pathway.

Disclosures: R. Gao: None. **J. Zhu:** None. **L. Liu:** None. **H. Kaku:** None. **N. Li:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.11/FF20

Topic: E.02. Cerebellum

Support: NIH NS113110
NIH NS112312
NIH NS131229
NIH NS132025
Simons Collaboration on the Global Brain
McKnight Foundation

Title: Functional connectivity of a cortico-cerebellar loop underlying motor planning

Authors: *J. ZHU, R. GAO, N. LI;
Baylor Col. of Med., Houston, TX

Abstract: The neocortex and cerebellum interact to mediate cognitive functions (Ito 2008; Schmahmann et al., 2019). It remains unknown how the two structures organize into functional networks to mediate specific behaviors. In mice planning directional licking based on short-term memory, preparatory activity instructing future movement depends on anterior lateral motor cortex (ALM) and the cerebellum (Gao et al., 2018). In previous work, we found that the cerebellar regions with conjunctive input-output connectivity with ALM selectively formed a functional closed loop with ALM to support motor planning of directional licking, whereas other cerebellar regions contributed little to motor planning despite input or output connectivity to ALM (Zhu, Hasanbegović, et al., 2023). Preparatory activity was selectively enriched in ALM and cerebellar conjunction regions. Here we examine the functional connectivity of cerebellar conjunction regions with ALM and how the specificity of ALM-cerebellar closed loop is established. First, we mapped functional connectivity from ALM to cerebellar cortex using ChR2 photoactivation of ALM layer 5b pyramidal-tract (PT) neurons innervating the pons while recording from distinct cerebellar regions (Sim1_KJ18-cre x Ai32 mice). Passive ALM

photoactivation readily evoked spikes in cerebellar regions innervated by mossy fibers arising from the ALM-recipient pontine nuclei. The light-evoked activity was correlated with the density of mossy fiber terminals, with the strongest activity observed outside of the conjunction regions. Next, we examined how distinct cerebellar regions were functionally coupled to ALM. We photostimulated Purkinje cells expressing ChR2 while recording from ALM (L7-cre x Ai32 and L7-cre x GtACR mice). Photostimulating distinct cerebellar regions produced activity changes in ALM, even in cerebellar regions that do not provide direct output to ALM. These light-induced activity changes suggest that broad regions of the cerebellum could influence ALM activity through either direct or indirect pathways. Yet, during motor planning, only perturbations in cerebellar conjunction regions affected preparatory activity in ALM and subsequent movements. These functional connectivity measurements failed to explain selective coupling between ALM and cerebellar conjunction regions. We hypothesize that a gating mechanism attenuates the flow of task-related information outside of the ALM-cerebellar functional network, while reciprocal communication between ALM and cerebellar conjunction regions maintain preparatory activity.

Disclosures: J. Zhu: None. **R. Gao:** None. **N. Li:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.12/FF21

Topic: E.02. Cerebellum

Support: NIH R15MH106957
NIH R15MH126404
DoD W81XWH-19-1-0249

Title: Different cortical networks are modulated by lateral vs. midline cerebellar tDCS

Authors: *J. DUST[1], L. C. RICE[3], M. M. LEE[4], M. R. RUBIN[1], M. PAKNEJAD[1], H. YOUNESIE[1], D. C. GAWLITZEK[1], B. D. BAUCOM[1], E. M. BARNES[1], C. J. STOODLEY[2]; [1]Dept. of Neurosci., [2]American Univ., American Univ., Washington, DC; [3]Kennedy Krieger Inst., Baltimore, MD; [4]Ctr. for Applied Brain and Cognitive Sciences, Tufts Univ., Tufts Univ., Medford, MA

Abstract: The cerebellum is engaged during a wide range of behaviors, from motor learning to working memory. The cerebellum supports these diverse functions via its vast interconnections with the cerebral cortex, and different functional subregions of the cerebellum emerge based on cerebro-cerebellar connectivity patterns. Evidence from preclinical studies indicates that the cerebellum modulates the coherence of functional networks in the cortex in a location-specific manner. We examined whether neuromodulation of right posterolateral cerebellar lobule VII (RVII) vs. the posterior midline differentially impacts resting-state functional connectivity (FC) in humans. We predicted that transcranial direct current stimulation (tDCS) targeting RVII

would affect FC in the interconnected fronto-parietal network (FPN) and posterior midline tDCS would impact default mode network (DMN) regions. Twenty-two young adults (mean 21±2.4 years, sex assigned at birth: 19 male, 3 female) received 20min of 1.5mA excitatory (anodal) or sham tDCS in separate neuroimaging sessions. A 7min resting-state fMRI scan was acquired following tDCS. Fourteen participants received tDCS targeting RVII while 8 received tDCS targeting the posterior midline. Multivariate pattern analysis (MVPA; CONN version 20b) was employed to compare post-anodal vs. post-sham FC using a within-subjects design. Results were thresholded at p<.005 with a False Discovery Rate (FDR) p<.05 cluster-level correction. The MVPA results were entered into *post hoc* seed-based analyses to further evaluate FC changes. Preliminary analyses were conducted within each group (RVII, posterior midline) separately to evaluate whether modulation of diverse cerebellar subregions differentially altered whole-brain FC. Results revealed that posterior midline tDCS modulated FC from the medial frontal pole, part of the DMN, while tDCS targeting RVII altered FC in lateral frontal regions that are part of fronto-parietal cognitive control networks (FPN). *Post hoc* analyses showed that posterior midline tDCS altered FC between the medial frontal pole (DMN) and the medial superior parietal lobule (DMN; T[7]=-5.93, p-FDR<.001) of the dorsal attention network. Modulation of RVII changed FC between the left lateral frontal pole (FPN) and precuneus (DMN; T[13]=-8.89, p-FDR<.001). In both cases, there was an increase in the level of anti-correlations between these regions. These findings indicate that neuromodulation of the lateral vs. posterior medial cerebellum alters FC in different cortical networks, consistent with the hypothesis that the cerebellum acts to coordinate communication between distant cortical regions.

Disclosures: J. Dust: None. **L.C. Rice:** None. **M.M. Lee:** None. **M.R. Rubin:** None. **M. Paknejad:** None. **H. Younesie:** None. **D.C. Gawlitzek:** None. **B.D. Baucom:** None. **E.M. Barnes:** None. **C.J. Stoodley:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.13/FF22

Topic: E.02. Cerebellum

Title: Reward modulation of brain-wide circuitry underlying motor learning and skilled performance

Authors: *X. GOFMAN[1], J. N. INGRAM[1], A. LAWEN[1], Y. KE[2], T. TABACHNIK[1], D. WOLPERT[1], A. JASANOFF[2], I. KAHN[1];
[1]Zuckerman Inst., Columbia Univ., New York, NY; [2]Dept. of Biol. Engin., MIT, CAMBRIDGE, MA

Abstract: The consensus view of motor learning emphasizes the distinct roles played by the motor cortex, basal ganglia, and cerebellum. The basal ganglia are associated with reinforcing goal-directed actions, while the cerebellum refines actions through supervised learning. Recent

findings challenge this separation by observing reward signals in the cerebellum. Understanding how reward dynamically influences activity within and between these motor circuits remains incomplete. Our study aims to investigate the interactions between the motor cortex, basal ganglia, and cerebellum and identify other regions involved in motor learning and performance by utilizing high-field fMRI and an MRI-compatible motor behavior paradigm in mice. Mice are trained to manipulate an isometric joystick, producing forepaw push and pull forces to obtain a reward. The training involves incremental steps with rewards given for successful controlled force application. As training progresses, force threshold and time-related parameters are adjusted. After achieving stable performance, an adaptation phase is introduced where the mice are required to learn to apply the force in the opposite direction. This phase relies on post-action feedback and reward evaluation to modify subsequent performance. The mice are scanned daily throughout the training period to identify specific signatures associated with actions and their dynamics during different stages of motor learning, allowing monitoring of the brain-wide responses from the naïve to expert states. The experimental design enables us to manipulate performance to identify distinct behavioral events, distinguish forelimb- from reward consumption-related responses, and equivalent rewarded forelimb actions (occurring within a trial) versus unrewarded forelimb actions (occurring during the ITI). Preliminary data of fMRI activation maps for a single forelimb motor action, both rewarded and non-rewarded, point toward motor-related regions, including the posterior motor cortex, somatosensory cortex, areas within the striatum, nucleus accumbens, anterior cingulate cortex, posterior parietal cortex, and cerebellum. While most brain regions display similar activation maps between the two event types, our preliminary analyses reveal differences in fMRI signal changes in several regions, including nucleus accumbens and motor cortex. Our goal is to employ fMRI as a screening tool to identify regions of interest in the context of motor learning and measure direct cellular activity from these regions to study the brain-wide processes underlying motor learning and its modulation by reward.

Disclosures: **X. Gofman:** None. **J.N. Ingram:** None. **A. Lawen:** None. **Y. Ke:** None. **T. Tabachnik:** None. **D. Wolpert:** None. **A. Jasanoff:** None. **I. Kahn:** None.

Poster

PSTR483. Cerebellum: Interactions with Other Brain Areas

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR483.14/FF23

Topic: H.10. Human Learning and Cognition

Support: Italian Ministry of Research PRIN 20208RB4N9_004

Title: Effective connectivity between cerebellum and contralateral parietal and prefrontal cortex during observation and imitation of complex bimanual action sequences

Authors: *A. ERRANTE[1,2], S. ZICCARELLI[1], C. RUSSO[1], L. FOGASSI[1];
[1]Dept. of Med. and Surgery, Univ. of Parma, Parma, Italy; [2]Neuroradiology unit, Univ. Hosp. of Parma, Parma, Italy

Abstract: Humans and monkey studies have shown that specific sectors of the cerebellum activate not only during the execution but also during the observation of hand actions, indicating its involvement in the Action Observation Network (AON). However, the role of the cerebellum in the imitation of unimanual and bimanual actions has not been deeply investigated. To address this issue, in the present study, healthy subjects were involved in an event-related fMRI task during which they had to observe bimanual action sequences (Origami-like paper folding) with the aim of imitating them, followed by the actual imitation of the previously observed sequences (Imitation condition). Control conditions included: (a) observation of bimanual action sequences, (b) observation of bimanual simple reaching movements, (c) observation of bimanual action sequences followed by execution of a free-choice bimanual sequence (Non-imitation condition), and (d) observation of videos showing natural scenarios followed by the execution of a free-choice bimanual action sequence. Participants' imitation performance was recorded, during scanning, by means of MR-compatible cameras, in order to calculate accuracy scores. Regression analyses were performed to investigate the possible relation between imitation scores and degree of activation of cortical areas and cerebellum. Furthermore, ROI-to-ROI and Seed-to-Voxels generalized psychophysiological interaction (gPPI) analyses were carried out, to investigate the effective connectivity between cerebellar and cortical areas during the imitation task. The results showed that: a) observation of action sequences during Imitation condition activate stronger parietal, premotor and prefrontal cortex, as well as lateral cerebellum, as compared to control observation conditions; b) the activation of intraparietal sulcus (IPS), ventral premotor cortex (PMv), dorsolateral prefrontal cortex (DLPFC) and cerebellar lobule VI is correlated with individual imitative performance accuracy; c) effective connectivity between cerebellum and IPS, and between IPS and DLPFC during observation to imitate predicts the subsequent imitative performance. These findings confirm the role of cerebellum in the internal simulation of observed action sequences, which allows, in concert with parietal and prefrontal cortex, to accurately imitate the observed model. From a translational clinical perspective, the knowledge of these cortico-cerebellar mechanisms could be crucial for imitation based improvement of motor functions in adult and pediatric patients with neurological disorders.

Disclosures: A. Errante: None. **S. Ziccarelli:** None. **C. Russo:** None. **L. Fogassi:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.01/FF24

Topic: E.03. Basal Ganglia

Support: Howard University BFPSAP grant (X.Z)
Karen Toffler Charitable Fund (X.Z)

Title: Harmaline toxicity on dorsal striatal neurons and its role in tremor

Authors: X. ZHAN, L. V. DO, L. ZOU, R. S. ZHAN, M. JONES, Jr., S. NAWAZ, *K. MANAYE;
Physiol. and Biophysics, Howard Univ. Col. of Med., Washington, DC

Abstract: Harmaline is one of the β-carboline derivative compounds that is widely distributed in the food chain and human tissues. Harmine, a dehydrogenated form of harmaline, appeared to have a higher concentration in the brain, and appear to be elevated in essential tremor (ET) and Parkinson's disease. Exogenous harmaline exposure in high concentration has myriad consequences, including inducing tremor, and causing neurodegeneration of Purkinje cells in the cerebellum. Harmaline-induced tremor is an established animal model for human ET, but its underlying mechanism is still controversial. One hypothesis posits that the inferior olive-cerebellum pathway is involved, and CaV3.1 T-type Ca^{2+} channel is a critical target of action. However, other accumulating evidence indicates that tremor can be generated without disturbing T-type channels. This implicates that additional neural circuits or molecular targets are involved. Using *in vitro* slice Ca^{2+}-imaging and patch clamping, we demonstrated that harmaline reduced intracellular Ca^{2+} and suppressed depolarization-induced spiking activity of medium spiny striatal neurons (MSN), and this effect of harmaline can be partially attenuated by sulpiride (50 μM). In addition, the frequency of spontaneous excitatory post-synaptic currents (sEPSCs) on MSNs were also significantly attenuated. Furthermore, the induced tremor in C57/BL6 mice by harmaline injections (i.p. 12.5 - 18 mg/kg) was also shown to be attenuated by sulpiride (20 mg/kg). This series of experiments suggests the dorsal striatum is a site of harmaline toxic action and might contribute to tremor generation. The findings also provide evidence that D2 signaling might be a part of the mechanism underlying essential tremor.

Disclosures: X. Zhan: None. **L.V. Do:** None. **L. Zou:** None. **R.S. Zhan:** None. **M. Jones:** None. **S. Nawaz:** None. **K. Manaye:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.02/FF25

Topic: E.03. Basal Ganglia

Title: Lambda-cyhalothrin exposure impairs DA-D2 mediated AKT/GSK-3β signaling and induces histological and ultrastructural changes in the nigrostriatal region

Authors: *A. KUMARI, A. SRIVASTAVA, V. K. KHANNA;
CSIR - Indian Inst. of Toxicology Res., Lucknow, India

Abstract: Introduction The sensitivity of the striatal dopaminergic system to lambda-cyhalothrin (LCT); a new generation type II synthetic pyrethroid pesticide, was earlier reported by us. Continuing the leads, the present study has been carried out to understand the involvement

of AKT/GSK-3β signaling in an attempt to identify molecular targets involved in LCT induced motor deficits. Further, histological and ultrastructural changes were also examined in the nigrostriatal region. **Materials and Method** Adult male *Wistar* rats (180±20gm) were obtained from CSIR-IITR, Lucknow and divided into four treatment groups. Rats in three groups were treated with LCT at any of the doses (0.5 or 1.0 or 3.0 mg/kg body weight, p.o.) for 45 days while fourth group animals were given corn oil identically and served as control. 24h after last dosing, rats were sacrificed, brains were removed and dissected to isolate substantia nigra and corpus striatum and processed for immunoblotting, histological and ultrastructural studies. A separate set of rats were used for behavioral studies. **Results** Rats treated with LCT exhibited decrease in motor activity along with decrease in the levels of dopamine (DA) receptor D2, p-AKT and p-GSK-3β and increase in β-arrestin1/2 in substantia nigra and corpus striatum as compared to controls. Further, decrease in the levels of dopamine and increase in DA turnover was also observed in LCT treated rats as compared to controls. These biochemical changes were associated histological alterations evident by the presence of degenerating pyknotic neurons with less Nissl substance in SNpc and corpus striatum of LCT treated rats. Ultrastructural examination of each region also visualized presence of degenerating neurons with irregular shape and highly electrodense cytoplasm containing swollen mitochondria, dilated ER-golgi network and damaged myelin sheath both in substantia nigra and corpus striatum of LCT treated rats. Degenerating synapses with fewer synaptic vesicles in presynaptic membrane, thickened PSD and blurred synaptic cleft were also visible in LCT treated rats. The presence of activated microglia with condensed nuclei and enlarged tertiary lysosomes and reactive astrocytes were also visualized in substantia nigra and corpus striatum of LCT treated rats as compared to controls. **Conclusion** Results indicate that LCT alters AKT/GSK-3β signaling and induces histological & ultrastructural changes in the nigrostriatal region of rats and these changes are associated with motor dysfunctions.

Disclosures: **A. Kumari:** None. **A. Srivastava:** None. **V.K. Khanna:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.03/GG1

Topic: E.03. Basal Ganglia

Title: The influence of anesthesia on dopamine signaling in the rat dorsal striatum

Authors: *G. E. ROSENBAUM[1], D. E. GINDER[4], M. P. GAINER[2], D. P. DABERKOW[3]; [1]Biol., [3]Eastern Washington Univ., [2]Eastern Washington Univ., Cheney, WA; [4]Washington State Univ., Washington State Univ., Pullman, WA

Abstract: The striatum is highly innervated by dopamine (DA) and is involved in reward learning and movement control. Fast-scan cyclic voltammetry (FSCV) is a technique that uses microelectrodes (diameter ~5 µm, length ~150 µm) to monitor DA signaling in the brain. FSCV

studies with anesthetized rodents commonly use urethane or isoflurane anesthesia; however, the possible influence of isoflurane anesthesia on DA signaling has not been thoroughly investigated. Male Sprague-Dawley rats (*rattus norvegicus*) 300-500 grams were anesthetized with urethane or isoflurane anesthesia. Once fully anesthetized, rats were secured in a stereotaxic apparatus where their skin and fascia were removed to allow for the drilling of small holes (1-2 mm in diameter) for electrode placement. The reference electrode, coated with Ag/AgCl, was placed just below dura, the FSCV electrode was placed in the dorsal striatum (+1.2 AP, +2.0 ML, -5.0 DV), and the stimulating electrode was placed above the medial forebrain bundle (-4.6 AP, +1.4 ML, -7.5 DV). Biphasic pulses (60 Hz, 60 pulses, 300 µA) were sent through the bipolar stimulating electrode to evoke DA release. Once consistent DA signals were observed, DA signals were stimulated and recorded for 1 hour after an intraperitoneal injection of saline. Preliminary data suggest an attenuation of the DA signals under isoflurane anesthesia when rat body temperature was not kept stable. Conversely, under urethane anesthesia, when temperature was carefully monitored and kept stable, DA signals remain relatively stable. Future directions involve carefully monitoring and keeping rat temperature stable under isoflurane anesthesia while monitoring DA with FSCV.

Disclosures: G.E. Rosenbaum: None. **D.E. Ginder:** None. **M.P. Gainer:** None. **D.P. Daberkow:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.04/Web Only

Topic: E.03. Basal Ganglia

Title: Subthalamic-pallidal beta coherence and gamma entrainment: Assessing STN-GP connectivity in Parkinson's Disease

Authors: *D. D. CUMMINS[1], S. S. SANDOVAL-PISTORIUS[2], S. CERNERA[2], R. FERNANDEZ[2], L. H. HAMMER[2], P. A. STARR[3];
[1]Mount Sinai Hlth. Syst., New York, NY; [2]Univ. of California San Francisco, San Francisco, CA; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Local field potential (LFP) recordings of the subthalamic nucleus (STN) and globus pallidus (GP) in Parkinson's Disease (PD) have revealed electrophysiological biomarkers that correlate with symptom states. Beta oscillations (13-30Hz) correlate with bradykinesia, while gamma oscillations (>40Hz) often correlate with pro-kinetic on-treatment states such as on levodopa and/or deep brain stimulation (DBS). Dual-target DBS, where electrodes are implanted in both the STN and GP, has enabled electrophysiological studies of STN-GP connectivity in humans, though this area of research remains largely unexplored. We assessed the effects of levodopa and DBS on basal ganglia electrophysiology in one PD patient with STN and GP leads connected to bilateral Percept PC DBS systems. We performed at-home simultaneous STN and

GP LFP recordings while ON and OFF levodopa. Power spectra and STN-GP magnitude-squared coherence were determined ON- and OFF-levodopa, and with STN-DBS, GP-DBS, and dual-target STN-GP-DBS.OFF-levodopa and OFF-DBS, a beta peak was present at bilateral STN and GP, coincident with prominent STN-GP beta coherence. Levodopa completely suppressed bilateral GP beta activity and suppressed STN-GP coherence, while decreasing STN beta. Levodopa suppressed low gamma activity (45-60Hz) at bilateral STN in the OFF-DBS state. Both OFF-levodopa and ON-levodopa, STN beta power was reduced with DBS at STN, while GP beta power was reduced by DBS at GP. The same reduction in beta power was not seen across targets (DBS at STN did not reduce GP beta power, or vice-versa).Finely tuned gamma (FTG) activity at half the stimulation frequency (62.5Hz) was seen in the STN during GP-DBS, ON- and OFF-levodopa. To assess the effects of movement on FTG activity, we recorded LFPs during instructed movement. We observed FTG activity in bilateral GP and STN during contralateral body movements while on GP-DBS and ON-levodopa. No FTG was seen with STN-DBS or STN-GP-DBS.In a rare opportunity to study STN-GP connectivity in a human patient, we used a commercially available bidirectional DBS system to show STN-GP beta coherence that is suppressed on levodopa, and GP-DBS and movement-induced gamma entrainment in the basal ganglia. Suppression of STN-GP beta coherence ON-levodopa agrees with early studies in the peri-operative setting, while showing this can be recorded chronically with modern commercial DBS systems. Ongoing work seeks to expand this study to additional individuals with PD receiving dual-target DBS.

Disclosures: D.D. Cummins: None. **S.S. Sandoval-Pistorius:** None. **S. Cernera:** None. **R. Fernandez:** None. **L.H. Hammer:** None. **P.A. Starr:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.05/GG2

Topic: E.03. Basal Ganglia

Support: NIH/NINDS R01 NS107734

Title: Differential role of context in long-term rescue of parkinsonian motor deficits by cell-specific pallidal stimulation vs. levodopa

Authors: *T. CHEUNG[1,2], G. SAMADJOPOULOS[2], A. KRISHNAN[3], U. KANG[2]; [1]NYU Langone Hlth., New York, NY; [2]Neurol. and Neurosci. Inst., New York Univ. Grossman Sch. of Med., New York, NY; [3]New York Univ., New York, NY

Abstract: In Parkinson's disease, degeneration of dopaminergic neurons leads to profound motor impairment including slowness of movement. Although treatments with the dopamine precursor levodopa (L-DOPA) dramatically improve motor symptoms, over time patients experience disabling motor fluctuations, including shortened therapeutic duration of action of L-

DOPA. Emergence of disabling motor fluctuation is associated with the decline of a component of L-DOPA's antiparkinsonian response, known as the long duration response (LDR). The LDR is a long-lasting motor improvement that builds up over days of L-DOPA treatment and persists long after L-DOPA plasma level has returned to baseline, gradually decaying over many days to weeks after discontinuation of L-DOPA. Despite LDR clinically accounting for 60-65% of the total motor benefits from L-DOPA and potentially countering motor fluctuation, LDR's mechanism remains unknown. Using a dual-task animal model of LDR, we recently showed that both L-DOPA-induced LDR and its decay involve experience-dependent and task-specific learning mediated by striatal projection neurons (Cheung et al., *PNAS* 2023). Here, we show that repeated optogenetic stimulation of parvalbumin-expressing neurons in the external globus pallidus (GPe PV^+ neurons) - part of the striatopallidal pathway - induced locomotion LDR, whereby locomotion deficits were rescued even when mice were tested in a later session without stimulation. Furthermore, we show that repeated pairings of L-DOPA with a specific open field also induced locomotion LDR. L-DOPA-induced LDR was evident in the paired open field, but not in a different, unpaired open field. Surprisingly, unlike LDR induced by L-DOPA, locomotion LDR induced by GPe PV^+ neuron stimulation generalized to the unpaired open field. Therefore, while locomotion LDR induced by L-DOPA was context selective, LDR induced by GPe PV^+ neuron stimulation was not. These results demonstrate the importance of context-dependent learning in LDR induced by dopaminergic treatment. Additionally, these results uncover previously unexplored effects of GPe deep brain stimulation, which may be both be beneficial (long-term motor rescue across different contexts) and detrimental (reduced context discrimination).

Disclosures: **T. Cheung:** None. **G. Samadjopoulos:** None. **A. Krishnan:** None. **U. Kang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amprion. F. Consulting Fees (e.g., advisory boards); NurrOn Pharmaceuticals Inc., UCB Biopharma SRL.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.06/GG3

Topic: E.03. Basal Ganglia

Support: NIH Grant R01MH121099
NIH Grant R01DA048096
NIH Grant R01MH124115
NIH Grant 5KL2TR001420

Title: Subsecond Changes in Dopamine Levels in the Putamen Anticipate Willful Action in Humans

Authors: *A. KOLAHI SOHRABI[1], A. JIANG[2], B. LIEBENOW[3], E. K. DIMARCO[3], A. W. LAXTON[4], S. B. TATTER[4], K. T. KISHIDA[1,2,4,3];
[1]Virginia Tech-Wake Forest Univ. Sch. of Biomed. Engin. and Sci., Blacksburg, VA; [2]Dept. of Physiol. and Pharmacol., [3]Neurosci. Grad. Program, [4]Dept. of Neurosurg., Wake Forest Univ. Sch. of Med., Winston Salem, NC

Abstract: The precise relationship between subsecond changes in nigrostriatal dopamine (DA) signals and movement is largely unexplored in humans. Characterization of this relationship may aid in the treatment of movement disorders, such as Parkinson's disease (PD) and related movement disorders. Recently, Bang et al. (2020) showed that subsecond DA fluctuations in human putamen increased prior to action initiation (i.e., a button press indicating a choice) in a manner that scaled with reaction time. Here, we applied similar methods to study the relationship between DA in human putamen and action onset in patients with PD. We hypothesized that DA in the human putamen will track action initiation recorded as a button press in a probabilistic decision-making task. To test this hypothesis, we performed fast-scan cyclic voltammetry in humans, as previously reported by our lab, in patients with PD who underwent deep brain stimulation electrode implantation surgery with the globus pallidus interna as the DBS target. This DBS electrode trajectory permits human voltammetry recording in the putamen. DA measurements were recorded while subjects (n=3) played a probabilistic reward and punishment task (Sands et al., 2023) where participants indicated their choices using button presses. DA timeseries data were measured with 10Hz frequency and during analysis were time-locked to the onset of task stimulus presentations that preceded button presses. Statistical significance ($p<0.05$) for z-scored DA concentration changes at each time point was then determined using a 1-sample t-test against a DA concentration change of zero. Reaction time distributions (RT: time between stimulus presentation and button press) were plotted against the average DA timeseries for each subject. Across the three participants' data, we observed heterogeneity in the relationship of dopamine to action initiation. In two of the participants there appears to be a significant ramping of dopamine leading into the peak of the RT distribution, but the quality of this rise is not the same in both participants. In the third participant, DA levels do not significantly rise leading into the peak of the RT distribution. Our results are consistent with the hypothesis that subsecond changes in DA levels in the putamen anticipate movement but may do so in a manner that reflects various individual specific factors including clinical diagnosis, symptom severity, or variations in the specific electrode placement. Future analyses will test these hypotheses to determine whether heterogeneity in PD symptoms may be reflected in the ability of subsecond changes in DA levels to anticipate action in humans.

Disclosures: A. Kolahi Sohrabi: None. **A. Jiang:** None. **B. Liebenow:** None. **E.K. DiMarco:** None. **A.W. Laxton:** None. **S.B. Tatter:** None. **K.T. Kishida:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.07/GG4

Topic: E.03. Basal Ganglia

Support: KAKENHI JP (20H0354920)
KAKENHI JP (20K20671)
KAKENHI JP ("Hyper-Adaptability" 20H05484)
KAKENHI JP ("Adaptation Circuit Census" 21H05241)

Title: Anterograde trans-synaptic visualization of the striatal neurons innervated by dopaminergic neurons in the substantia nigra pars compacta in mice

Authors: *F. KARUBE, Y. YANG, F. FUJIYAMA;
Hokkaido Univ., Sapporo, Japan

Abstract: Recently, large diversity of dopaminergic (DA) neurons in the mesencephalon has been reported using gene expression and neural tracing analyses. As similar to other brain regions, molecular diversity of substantia nigra par compacta (SNc) DA neurons are correlated with topographical projection to the striatum. In another aspect, DA release is known to be volume transmission, whereas around 40% of axon terminals are estimated as ones with synaptic structure. It is yet uncovered whether diversity of DA neurons relates to innervation patterns on the striatal neuron types. To uncover this question, we examined anterograde trans-synaptic adeno-associated viral vector serotype 1 (AAV1) to investigate whether postsynaptic neurons receiving DA innervation are labeled. After AAV1-hSyn-Cre injection to SNc, expression of Cre was observed in the striatal neurons, both interneurons as well as medium spiny neurons (MSNs). Next, one week after AAV1 injection to SNc, Cre-driven AAVs expressing RFP/GFP were injected into the SNc and striatum. As the result, dense axonal arbors and neurons expressed fluorophores in the striatum. Using immunofluorescent staining for several transporters of neurotransmitters, almost all of axons expressed dopamine transporter (DAT), indicating their dopaminergic nature. Cre-driven fluorophore expression visualized striatal neurons containing both MSNs and interneurons. Labeled interneurons co-expressed one of interneuron markers. Choline acetyl transferase (ChAT) or parvalbumin expressing interneurons were more frequently expressed Cre than others. The total proportion of interneurons labeled by the above method reached more than 20% of all labeled neurons. Thus, it will be possible that trans-synaptic labeling can be dominant than retrograde labeling of MSNs. Subcellular localization of appositions of DAT-expressing axonal boutons on the labeled striatal neurons was analyzed. In some interneurons, including cholinergic neurons, a single DAT-expressing axons formed multiple appositions on the soma and proximal dendrites. It suggests that irrespective of diverse and dense axonal arborization of a single SNc DA neurons, a small number of DA neurons could have large effect on single striatal neurons.

Disclosures: F. Karube: None. **Y. Yang:** None. **F. Fujiyama:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.08/GG5

Topic: E.03. Basal Ganglia

Title: Comparison of unilateral and bilateral theta burst deep brain stimulation

Authors: *Y. SUN[1], J. NATARAJ[3], S. SEYYEDMOUSAVI[2], T. D. SANGER[4];
[1]Univ. of California Irvine, Mission Viejo, CA; [2]Univ. of California Irvine, Irvine, CA; [3]EECS, Univ. of California, Irvine, Irvine, CA; [4]Electrical Engin., UCI, Redondo Beach, CA

Abstract: Deep brain stimulation (DBS) is a neuromodulatory intervention that is utilized to treat movement disorders such as Parkinson's disease and pediatric dystonia. DBS typically involves the delivery of continuous pulse trains to bilateral targets, which is thought to attenuate signal abnormalities in motor circuits, though the mechanism of action is currently unknown. However, there is some evidence that intermittent DBS (iDBS) may provide the same or increased treatment efficacy, while reducing the occurrence or severity of side effects in patients with incomplete response to continuous DBS. Theta burst stimulation (TBS) is a type of iDBS that combines periods of 100 msec pulses between 50 to 250 Hz and periods of 100 msec off to form a 5 Hz burst. Previous studies reported theta-burst deep brain stimulation on basal ganglia (BG) and/or thalamus generated better treatment on a subset of clinic patients who have movement disorders like childhood dystonia with fewer side effects. This study aims to further understand the different treatment performances of unilateral TBS and bilateral TBS by analyzing intercranial recorded signal. This study investigates the effects of TBS with dystonic children underdoing a staged DBS procedure with temporary depth electrodes implanted in the basal ganglia and various thalamic nuclei. After one week of testing and recording in an inpatient neuromodulation unit to determine optimal DBS targets and settings for continuous DBS, the electrodes are then removed. Frequencies of TBS pules is the same or close to frequencies of best clinic continuous DBS setting, which depend on different patients and brain areas. The intercranial recorded signal collected from the temporary stimulation can help to determine the modulation of brain activity during different DBS settings. Based on the evoked potentials' comparison results, the evoked potential patterns, which are detected above the stimulation frequencies, vary not only with the simulation mode but also with the simulation voltage. However, the best clinic setting did not generate the greatest electrophysiological effect. Meanwhile, the responses of BG and Thalamic to unilateral TBS and bilateral TBS in a long-time scale were observed that bilateral TBS produced different response signals in the low frequency region than unilateral TBS. The best clinic setting might not generate the largest evoked potentials in target areas. Compared with unilateral TBS, bilateral TBS yielded better treatment results, which may be due to the low frequency response signal.

Disclosures: Y. Sun: None. **J. Nataraj:** None. **S. Seyyedmousavi:** None. **T.D. Sanger:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.09/GG6

Topic: E.03. Basal Ganglia

Support: Augusta University

Title: Regional manipulation of striatal circuit shapes motor learning

Authors: *C. CRYAN[1], B. MUNTEAN[2];
[1]Augusta Univ. Neurosci. Grad. Program, Augusta, GA; [2]Med. Col. of Georgia at Augusta Univ., Augusta, GA

Abstract: Motor learning requires repeated trials to make a task autonomous. The dorsal striatum, which divides into dorsomedial (DMS) and dorsolateral (DLS) regions, plays an essential role in initiating changes in motor function. Both dorsal regions play a role in coordination of movements and are modulated by dopamine signaling on direct or indirect medium spiny neurons (dMSNs and iMSNs, respectively). While DMS is thought to be important in early phases of learning and DLS in later phases, the overlap in function of these regions has yet to be fully uncovered. Moreover, despite a relatively extensive understanding of dopamine release properties, the interrogation of dopamine signals to downstream second messengers by dorsal MSNs remains in its infancy. Here, we tackled both issues by recording dopamine signaling in acute brain slices and applying chemogenetics to probe striatal circuitry in mice during motor learning. A D2-cAMPer-cre mouse was used for brain slice experiments, allowing real-time recording of the circuitry. We observed striking differences in iMSN dopamine signaling between DMS and DLS regions. The data suggests motor learning requires differential decoding of dopamine between the dorsal regions. We therefore hypothesized that regional modulation of dopamine signal integration, via chemogenetics, would modify motor learning. We performed stereotaxic injections of a Cre-dependent inhibitory DREADD (AAV-hSyn-DIO-hM4D(Gi)-mCherry) in the dorsal striatum of A2a-Cre mice, which selectively expresses Cre in iMSNs. Two cohorts were used, one injected bilaterally in DLS, one injected with one hemisphere DMS and one hemisphere DLS. Using the rotarod to test striatal-dependent motor learning, we observed variances in learning curves which reveal unique contributions of DMS and DLS. Inhibition of both DMS and DLS appear to decrease learning, where DLS inhibition does not alter learning. Combining the data from acute slices and behavior, our results demonstrate the interplay between dorsal striatal regions in learning and maintaining motor skills.

Disclosures: C. Cryan: None. **B. Muntean:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.10/GG7

Topic: E.03. Basal Ganglia

Title: Dorsolateral striatum dopamine concentrations dip and rise with running initiation and cessation regardless of whether the running is forced or voluntary

Authors: *M. RAHMAN[1], I. KONDRATYEV[1], R. Y. MARK[1], Y. YANG[2], I. T. ELLWOOD[1]; [1]Dept. of Neurobio. and Behavior, [2]Cornell Univ., Ithaca, NY

Abstract: Dopamine in the dorsolateral striatum (DLS) is essential for movement, but its precise role in allowing and inhibiting ongoing movements, as well as overall motor vigor, remains controversial. Current evidence varies whether dopamine concentrations increase or decrease during movement initiation and cessation. One possible reason for this could be variation in task design and presence or absence of reward. In this study, we recorded DLS dopamine concentrations using fluorescent dopamine indicators in real-time in mice performing a range of treadmill tasks to measure dopamine levels during motor initiation, cessation and ongoing activity. Mice either ran voluntarily, were rewarded for running or were forced to run on a driven treadmill. We found a remarkably consistent pattern of dopamine concentration decreases as mice initiated running and increases when they stopped. The largest changes we observed across conditions were a more pronounced and time-locked dip and rise in the driven running condition. We discuss the connection of these surprising findings with existing theories of motor vigor and reinforcement.

Disclosures: M. Rahman: None. **I. Kondratyev:** None. **R.Y. Mark:** None. **Y. Yang:** None. **I.T. Ellwood:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.11/GG8

Topic: E.03. Basal Ganglia

Title: An in vivo patch-clamp and modeling study of K-ATP Channels in midbrain dopamine neurons

Authors: *R. EGGER[1], C. J. KNOWLTON[2], C. C. CANAVIER[2], J. ROEPER[1]; [1]Inst. of Neurophysiol., Goethe-University Frankfurt, Frankfurt am Main, Germany; [2]LSU Hlth. Sci. Ctr., LSU Hlth. Sci. Ctr. New Orleans: Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: We have recently established deep in vivo patch-clamp recordings of dopamine (DA) midbrain neurons in mice (Otomo et al. 2020) and now apply this approach to study the functional role of ATP-sensitive potassium (K-ATP) channels in these neurons. As we have previously demonstrated, the expression of K-ATP channels in DA neurons in the medial substantia nigra (SN) are required for in vivo burst firing and in turn for novelty-induced exploratory behavior (Schiemann et al. 2012). However, the moment-to-moment contribution of K-ATP channels to ongoing in vivo activity of DA neurons has not yet been explored. To

address this question, we added 100 µM of the K-ATP channel blocker tolbutamide to the internal pipette solution to inhibit these channels selectively in the target cell in vivo. Previous control experiments comparing electrical in vivo activity in on-cell and whole-cell mode demonstrated stable firing pattern and gave no evidence that in vivo whole-cell patching *per se* altered intrinsic K-ATP channel activity (Otomo et al. 2020). When dialyzing tolbutamide into DA SN neurons, the minimal voltage of the interspike intervals (ISIs) significantly depolarized after 3min of wash-in in n = 16/47 cells. Furthermore, some cells (n =9) went into a depolarization block, only being able to fire a few spikelets, after wash-in or quickly after reaching the whole-cell configuration. With these cells we were then able to perform a rescue experiment by injecting a constant negative holding current of -50 to -200pA, to mimic a leak current (K-ATP), resulting in an oscillatory rebound bursting activity. These results, consistent with our previous study (Knowlton et al. 2018), show a new dimension of the heterogeneity of K-ATP channel function in midbrain DA neurons. In addition, the smaller spikes and tendency to enter depolarization block in tolbutamide are predicted by our Markov models of NaV1.2 (Knowlton et al 2021). Similar to previously reported for pancreatic alpha-cells (Göpel et al. 2000) open K-ATP channels in some DA neurons might be necessary for action potential firing.

Disclosures: **R. Egger:** None. **C.J. Knowlton:** None. **C.C. Canavier:** None. **J. Roeper:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.12/GG9

Topic: E.03. Basal Ganglia

Title: Projection-specific uni-directional organisation of dendritic dopamine transmission in the midbrain

Authors: *N. HAMMER[1], G. TIAN[2], K. BEIER[2], J. ROEPER[1];
[1]Inst. of Neurophysiol., Goethe Univ., Frankfurt, Germany; [2]Physiol. & Biophysics, Univ. of California, Irvine, Irvine, CA

Abstract: Most midbrain dopamine (DA) neurons are inhibited by activation of somato-dendritic D2-autoreceptors (D2R) (Lacey et al., 1987). The presence of genuine synaptic D2R signalling between pre- and postsynaptic DA neurons was demonstrated in midbrain slices in vitro (Beckstead et al., 2004). In addition, spontaneous D2R-mediated IPSCs were recorded in DA neurons showing functional dendro-dendritic vesicular DA release between midbrain DA neurons (Gantz et al., 2013). Our previous studies reported different D2R and GIRK2 expression levels in midbrain DA neurons projecting to distinct target areas (Lammel et al., 2008), but the degree of synaptic and extrasynaptic D2R signalling within and across these DA subpopulations is currently unknown.Therefore, we recorded electrically evoked, D2R-mediated, sulpiride-sensitive, slow inhibitory postsynaptic currents (eIPSCs) in retrogradely identified DA neurons in vitro. We observed significant differences in peak D2R-sIPSC amplitudes between DA

neurons projecting lateral shell of nucleus accumbens (lNAcc), dorsomedial striatum (DMS) and dorsolateral striatum (DLS). Mean eIPSC amplitudes of lNAcc-projecting DA neurons displayed much larger synaptic currents compared to DA neurons projecting to the dorsal striatum (lNAcc: 27.1±2.5pA, n=21, N=9; DMS: 16.3±1.4pA, n = 23, N=8; DLS: 12.5±1.3pA, n=18, N=7). Contrary to the eIPSC the pharmacological D2R activation using quinpirole showed a significantly lager current in cells projecting to the dorsal striatum compared to lNAcc projecting cells (lNacc: 58.6±8.2pA, n=10, N=5; DMS: 87.7±10.9pA, n=10, N=4; DLS: 76.5±7.2pA, n=12, N=4).Using a retrograde AAV9-based approach in DAT-cre mice, we optogenetically stimulated presynaptic DA neurons projecting to DLS while recording optically-evoked, sulpiride-sensitive D2-IPSC (oIPSC) in DA neurons projecting to lNAcc ($lNAcc_{DLS}$:14.9±3.7pA, n=13,N=4). When reversing pre- and postsynaptic DA neurons, little to no oIPSCs were recorded (DLS_{lNAcc}:3.6±1.4pA, n=9, N=3).We currently are using cell-type specific Tracing the Relationships between Inputs and Outputs (cTRIO; Beier et al., 2015), modified to explore local connectivity relationships (Beier, 2022) to mark specific DA-DA connections for physiological recordings.These results demonstrate a uni-directional organisation of dendritic dopamine transmission in the midbrain among projection-identified DA subpopulations. Given that dorsal striatum projecting DA neurons synapse onto nucleus accumbens projecting DA neurons, it provides a microcircuit for fast sensorimotor to limbic feedback.

Disclosures: **N. Hammer:** None. **G. Tian:** None. **K. Beier:** None. **J. Roeper:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.13/GG10

Topic: E.03. Basal Ganglia

Title: Effects of $Ca_v1.3$-inhibition on DA SN neuron in vivo firing and explore - exploit behavior in mice

Authors: *J. BOEHM[1], P. VOGEL[1], N. J. ORTNER[2], J. STRIESSNIG[2], J. ROEPER[1]; [1]Goethe-University Frankfurt a.M., Frankfurt a.M., Germany; [2]Pharmacol. and Toxicology, Univ. of Innsbruck, Innsbruck, Austria

Abstract: The Dihydropyridine (DHP)-sensitive L-Type calcium channels $Ca_v1.3$ and $Ca_v1.2$ play diverse roles in physiological processes, such as enhancing pacemaking in the heart. These channels are also present in dopamine (DA) neurons of the substantia nigra (SN). Our previous studies demonstrated that $Ca_v1.3$ channels selectively act as linear full-range amplifiers of firing rates (FR) in lateral DA SN neurons projecting to the dorsolateral striatum. Furthermore, we have shown that the FR amplifier function of $Ca_v1.3$ channels is dampened by clinically relevant concentrations of isradipine (ISR), both in vitro using acute brain slices and in vivo in anesthetized mice (Shin et al., 2022, Science Advances).
In this study, we extended our investigations to observe the in vivo effects of ISR in awake and

freely moving mice during open field behavior using chronic extracellular recordings. Consistently, we found that 15 minutes after administering systemic ISR injections (ISR = 3mg/kg BW) to $Ca_v1.2DHP^{-/-}$ mice, where DHPs exclusively target $Ca_v1.3$ channels, there was a significant 24% decrease in mean FR of lateral pharmacologically identified DA SN neurons (>50% firing rate reduction after Quinpirole i.p. injection) compared to Vehicle injections (n=28, N=3; mean FR baseline = 4.97 Hz compared to mean FR ISR = 3.80 Hz). Additionally, we observed a positive correlation between the FR reduction and the baseline firing rate of the DA lSN neuron (ΔHz-slope = 0.29).
As a previous study by Koralek and Costa (Koralek & Costa, 2021, Science Advances) suggested a crucial role of electrical activity in DA SN neurons in selectively invigorating exploitative behavior in mice, we hypothesized that an ISR-induced decrease in FR of lSN neurons might influence exploitative and/or explorative behavior. To investigate this hypothesis, we trained a group of mice (6 male wildtype and 6 male and female $Ca_v1.2DHP^{-/-}$ mice) to perform a two-poke sequence learning task, where rewarded exploitative action sequences were distinguished from other explorative actions. Following initial pre-training, mice were required to learn a specific two-poke sequence in a three-port operant chamber to receive a sugar-water reward in a central reward-port. The mice successfully learned the task within 10 days of training and reached plateau performance characterized by stable exploitative poking of the target sequence, achieving an average of 10 rewards/ minute by the end of the training period. At this stage, we administered ISR or VEH i.p. injections 15 minutes prior to placing the animals in the operant chamber and opened an additional exploration port. We are currently analyzing the results of these experiments.

Disclosures: **J. Boehm:** None. **P. Vogel:** None. **N.J. Ortner:** None. **J. Striessnig:** None. **J. Roeper:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.14/GG11

Topic: E.03. Basal Ganglia

Support: NSF IOS1655365
NSF IOS2011998

Title: Reciprocal inhibition between D1 medium spiny neurons controls specific types of task-switching behaviors in mice.

Authors: *M. PETROCCIONE, N. AFFINNIH, A. SCIMEMI;
Univ. At Albany - SUNY, Albany, NY

Abstract: D1-receptor expressing medium spiny neurons (D1-MSNs) form a dense network of inhibitory synaptic connections with D1- and D2-MSNs. Although previous work has shown that

D1-D2 inhibition enhances activation of the direct pathway, implicated in the execution of reward-seeking behaviors, little is known about the role of homosynaptic reciprocal inhibition between D1-MSNs. One hypothesis that has been proposed, but has not been fully tested, is that this might control the activation of neuronal ensembles implicated with the activation of different motor outputs. The experimental challenge to verify this comes from the fact that it is technically difficult to modulate D1-D1 inhibition without also affecting D1-D2 inhibition, as these rely on the activity of inhibitory synapses formed by the same cohort of cells (i.e., D1-MSNs). We serendipitously found that a specific type of neuronal glutamate transporters is expressed pre-synaptically at D1-D1 but not D1-D2 synapses in the striatum. This provides us with a powerful and innovative tool to examine how D1-D1 inhibition shapes the execution of probabilistic task-switching reward-based behaviors. Together, our findings identify some important molecular and cellular mechanisms implicated with behavior flexibility in mice.

Disclosures: M. Petroccione: None. **N. Affinnih:** None. **A. Scimemi:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.15/GG12

Topic: E.03. Basal Ganglia

Support: ZIA-AA000421
ZIA-EY000511

Title: Circuits underlying dopamine transmission during visual learning

Authors: *H. GOLDBACH[1,2], E. SWANSON[1], M. AUTHEMENT[1], K. ELLIOTT[3], J. KWON[1], S. PREUSS[3], C. MEJIAS-APONTE[3], C. GERFEN[1], C. QUAIA[3], V. A. ALVAREZ[1], R. J. KRAUZLIS[3];
[1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Brown Univ., Providence, RI; [3]Natl. Eye Inst., Bethesda, MD

Abstract: The primary input area of the basal ganglia, the striatum, plays a role in integrating signals from the cortex, midbrain, and thalamus to make associations between stimuli, actions, and rewards. The canonical view has been that midbrain activity drives all dopamine signals in the striatum. However, recent findings have forced the field to reconsider this viewpoint: cortical and thalamic inputs to the striatum can also produce large, local dopamine signals indirectly through striatal cholinergic interneurons. Here, we aimed to determine how dopamine, specifically cholinergic-evoked dopamine, may be involved in visual learning. This would reveal a novel mechanism for learning specific associations that is not explicable with current models and would be consistent with recent evidence linking visual cortico-striatal circuits to visual learning. Mice injected with dLight1.2 in the dorsomedial striatum were trained on a unilateral orientation-change detection task requiring them to report a stimulus change by licking a spout.

We saw that while all mice had dopamine responses to the stimulus onset ("wait cue"), only mice that learned that task with a high degree of proficiency developed a dopamine response to the stimulus change ("go cue"). We then used ex vivo voltammetry to measure electrically and optogenetically-evoked dopamine transients in trained animals. We found striking differences in electrically evoked striatal dopamine release that depended on visual task training, which could be attributed to the cholinergic-evoked component of the overall dopamine signal. Interestingly, visual corticostriatal inputs failed to evoke dopamine transients through cholinergic interneurons in either naive or trained animals. The inability to evoke dopamine signals by visual corticostriatal inputs was explained by a lack of direct synaptic connections onto cholinergic interneurons, as measured with cell-attached electrophysiology. Similarly, somatosensory and auditory cortical inputs could not evoke striatal dopamine for the same reason. However, frontal cortical inputs reliably made direct synaptic connections onto cholinergic interneurons, suggesting that these inputs could serve as potential mediators of dopamine signaling in this striatal sub-region. These findings provide surprising information regarding corticostriatal connectivity and the neural circuits involved in visual learning and sensory perception.

Disclosures: **H. Goldbach:** None. **E. Swanson:** None. **M. Authement:** None. **K. Elliott:** None. **J. Kwon:** None. **S. Preuss:** None. **C. Mejias-Aponte:** None. **C. Gerfen:** None. **C. Quaia:** None. **V.A. Alvarez:** None. **R.J. Krauzlis:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.16/GG13

Topic: E.03. Basal Ganglia

Support: IOS1655365
IOS2011998

Title: Modulation of dopamine release by neuronal glutamate transporters

Authors: *N. AFFINNIH[1], M. RODRIGUEZ[2], A. SCIMEMI[3];
[1]State Univ. of New York, Albany, Albany, NY; [2]St. George's Univ., West Indies, Grenada; [3]Biol., SUNY Albany, Albany, NY

Abstract: Dopamine release in the basal ganglia controls reward-based behaviors and habit learning. In the striatum, dopamine release is self-regulated and subject to modulation by other neurotransmitters with receptors located in the axonal projections of dopaminergic neurons. These include the neurotransmitters glutamate, GABA, and acetylcholine. Recent data from our lab show that the neuronal glutamate transporter EAAC1, which is abundantly expressed in the striatum, limits glutamate spillover onto D1 receptor expressing medium spiny neurons (D1-MSNs) and enhances GABA release at reciprocal inhibitory synapses formed between D1-MSNs. As a result, mice that do not express EAAC1 in D1-MSNs show altered action switching

behaviors in a probabilistic reward-base lever press test. These findings raise the possibility that, by altering glutamatergic and reciprocal inhibition among D1-MSNs, EAAC1 might also change dopamine release through currently unknown circuit mechanisms. To test this, we performed a series of imaging experiments *in vitro* using the dopamine sensor dLight. The results showed that dopamine release is increased in the dorsolateral striatum (DLS) of EAAC1$^{-/-}$ mice. This effect is not due to changes in the number or density of dopaminergic neurons projecting to the DLS. Instead, it can be accounted for by changes in the cholinergic modulation of dopamine release. Together, these findings provide novel insights into the functional implications of glutamate transporters and excitation/inhibition coordination on the functional properties of striatal circuits and on the execution of reward-based behaviors.

Disclosures: **N. Affinnih:** None. **M. Rodriguez:** None. **A. Scimemi:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.17/GG14

Topic: E.03. Basal Ganglia

Support: NIH BRAIN Initiative Grant R00NS112417
APDA Grant 2021APDA00RG00000209666

Title: Comparing tonic and phasic calcium in the dendrites of vulnerable midbrain neurons

Authors: *R. Y.-T. CHEN, R. C. EVANS;
Neurosci., Georgetown Univ., Washington DC, DC

Abstract: Several midbrain nuclei degenerate in Parkinson's Disease (PD). Many of these nuclei share the common characteristics that are thought to contribute to their selective vulnerability, including pacemaking activity and high levels of calcium influx. Specifically, the cholinergic neurons of the pedunculopontine nucleus (PPN) and the dopaminergic neurons of the substantia nigra pars compacta (SNc) both degenerate in PD. It is well established that the low-threshold L-type calcium current is a main contributor to tonic calcium in SNc dopaminergic neurons, and is hypothesized to contribute to their selective vulnerability. However, it is not yet clear whether the vulnerable PPN cholinergic neurons share this property. Therefore, we used two-photon dendritic calcium imaging and whole-cell electrophysiology to evaluate the role of L-type calcium channels in the tonic and phasic activity of PPN neurons and the corresponding calcium signal. We found that blocking L-type channels with nifedipine significantly reduces tonic firing rate and dendritic calcium levels in SNc neurons, but not in PPN neurons. However, blocking sodium channels with tetrodotoxin significantly reduces dendritic calcium levels in PPN neurons. This indicates that pacemaking generates a significant calcium load in PPN neurons, but this tonic dendritic calcium is not primarily mediated by influx through L-type calcium channels. In addition, we found that nifedipine significantly reduces calcium influx during phasic firing in

PPN cholinergic neurons, suggesting that PPN neurons mainly express high-threshold L-type calcium channels. Together, these findings show that L-type calcium channels play different roles in the activity of SNc and PPN neurons, and suggest that low-threshold L-type channels are not likely to be the main cause of vulnerability to neurodegeneration in PPN cholinergic neurons.

Disclosures: R.Y. Chen: None. **R.C. Evans:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.18/GG15

Topic: E.03. Basal Ganglia

Support: UH3- NS100553
UG3-NS130202
R01-NS119520
R01-NS124563

Title: Subthalamic nucleus single-unit activity in humans with PD encode both force magnitude and rate of change during a sustained grip-force task

Authors: *J. W. OLSON[1], S. WAHID[2], D. KUHMAN[3], Z. T. IRWIN[4], C. L. GONZALEZ[5], S. BLACK[2], B. L. GUTHRIE[6], T. WICHMANN[7], H. C. WALKER[2];
[1]Neurol., Univ. of Alabama, Birmingham, Birmingham, AL; [2]Neurol., [3]Physical Therapy, [4]Neurosurg., Univ. of Alabama at Birmingham, Birmingham, AL; [5]Neurol., [6]Neurosurg., UAB, Birmingham, AL; [7]Emory Univ. Sch. Med., Atlanta, GA

Abstract: The subthalamic nucleus (STN) is a primary target for neuromodulation therapies for Parkinson disease (PD), yet its function in sensorimotor neural circuits is not completely understood. Here we recorded single-unit activity from the dorsolateral STN in human patients with PD during deep brain stimulation surgery to investigate the role of STN in simple motor behaviors in the contralateral hand. We studied single-unit discharge patterns during a cued isometric sustained grip-force task. Although we found diverse and abundant changes in the instantaneous discharge frequency of STN units after the onset of the applied force, we found minimal if any changes during motor preparation. During sustained force, (12/21) units had a change in firing rate, exhibiting either a linear relationship between discharge frequency and force magnitude (7/21) and/or a shift from baseline independent of force magnitude (9/21). Units with the latter type of response were located more dorsally, anteriorly, and laterally. The most pronounced changes in single unit activity occurred during the dynamic phase (squeeze/release) of the grip force. During squeeze, 6/21 (4/21) units increased (decreased) activity with median latency of 56.5 ms (-69.5 ms) from squeeze onset. During release, 5/21 (8/21) units increased (decreased) activity with median latency of 153.0 ms (58.0 ms) from release onset. Our findings provide evidence that dorsolateral STN neurons primarily encode the rate of change in force

(yank) and/or force magnitude, and to a lesser extent, motor preparation. This suggests a role in either sensory feedback and/or the control/refinement of motor behaviors that have already been initiated.

Disclosures: J.W. Olson: None. **S. Wahid:** None. **D. Kuhman:** None. **Z.T. Irwin:** None. **C.L. Gonzalez:** None. **S. Black:** None. **B.L. Guthrie:** None. **T. Wichmann:** None. **H.C. Walker:** Other; Dr. Walker serves on the scientific advisory board for Varian..

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.19/GG16

Topic: E.03. Basal Ganglia

Support: ZIA AG000928

Title: Sex-specific behavioral effect of impaired glutamatergic input to aldehyde dehydrogenase 1A1-positive nigrostriatal dopaminergic neurons in mice

Authors: *K. CARMICHAEL, H. CAI;
Transgenic Section, Lab. of Neurogenetics, Natl. Inst. on Aging, NIH, Bethesda, MD

Abstract: The ventrolateral *substantia nigra pars compacta* in individuals with Parkinson's disease (PD) display a preferential degeneration of a subpopulation of dopaminergic neurons (DANs) that selectively express aldehyde dehydrogenase 1A1 (ALDH1A1). Although studies suggest a role of these neurons in the regulation of locomotor skill acquisition in mice, how their activity is regulated by presynaptic inputs remains unknown. To explore the behavioral role of glutamatergic input, we used an inducible Cre mouse model to knock out *Grin1*, a gene coding for a critical N-methyl-D-aspartate receptor (NMDAR) subunit, in ALDH1A1-positive (ALDH1A1+) midbrain DANs in fully developed mice (Grin1 KO). We then investigated the effect of impaired NMDAR-mediated glutamatergic input on open field locomotion, rotarod motor learning, operant learning, and free feeding activity post-food restriction. Neither female nor male 3-4 month-old Grin1 KO mice showed deficits in open field locomotion and rotarod motor learning relative to controls. Since changes in nigrostriatal DANs are associated with aging, open field and rotarod performance was also assessed in 1 year-old mice. Again, Grin1 KO mice showed no deficits in locomotion or motor learning. Conversely, lever pressing performance during fixed and progressive ratio operant tasks suggests a sex-specific effect associated with the dysfunction of NMDAR-mediated glutamatergic input to ALDH1A1+ midbrain DANs in 3-4 month-old mice. Grin1 KO female (but not male) mice earned more rewards during fixed and progressive ratio schedules relative to controls, indicating a potential excessive eating abnormality. We then used FED3, a home cage feeding device to quantify pellet consumption following food restriction in the same cohort of mice. While there was a modest increase in pellet consumption in female (but not male) mice following food restriction, the

normalized body weights of Grin1 KO female mice rapidly increased and then surpassed baseline weights after a few days. The findings indicate that although impaired NMDAR-mediated glutamatergic regulation of ALDH1A1+ midbrain DANs may not affect spontaneous locomotion or motor learning, it is related to female-specific increases in both motivation to work for a reward and body weight following food restriction. These sex-specific behavioral differences highlight the importance of considering the role of sex in animal models of disease. Going forward, a better understanding of how ALDH1A1+ DANs integrate diverse glutamatergic inputs may provide insight into the consequences of the ALDH1A1+ DAN loss in PD-related non-motor symptoms.

Disclosures: K. Carmichael: None. **H. Cai:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.20/GG17

Topic: E.03. Basal Ganglia

Support: Department of Biological Sciences at Carnegie Mellon University
NIG Grant R01NS117058
NIH Grant R01NS104835
NIH Grant R01NS101016

Title: Isolation of a basal ganglia-receiving brainstem population and its role in motor rescue of a Parkinsonian mouse model

Authors: *M. D. CUNDIFF[1], A. H. GITTIS[2];
[1]Biol. Sci., [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Parkinson's disease (PD) is a neurodegenerative disease that is a result of the degeneration of dopamine neurons. Areas of intervention for PD treatment are the basal ganglia and the mesencephalic locomotor region (MLR). The MLR is specifically linked to the initiation and modulation of posture, gait, and speed. Previously, we have shown that excitation or inhibition of specific cell types within the basal ganglia restores movement in a dopamine-depleted mouse model. Insight into the mechanism downstream of this rescue is still poorly understood. Of major interest is the projection from the substantia nigra pars reticulate (SNr) of the basal ganglia to the MLR and the role this connection plays in movement rescue. Using viral tracing techniques, we can analyze this projection at a cell-type specific level. We used a dopamine-depletion mouse model to investigate the mechanism for motor rescue via the basal ganglia targeted MLR neurons (MLRSNr). We have been able to show that the MLRSNr population is composed of multiple cell types and shows potential in being required to persistently rescue motor function of a Parkinsonian mouse model. <!--EndFragment-->

Disclosures: M.D. Cundiff: None. **A.H. Gittis:** None.

Poster

PSTR484. Basal Ganglia: Dopamine

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR484.21/GG18

Topic: E.03. Basal Ganglia

Support: NIH Grant DP2NS105553
NIH Grant R01MH130658
Parkinson's Foundation
American Parkinson's Disease Association

Title: A permissive role for dopamine in the expression of vigorous movements

Authors: H. LIU[1], R. MELANI[1], M. MALTESE[1], A. SANKARAMANCHI[2], J. MARTIN[1], ***N. X. TRITSCH**[1];
[1]NYU Grossman Sch. of Med., New York, NY; [2]Albert Einstein Col. of Med., Albert Einstein Col. of Med. Dominick P. Purpura Dept. of Neurosci., Bronx, NY

Abstract: Dopamine is essential for the production of vigorous movements, but how dopamine modifies the gain of motor commands remains unclear. We developed a dexterous motor task in which head-restrained male and female mice self-initiate fast and large-amplitude lever pushes with their left forelimb to earn rewards. We show that this task is goal-directed and depends on cortico-striatal circuits in the hemisphere contralateral to the limb used to push the lever. We find that unilateral loss of midbrain dopamine neurons reduces the speed and amplitude of lever pushes, and that levodopa treatment rapidly restores motor vigor, consistent with parkinsonian bradykinesia. Photometry recordings of striatal dopamine levels indicate that the therapeutic efficacy of levodopa does not require phasic dopamine release. In dopamine-intact mice, brief optogenetic stimulation or inhibition of midbrain dopamine neurons immediately prior to or during lever pushes is also insufficient to increase the speed and amplitude of forelimb movements. Together, our data show that phasic dopamine transients are unlikely to specify the vigor of forelimb movements online as they are being executed, and suggest instead that dopamine plays a permissive role in the selection and production of vigorous movements. Our findings have important implications for our understanding of how the basal ganglia contribute to motor control under physiological conditions and in Parkinson’s disease.

Disclosures: H. Liu: None. **R. Melani:** None. **M. Maltese:** None. **A. Sankaramanchi:** None. **J. Martin:** None. **N.X. Tritsch:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.01/GG19

Topic: E.04. Voluntary Movements

Support: This research was sponsored by the U.S. Army Research Laboratory and was accomplished under Cooperative Agreement Number W911NF-21-2-0187.

Title: Visual Search in Virtual Reality: Using co-recording of EEG, eye tracking, and head movements to explore the movement-related potential associated with participant responses

Authors: *G. WILSON[1], A. MADISON[2], C. CALLAHAN-FLINTOFT[2], A. J. RIES[2]; [1]US Air Force Acad., USAF Academy, CO; [2]DEVCOM Army Res. Lab., Aberdeen Proving Ground, MD

Abstract: Visual Search in Virtual Reality: Using co-recording of EEG, eye tracking, and head movements to explore the movement-related potential associated with participant responses Gabby Wilson[1], Anna Madison[1,2], Chloe Callahan-Flintoft[2], and Anthony J. Ries[1,2]1. Warfighter Effectiveness Research Center, U.S. Air Force Academy, CO2. DEVCOM Army Research Lab, Aberdeen Proving Ground, MD

Studies exploring the preparation, execution, and control of voluntary body movements have sought to clarify the cortical generators and neuronal mechanisms of movement-related potential (MRP) in the electroencephalographic (EEG) signal. Previous studies exploring MRPs, however, control for ocular and other muscle artifacts by using paradigms that maintain a fixed eye and head position or by having participants imagine executing a motor response. As a result, MRPs have been understudied in the context of more naturalistic visual behaviors, where information is selected by moving the eyes and head. In this study, participants completed a simple virtual reality search task where they located a tilted Gabor patch varying in spatial frequency, and reported the orientation of the Gabor by pressing the trigger buttons on an Xbox controller. During the task, we co-recorded EEG with eye and head tracking to allow participants to freely move their eyes and head. We used state-of-the-art data processing techniques, such as independent component analysis and deconvolution modeling, to minimize the impact of artifacts and overcome issues inherent with free-viewing EEG experiments. We isolated the pre-execution MRP by creating event-related potentials time-locked to participant responses via button press. At both left (C1, C3) and right (C2, C4) central electrodes, we observed a negativity increase starting around 400 ms prior to the button press, which is consistent with the negativity slope component of the MRP. We also observed a positive deflection peaking around 200 ms prior to a button press, which is consistent with pre-movement positivity. Overall, our results demonstrate that MRPs can be isolated from EEG data with naturalistic search behaviors involving eye and head movements, thereby increasing our understanding of motor behavior in the context of real world activities.

Disclosures: G. Wilson: None. **A. Madison:** None. **C. Callahan-Flintoft:** None. **A.J. Ries:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.02/GG20

Topic: E.04. Voluntary Movements

Support: NIH T32-NS082128-06

Title: Unique Neural Mechanisms Underlying Speed Control of Low-Force Ballistic Contractions

Authors: *J. KIM[1], S. DELMAS[1], Y. CHOI[1], J. C. HUBBARD[1], M. WEINTRAUB[1], F. ARABATZI[3], B. YACOUBI[1], E. A. CHRISTOU[1,2];
[1]Dept. of Applied Physiol. and Kinesiology, [2]Dept. of Neurology, Norman Fixel Inst. of Neurolog. Disorders, Univ. of Florida, Gainesville, FL; [3]Sch. of Physical Educ. and Sports Sci. (Serres), Aristotle Univ. of Thessaloniki, Serres, Greece

Abstract: Ballistic contractions are voluntary contractions performed with maximal velocity and short muscle contraction time. A popular theory on the control of ballistic contractions (speed-control hypothesis) suggests that the rate of force development (RFD) is dictated by the force amplitude, because the time to peak force (TPF) remained constant regardless of force amplitude changes. This constant TPF resulted in a linear relationship between the RFD and force amplitude. Freund and Büdingen's observations in 1978 were instrumental in formulating the speed-control hypothesis, which offers a framework for understanding the mechanisms underlying ballistic contractions. However, this hypothesis presents a key limitation: it fails to account for force levels below 20% of maximum. Here, we performed a study examining the relationship between RFD and force amplitude from 2 to 85% maximum and the underlying structure of muscle activity in 18 young adults. Participants exerted ballistic index finger abduction for 50 trials in each of 7 randomly assigned force levels (2, 5, 15, 30, 50, 70 & 85% maximum). We quantified the TPF, RFD, Time from EMG onset to Peak EMG (TP-EMG), EMG Duration, Rate of EMG Development (RED; Peak EMG divided by TP-EMG), and integrated EMG (iEMG). Contrary to the speed-control hypothesis, we found that TPF was not constant but significantly varied from 2 to 85% maximum. Further, the RFD slope from 2-15% maximum was greater than the RFD slope from 30-85% maximum. The longer TPF at low force levels (15 to 2% maximum) was associated with the variability of EMG Duration, whereas the longer TPF with higher force levels (30 to 85% maximum) was associated with iEMG. Thus, the regulation of TPF for low and high force levels was different, suggesting that neuronal variability is critical for low force levels and neuronal amplitude for high force levels. These findings present compelling new evidence highlighting the limitations of the speed-control hypothesis in explaining the velocity of ballistic contractions at extremely low force levels. Instead, our data suggest that these low-force ballistic contractions may be governed by distinct elements of neural drive underscoring the need for a new theoretical framework.

Disclosures: J. Kim: None. **S. Delmas:** None. **Y. Choi:** None. **J.C. Hubbard:** None. **M. Weintraub:** None. **F. Arabatzi:** None. **B. Yacoubi:** None. **E.A. Christou:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.03/HH1

Topic: E.04. Voluntary Movements

Support: NIH CTSA UL1 award 2U1LTR002378

Title: Evaluating the reliability of using Google MediaPipe for human hand pose analysis for assessing hand dexterity function in the healthy and stroke

Authors: *R. BAJAJ[1], P. IHEJIRIKA[1], V. ROWE[2], J. XU[1];
[1]Univ. of Georgia, Athens, GA; [2]Georgia State Univ., Atlanta, GA

Abstract: The human hand possesses a large repertoire of dexterous movement, which can be seriously compromised and difficult to restore after an injury such as stroke. Video-based hand pose estimation has emerged as a prominent area of research and development that offers a promising tool to capture this large repertoire of hand movement and accurately detect hand impairment after injury (Charry-Allen et al., 2023). Our aim in this project is to quantitatively assess the reliability of using one of these tools, Google MediaPipe (Lugaresi et al., 2019), to assess kinematic differences between healthy participants and persons with stroke.
Healthy (N=15) and stroke (N=2) participants were video recorded while performing a standard upper extremity function test, the Action Research Arm Test (ARAT) and the Fugl-Meyer Assessment for the Upper Extremity (FMA-UE). A cohort of occupational therapists (N = 4) performed the FMA-UE and ARAT tasks by simulating stroke impairments. The FMA-UE involves upper extremity single- or multi-joint movements used to reach and control a starting position, and to grasp objects and hold them against resistance. The ARAT test utilizes four subscales (grasp, grip, pinch, movement). Video recording was done using a 3-camera setup: right, left, and at the center facing the participant. All cameras were placed at an angle where the maximum view of arms and shoulders of the participant was permitted. The videos were then clipped into single tasks and run through MediaPipe, which estimated key points for upper limb joints. A Kalman Filter algorithm was used for data smoothing. We then examined the distributions of joint angles and key-points for task-involved and non-involved joints to assess the reliability of the estimated key-points.
Our results showed relatively small level of noise introduced by MediaPipe for the stationary joints (SD ~ 0.65 – 3.21°) in wrist and fingers. Range of motion (ROM) for task-involved joints showed consistent trajectories across three repetitions (SD ~10°). Moreover, our tools can sensitively detect the differences between stroke and healthy participants' shoulder, elbow, wrist, and finger joint angles deviations from normal in various tasks in ARAT and FMA-UE, showing reduced ROM, delayed action initiation, and prolonged motion trajectories in stroke survivors compared to healthy participants. We also noticed some remarkable errors in the key-point estimates output from MediaPipe due to skin color and video recording environment that

requires substantial post-processing. Our results indicate that MediaPipe can be a promising tool for hand kinematic estimation in healthy individuals and those with motor impairments.

Disclosures: **R. Bajaj:** None. **P. Ihejirika:** None. **V. Rowe:** None. **J. Xu:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.04/HH2

Topic: E.04. Voluntary Movements

Title: Grip force control when manipulating mechanically coupled objects with both hands

Authors: *A. S. NAIK, S. AMBIKE;
Hlth. and Kinesiology, Purdue Univ., West Lafayette, IN

Abstract: The influential dynamic dominance theory explains handedness in humans. It focuses on arm movements and posits that in right-handed people, the left hemisphere specializes in predictive control for coordinated dominant (right) arm movements, and the right hemisphere specializes in impedance control to stabilize the position of the non-dominant (left) arm. However, this theory has not been applied to bimanual object manipulation. We studied if grip-load force coupling in both hands aligns with the predictions of the dynamic dominance theory. Healthy young right-handed adults (N = 24) held one object in each hand using pinch grasps. Both objects were connected by a spring. Force sensors on each object measured digit forces and a tracker recorded object movements. Participants tracked a vertically oscillating target on a computer screen by moving one object while keeping the other object static (order balanced across hand). The changing length of the spring due to the movement of the tracking hand induced disturbing loads on both objects. An inertial load arose only on the moving object from the movement of the tracking hand. We quantified grip force (G) and the inertial (I) and the spring (S) loads for each hand. We assessed G-I and G-S coupling using cross-recurrence quantification analysis. The analysis yields trapping time (TT) which quantifies the average duration that one time series is trapped compared to the other; lower TT implies stronger coupling. We predicted that the right hand, under predictive control, would exhibit stronger grip-inertia (G-I) force coupling to counteract load force arising from object's movements. The left hand, under impedance control, would demonstrate superior grip-spring (G-S) force coupling to counteract external disturbances caused by the spring. We fit mixed-effects models to TT with *hand* and *load type* as fixed effects ($p<.05$) to test these hypotheses. The right hand had stronger coupling (TT=18±1 ms) compared to the left hand (TT=20±1 ms, main effect of *hand*; $p<.01$). Both hands exhibited stronger G-I coupling (TT=13±1 ms) compared to G-S coupling (TT=27±1 ms, main effect of *load type*; $p<.01$). Contrary to dynamic dominance theory, our results suggest global dominance of the right hand during object manipulation, as it is more responsive to both load types compared to the left hand. Additionally, the motor system prioritized loads from limb movements over external perturbations, as both hands were more responsive to I than S. This

study enhances our understanding of bimanual prehension and provides insights for future investigations on inter-hemispheric differences in prehension control using imaging and modeling approaches.

Disclosures: A.S. Naik: None. **S. Ambike:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.05/HH3

Topic: E.04. Voluntary Movements

Support: NIH U01NS123125
NIH R01NS125270
NIH T32NS086749

Title: Tactile sensory input improves grasp force classification using specific neural modes

Authors: *G. BLUMENTHAL[1], B. M. DEKLEVA[1], A. R. SOBINOV[2], S. M. CHASE[3], S. BENSMAIA[2], J. L. COLLINGER[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Univ. of Chicago, Chicago, IL; [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Tactile sensory feedback from the hands enables precise grasping and force control during object manipulation. However, it remains unclear how this sensory feedback modulates the cortical dynamics of motor cortex (M1) during grasping actions. To investigate this, we compared the cortical dynamics of M1 during grasp between human subjects with cervical spinal cord injury (SCI) absent of afferent input from the hands, and uninjured non-human primates (NHPs) with intact afferent input from the hands. Three human participants with cervical spinal cord injury and intracortical microelectrode arrays implanted in the M1 hand region were instructed to attempt to grasp an object while observing a virtual hand performing the behavior. Visual feedback of force was provided to coordinate the timing of the grasp. M1 activity was recorded as participants attempted grasps that varied in target force. Similarly, two uninjured NHPs with intracortical microelectrode arrays implanted in the M1 hand region were trained to grasp a force plate. Animals were cued to the timing and force target of the grasp using visual indicators while M1 activity and force output were recorded. Using dimensionality reduction, we identified distinct neural modes that were qualitatively similar in both human and NHP data and were uniquely modulated during specific phases of the grasp, including transient responses at onset and offset of grasp and a tonic neural mode during the hold phase. For both human and NHP data, target force was accurately classified above chance using all neural modes as input features. Using individual neural modes to classify target force, we found that the tonic holding mode was the most informative about target force in both human and NHP subjects. However, the tonic holding mode in humans (without tactile input) classified force targets only 30-60% as

well as using all human neural modes, whereas the holding mode in NHP data (with tactile input) classified force targets nearly as well as using all NHP neural modes. These results suggest that while force information is represented in M1 in the absence of tactile input, tactile input enhances force encoding and is likely beneficial for brain computer interface-based therapies to restore grasp force control.

Disclosures: G. Blumenthal: None. **B.M. Dekleva:** F. Consulting Fees (e.g., advisory boards); Blackrock Microsystems. **A.R. Sobinov:** None. **S.M. Chase:** None. **S. Bensmaia:** None. **J.L. Collinger:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.06/HH4

Topic: E.04. Voluntary Movements

Support: OHSU Medical Research Foundation: New Investigator Grant 4321

Title: Stereotyped Repetition Hampers Motor Flexibility

Authors: *C. A. SAGER, E. DIAMOND, R. HULSEY-VINCENT, M. MARNEWECK; Univ. of Oregon, Eugene, OR

Abstract: Motor planning is critical for the smooth and efficient execution of upper limb interactions with objects in our environment. Motor planning is thought to rely on integrating sensory information from multiple channels for target localization and inference of object properties and on predictive internal models (or sensorimotor memories) derived from previous similar experiences. When sensory processing is unavailable or noisy, as is the case in aging, Bayesian integration principles would predict a down-weighting of sensory processing and an up-weighting of predictive internal models to achieve a task goal. In these instances of sensory uncertainty, the central nervous system compensates by issuing stereotyped motor commands as a result of generating and using overgeneralized predictive models (e.g., similar grip forces independent of varying object properties). Here we test the hypothesis that repeating motor behaviors hampers motor flexibility with an object manipulation task that relies on sensorimotor memories to minimize tilt of an object with a visually concealed asymmetric mass distribution that could switch between the left and right. We manipulate the number of trial repetitions in generating the same force and torque control for an object with a given mass distribution before we switch the mass distribution to the other side. Consistent with our hypothesis, increasing the number of trials in manipulating an object with a given mass distribution increases errors on the trials following a mass distribution switch. This hampering effect of repetition on motor flexibility is seen in younger and older healthy adults, despite there being group differences in timing of force generation suggestive of age-related compensatory strategies for sensory processing deficits. Together, these results suggest that motor control errors due to motor

inflexibility arise from repetitive stereotyped motor behavior independent of sensory processing deficits, both of which are typically observed with advancing age.

Disclosures: C.A. Sager: None. **E. Diamond:** None. **R. Hulsey-Vincent:** None. **M. Marneweck:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.07/HH5

Topic: E.04. Voluntary Movements

Support: NIH Grant R21AR081636

Title: Independent control of manipulation and grasp forces for dexterous manipulation

Authors: *W. NOLL, Y.-H. WU, M. SANTELLO;
Arizona State Univ., Tempe, AZ

Abstract: Human dexterity relies on complex sensorimotor interactions. Research on dexterous manipulation has provided significant insights over the past four decades on humans' ability to build sensorimotor memory for anticipatory control of digit forces, as well as reactive control to rapidly adjust forces in response to motor execution errors. However, most tasks or analytical tools used in previous research are not suitable to address how digit forces are coordinated to simultaneously attain two critical task goals: to prevent the object from slipping and control object position and orientation (pose). In our recent work, we addressed this question by using a force analysis tool developed for robotic applications, the manipulation and grasp force decomposition (MGFD).[1] MGFD decomposes digit forces into Grasp Force (FG), the force required to prevent object slip, and Manipulation Force (FM), the force to control object pose. We had found that FG is controlled in a feedforward fashion, whereas FM requires sensory feedback acquired throughout object lift.[1] The present study addressed the extent to which FM and FG can be selectively modulated when changes in object properties (mass or external torque) challenge object slip prevention or pose control.
We asked 20 participants (10 females) to perform a dexterous object manipulation task that requires simultaneous object slip prevention and pose control using a precision grip. We instructed participants to reach and grasp an inverted-T shape object using the thumb and index fingertip, lift the object vertically while preventing tilt of the object in any direction, hold the object in midair, and replace the object back on the table. We systematically changed the object's mass distribution to vary the requirements for object slip prevention or pose control. This was accomplished by inserting two different weights at the same location at the bottom of the object (change in object mass) or moving the same weight to a different moment arm (change in external torque).
We found that FM was selectively modulated to changes in the object pose control requirement

($p < 0.001$), but not to changes in the object slip prevention requirement ($p = 0.95$). In contrast, FG was modulated to changes in both object mass and torque ($p < 0.05$). These findings indicate that (1) dexterous control of object pose is accomplished by modulation of FM, whereas (2) modulation of FG occurs even when object mass remains constant. Further work is needed to address the sensory mechanisms responsible for the independent modulation of FM and FG to task requirements.
Ref. 1. Wu Y, Santello M. Performance of dexterous object manipulation is enhanced by grasp force modulation.

Disclosures: **W. Noll:** None. **Y. Wu:** None. **M. Santello:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.08/HH6

Topic: E.04. Voluntary Movements

Support: NSERC Grant RGPIN-2022-05050 to RLW

Title: Sensorimotor Adaptation to Cross-Modal Conflict Reveals the Nature of Object Representations for Grasping

Authors: M. WONG, C. LEWIS, A. TAN, ***R. L. WHITWELL**;
The Univ. of Western Ontario, London, ON, Canada

Abstract: When reaching for a goal object, the hand's in-flight aperture (grasp aperture) scales to the size of the target. The hand's scaling follows a pre-movement, just-in-time visual analysis of the surface structural and egocentric spatial features of the target. Two theoretical positions provide conflicting accounts of what this analysis entails: The classic 'dual channel theory' (DCT) proposes a transformation of target size to grasp aperture, whereas the 'double pointing theory' (DPT) posits that target grasp points on the object's surface are transformed to finger-trajectories. This study tested the DCT and DPT using a classic grasp adaptation paradigm, in which unconscious adjustments in grasp aperture to a haptic target that is smaller than its visual, virtual counterpart persist for several reaches after the sizes are matched again (the after-effect). The DCT and DPT suggest different sources of object information for grasp adaptation; the DCT suggests object size, whereas the DPT suggests grasp points. In this study, 38 human participants underwent grasp adaptation while reaching for visible virtual targets while their hand movement was tracked using optoelectronic cameras. We tested the generalizability of the after-effect to novel target positions and orientations while keeping the size of the visible virtual target the same. The DCT predicts generalization, because the target's visual size remained the same. The DPT predicts no generalization, because the novel orientations and positions yield novel grasp points and resultant egocentric finger-trajectories. The results confirmed the smaller haptic sizes induced reductions in grasp aperture ($p<.001$). Crucially, an after-effect was observed only when

the target orientation and position remained the same ($p<.001$); adaptation failed to generalize to novel target orientation and position ($p>.82$). These findings support the DPT and suggest an analysis of target grasp points is more biologically-relevant approach for reaching and grasping applications in computer vision, robotics, and neuroprosthetics.

Disclosures: M. Wong: None. **C. Lewis:** None. **A. Tan:** None. **R.L. Whitwell:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.09/HH7

Topic: E.04. Voluntary Movements

Support: NSF Grant 1910939

Title: Tool incorporation and user strategy during human-robot teleoperation is impacted by robot dynamics

Authors: *J. CARDUCCI, J. BROWN;
Johns Hopkins Univ., BALTIMORE, MD

Abstract: Manipulating an environment remotely with a robotic teleoperator introduces novel electromechanical (EM) dynamics between user and environment. While considerable effort has focused on minimizing these dynamics, there is limited research into understanding their impact on a user's internal model and resulting motor control strategy. Here we investigate to what degree, if any, the dynamics of the teleoperator influence task behavior and tool incorporation. Our teleoperator testbed features three distinct transmissions between the leader and follower sides of the device. The leader side is controlled by user input via wrist pronation/supination, and the follower side is connected to a virtual environment. A virtual object tracking task is rendered by a rotary motor. A Rigid transmission is created with a rigid rod that mechanically couples the leader and follower without scaling. A Unilateral transmission is created with two rotary motors that couple the leader and follower without force feedback to the user. A Bilateral transmission is created with two rotary motors that couple the leader and follower while providing force feedback to the user. N=30 adult participants rotated a virtual disk in a viscoelastic virtual environment through counterbalanced presentation of each transmission. Users tracked targets oscillating at pre-defined frequencies, randomly selected from 0.55, 0.85, 1.15, 1.55, 1.85, 2.05, and 2.35 Hz, with an initial training frequency at 1.25 Hz. After session completion, trajectories of the target, leader, and follower were decomposed into components, intensity and delay, for all frequencies. We hypothesize that the different transmissions would result in different tracking performance based on the differences in dynamics. We also hypothesize that the dynamics influence the target speed where performance drops fastest. From two-way ANOVA and post-hoc Tukey tests, we have found that transmission dynamics have an impact ($F(2) = 12.659$, $p < 0.001$) on output intensity, with the rigid condition being different than the unilateral or bilateral

condition ($p < 0.001$) but no significance between the latter two conditions ($p = 0.998$). Significant interaction exists in user input intensity between transmission and speed ($F(12) = 0.885$, $p = 0.562$). From our findings, the gold standard of rigid transmission is reaffirmed. When the user adjusts for dynamics, the speed where user input drops most differs between transmissions. One possible explanation for post-adjustment differences is that mismatch between predicted and delayed/scaled sensory feedbacks induces the user to invert robot dynamics and match limb impedance to minimize error.

Disclosures: J. Carducci: None. **J. Brown:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.10/HH8

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 18H05287
JSPS KAKENHI 18H04038
JSPS KAKENHI 18K19767
JSPS KAKENHI 20H05489
JSPS KAKENHI 20H05714
JSPS KAKENHI 18K13378
JSPS KAKENHI 21K1857
JSPS KAKENHI 23K16631

Title: Cortical origins of the ipsilateral corticospinal tract in humans based on diffusion fiber tractography

Authors: *N. USUDA[1], S. K. SUGAWARA[2], Y. NISHIMURA[3];
[1]Tokyo Met. Inst. Med. Sci., Setagaya-ku, Tokyo, Japan; [2]Tokyo Metropolitan Inst. of Med. Sci., Setagaya, Japan; [3]Neural Prosthetics Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan

Abstract: Cortical activation in bilateral motor-related areas involves in the recovery of movement in paralyzed limbs after stroke. This suggests that the spared ipsilateral corticospinal tract (iCST)s, which descends the ipsilateral ventral or lateral funiculus in the spinal cord, contributes to functional recovery. Macaque monkey iCSTs are known to arise from multiple regions of cortical motor- and somatosensory-related areas (Toyoshima and Sakai, 1982), but this is still unknown in humans. The purpose of this study was to estimate the cortical origins of iCST, the proportions among each cortical origin and the proportion between ventral and lateral funiculus at the cervical level based on diffusion-weighted imaging (DWI) in humans.To define the candidate cortical origins of iCST, we reviewed anatomical and electrophysiological studies that explored cells degenerated in cortical areas after spinal cord injury and examined functional

mapping using intraoperative cortical electrical stimulation. Based on the review, primary motor cortex (M1), dorsal premotor cortex (PMd), ventral PM (PMv), supplementary motor area (SMA), pre-SMA, primary somatosensory cortex (S1), Brodmann area 5 (BA5), caudal cingulate zone (CCZ), posterior rostral cingulate zone (RCZp), and anterior RCZ (RCZa) were defined as candidates. In addition, we defined BA 8 as a negative control. Twenty-nine healthy volunteers participated in the 3T MRI experiments. T1-weighted image and DWI were measured covering through the whole-brain to the upper cervical cord. Streamlines were delineated from candidate areas to ventral and lateral funiculus in ipsilateral side at C1 level. The density of streamlines arising from each cortical origin was quantified, and areas significantly higher than that from BA 8 were defined as the iCST origins. We found that the densities of streamlines from M1, PMd, PMv, SMA, preSMA, S1 and BA5 were significantly higher than that from BA8. Therefore, we defined these seven areas as the origin of iCSTs in humans. The proportion of ipsilateral corticospinal (iCS) streamlines among cortical origins was calculated by counting the number of streamlines. 72% originated from M1, 14% from S1, 8% from SMA, and the rest from four areas. Regardless of cortical origins, almost 75% of iCST passed on the lateral funiculus. Thus, human iCSTs arise mainly from M1 and pass to the lateral funiculus of spinal cord, which is consistent with macaque monkeys (Toyoshima and Sakai, 1982). Our results may help to improve the prognosis of motor recovery in patients with spinal cord injury and stroke and provide insight into the mechanism of congenital mirror movements.

Disclosures: **N. Usuda:** None. **S.K. Sugawara:** None. **Y. Nishimura:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.11/HH9

Topic: E.04. Voluntary Movements

Title: Spatiotemporal coordination of fingertip forces during isometric finger individuation reveals individual and group differences across healthy and stroke hands

Authors: *P. IHEJIRIKA[1], M. ROSENBERG[3], L. H. TING[4], J. XU[2];
[2]Kinesiology, [1]Univ. of Georgia, Athens, GA; [3]Biomed. Engin., Emory Univ., Atlanta, GA;
[4]Biomed. Engin., Emory Univ. Georgia Tech., Atlanta, GA

Abstract: The complex, dynamic and variable nature of the human hand movement contributes to difficulties in injury assessment and design of effective rehabilitation strategies. Previous investigations into hand function have revealed much about the complexity of static hand postures (Yan & Bensmaia, 2020) and the loss of the complexity after stroke (Xu et al, 2021), but yet to explore the spatiotemporal structures of multi-finger coordination. Investigating these structures remains a challenge because it is difficult to quantify across individuals, diseases, and tasks. We use a data driven approach to analyze the trajectories of isometric fingertip forces using Principal Components Analysis (PCA). We hypothesized that spatiotemporal coordination

structures underling paretic hand movement will show 1) less similarity than those underling healthy and non-paretic hands, 2) more individual-level variability than those underlying healthy hand movement, and 3) less group-level variability than those underlying healthy hands. Isometric 3D force data was recorded from all five fingertips simultaneously from the right hand of 20 healthy controls, and both paretic (P) and non-paretic (NP) hand of 6 stroke survivors. Participants were instructed to control a dot in a virtual space to move towards a target in
one of 6 directions (+/- XYZ directions) at one of 4 force ranges (0.2,0.4,0.6,0.8) using one finger at a time while keeping other fingers inactive (Xu et al., 2021). A single set of PCs were computed over the entire force trajectories for all participants and all trials. The analyses produced group and subject specific spatiotemporal structures that were projected into a common low-dimensional space (PC1-3), producing traces over time for individuals and groups (Healthy, NP, P). We compared these traces between groups for each finger (Thumb, Index, Middle, Ring, Pinky). Results are currently qualitative and largely support our hypotheses. The first three PC trajectories for the Healthy/NP occupy a different and larger region in the latent space compared to the Paretic group. Consistent with our hypothesis 2), although finger PC trajectories for each individual in the Healthy group formed distinct clusters in the latent space, they appeared to present similar structures, thus less individual differences than the Paretic hands. Lastly, consistent with our hypothesis 3), the overall group-level finger force PC trajectories for the Paretic hands are compressed compared to those in NP/Healthy groups. Our results indicate that this is a promising approach to reveal finger force spatiotemporal structural differences across individuals and disease in a complex task.

Disclosures: **P. Ihejirika:** None. **M. Rosenberg:** None. **L.H. Ting:** None. **J. Xu:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.12/HH10

Topic: E.04. Voluntary Movements

Support: ISF Grant 1634/19
BSF Grant 2021323

Title: Direction-dependent neural control of finger dexterity in humans

Authors: O. RAJCHERT[1], S. OFIR-GEVA[4], *Y. MELUL[2], M. KHOURY-MIREB[3], N. SOROKER[4], **F. MAWASE**[3];
[1]Biomed. Engin., Technion Israel Inst. of Technol., Kiryat Motzkin, Israel; [3]Biomed. Engin., [2]Technion Israel Inst. of Technol., Haifa, Israel; [4]Loewenstein Rehabil. Hosp., Raanana, Israel

Abstract: Humans, more than all other species, skillfully flex and extend their fingers to performdelicate motor tasks. This unique dexterous ability is a product of the complex

anatomicalproperties of the human hand and the neural mechanisms that control it. Yet, the neuralbasis that underlies human dexterous hand movement remains unclear. Here wecharacterized *individuation* (fine control) and *strength* (gross control) during flexion andextension finger movements, isolated the peripheral passive mechanical couplingcomponent from the central neuromuscular activity involved in dexterity and then appliedvoxel-based lesion mapping in first-event sub-acute stroke patients to investigate thecausal link between the neural substrates and the behavioral aspects of finger dexterity.We found substantial differences in dexterous behavior, favoring finger flexion overextension. These differences were not caused by peripheral factors but rather were drivenby central origins. Lesion-symptom mapping identified a critical brain region for fingerindividuation within the primary sensory-motor cortex (M1, S1), the premotor cortex(PMC), and the corticospinal (CST) fibers that descend from them. Although there was agreat deal of overlap between individuated flexion and extension movements, we wereable to identify distinct areas within this region that were associated exclusively with fingerflexion. This flexion biased differential premotor and motor cortical organization wasassociated with the finger individuation component, but not with finger strength. Fromthese results we propose a neuroanatomical model that summarizes the distinctionsbetween individuation and strength and between finger movement in flexion andextension, revealed in human manual dexterity.

Disclosures: O. Rajchert: None. **S. Ofir-Geva:** None. **Y. Melul:** None. **M. Khoury-Mireb:** None. **N. Soroker:** None. **F. Mawase:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.13/HH11

Topic: E.04. Voluntary Movements

Support: Canadian Institutes of Health Research
SDSU Summer Undergraduate Research Program

Title: Hand and finger movement strategies for single-shot tactile search

Authors: *S. RESCHECHTKO[1], W. PANGAN[1], A. PRUSZYNSKI[2];
[1]Exercise & Nutritional Sci., San Diego State Univ., San Diego, CA; [2]Physiol. & Pharmacol., Univ. of Western Ontario, London, ON, Canada

Abstract: We often use touch to guide action, for example when we search for the edge of a roll of tape by moving our fingers across it. In this study, we investigated how people move their fingers when tasked with detecting features that are a few microns (um) tall, which is close to the limit of sensory ability. 30 human participants performed tactile detection tests using their index or little fingers in a two-alternative forced choice paradigm. Participants tried to feel features of 2, 6, and 10 um height and 500 um diameter. Each participant performed 23 trial repetitions per

height, with heights randomly interleaved. During each trial, participants moved a finger across two silica wafers and then reported which one had the feature; they were only allowed to feel each wafer once during each trial. We also recorded the contact forces that participants used during these tests. Participants performed better when using their index fingers than their little fingers. For smaller features, participants also performed better when they moved their fingers in an anterior-posterior direction (along the long axis of the finger) rather than medial-lateral direction. We found that participants used similar contact forces during both directions with their index fingers, but they used higher contact forces during (less successful) medial-lateral exploration with their little fingers. Our results suggest that, in addition to mechanoreceptor density, finger-specific dexterity and movement strategy plays an important role in tactile sensory ability.

Disclosures: S. Reschechtko: None. **W. Pangan:** None. **A. Pruszynski:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.14/HH12

Topic: E.04. Voluntary Movements

Support: NSERC Grant DG RGPIN-2019-05944

Title: Contextual scaling of rapid corrective motor responses in object manipulation

Authors: *M. R. MCGARITY-SHIPLEY[1], O. C. SCOTEN[2], J. P. GALLIVAN[1,2,3], J. R. FLANAGAN[1,2];
[1]Ctr. for Neurosci. Studies, [2]Dept. of Psychology, [3]Dept. of Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada

Abstract: The ability to skillfully manipulate objects is supported by predictions of object dynamics, as well as a multitude of corrective responses that are initiated when predictions are erroneous. Past work has demonstrated that, when an object is unexpectedly heavy—and liftoff does not occur as anticipated—the sensorimotor system triggers a rapid corrective response that involves an increase in vertical force that is terminated when liftoff is signaled via sensory feedback. We have recently found evidence that this corrective lifting response is highly sophisticated; when repeatedly lifting an object that occasionally and unpredictably increases weight in a single catch trial, the gain of the response—as measure by the rate of change of the force increase—adapts to the magnitude of the weight change. Our previous work has only examined these corrective responses when tracking a single object's weight. Real-world action tasks, by contrast, often involve interacting with multiple objects, which requires encoding and keeping track of their various possible weight states. Here we asked whether the sensorimotor system can simultaneously keep track of the weight histories of two objects, such that the corrective lifting response is intelligently tuned to the weight history of each object. Participants

used a 3-D robotic manipulandum with an augmented reality system to lift two visually distinct objects in alternation. The two objects had the same baseline weight (2N) but different 'catch' weights (6 and 9N) on occasional and unpredictable catch trials. We found that the gain of the corrective lifting response on catch trials differed for the two objects and scaled with the size of the catch weight. Our findings show that this rapid, corrective response can flexibly adapt to the potential weight states of two independent objects, thereby demonstrating impressive adaptability across contexts.

Disclosures: M.R. McGarity-Shipley: None. **O.C. Scoten:** None. **J.P. Gallivan:** None. **J.R. Flanagan:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.15/HH13

Topic: E.04. Voluntary Movements

Support: Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 416228727 – SFB 1410.

Title: From giver to receiver: Investigating the influence of object mass and giver's behavior on receiver's response in joint handover actions

Authors: *L. KOPNARSKI, J. RUDISCH, C. VOELCKER-REHAGE;
Neuromotor behavior and exercise, Univ. of Muenster, Münster, Germany

Abstract: Handovers are joint actions describing the transfer of an object between two persons. Accurate anticipatory knowledge of object properties might facilitate handover actions as this information can inform response-planning by the receiver. Our aims were, thus, to investigate i) whether the weight of an object can be anticipated from observing the movement kinematics of the giver; and ii) whether receivers adapt their action execution to this anticipated weight. Forty healthy participants (31 female, 9 male) aged 22.6 ± 2.5 years completed the experiment. A 3D motion capture system, as well as a self-constructed test object for grip force measurement, was used to record both, the participants' motion data and their applied grip forces. Two different object sizes (small, large) and three different object weights (light, medium, heavy) were used. The experiment was divided into four blocks; the condition object size varied between blocks and the condition object weight varied pseudo-randomly within blocks. Participants had no knowledge about the object weight before each trial. The parameters lifting delay and maximum lifting velocity were analyzed for the giver. Maximum grip aperture and initial grip force scaling were analyzed for the receiver. For the giver we found a significant effect for object weight on the lifting delay ($F(1,13) = 141.02$, $p < .05$, $\eta^2 = 0.27$) as well as on maximum lifting velocity ($F(1,61) = 60.28$, $p < .05$, $\eta^2 = 0.05$). Regarding the receiver, we observed a significant effect for object weight on the maximum grip aperture ($F(1,94) = 3.32$, $p < .05$, $\eta^2 = 0.00$), but not on

initial grip force scaling ($F(1,94) = 0.58$, $p = .56$, $\eta^2 = 0.00$). The results are consistent across both object sizes and showed that the object weight influenced observable parameters of the giver kinematics. However, receivers seemed not to use this information to adjust their own action executions to the object weight. We propose to investigate in future experiments whether receivers in handover actions are generally able to produce an adequate initial grip force scaling by performing a similar experiment with blocked weights.

Disclosures: **L. Kopnarski:** None. **J. Rudisch:** None. **C. Voelcker-Rehage:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.16/HH14

Topic: E.04. Voluntary Movements

Support: DFG Grant 416228727—SFB 1410

Title: A cohort study on the development of bimanual force-control in highly aged individuals with and without mild cognitive impairments

Authors: *J. RUDISCH, S. FRÖHLICH, C. VOELCKER-REHAGE;
Dept. of Neuromotor Behavior and Exercise, Univ. of Münster, Münster, Germany

Abstract: Bimanual coordination affords task-specific intra- and interhemispheric information processing in predominantly frontocentral brain networks. In role-differentiated bimanual tasks (RDBT) inhibition of crosstalk is necessary to avoid involuntary movements (e.g., when one hand manipulates and the other one stabilizes). Age-related cognitive decline might affect both, interhemispheric information exchange and inhibition of crosstalk, thus affecting RDBT performance. In this study, we investigated an RDBT in right-handed community-dwelling older adults (79 - 92 years) that are cognitively healthy (CHI: n = 76) or present mild cognitive impairments (MCI: n = 57). Participants performed a force-tracking task where they had to match a constant target force with their left (stabilizing role) and a sine-wave shaped force with their right hand (manipulating role). We were interested how the ability to produce a constant force was affected by age-related cognitive impairments. We therefore computed two measures of variability: 1. Coefficient of variation (COV) to investigate the magnitude; and 2. Detrended fluctuation analysis (DFA) to investigate the time-dependent structure of variability, with lower DFA scaling coefficients (DFA-α) indicating a more complex variability structure. Through the prospective cohort sequential study design, we followed up participants up to 3 times over 24 months. We used linear mixed effects modelling to investigate the effect of measurement time point, cognitive status, and sex on COV and DFA-α. For COV, we found only a significant effect for sex, with females showing higher COV as compared to males ($t(130) = 2.67$, $p = .009$, $r = 0.23$). Likewise, we found a significant effect of sex on DFA with females showing higher DFA-α than males ($t(129) = 2.65$, $p = .009$, $r = 0.23$). Additionally, we found a group x timepoint

interaction with MCI showing a reduction of DFA-α over time whereas CHI did not. The effect did not reach significance level, however ($t(151) = -1.40$, $p = .165$, $r = 0.11$). In young adults, we have previously shown that an RDBT leads to a decreased complexity in the stabilizing hand as compared to a task where both hands maintained a constant force. This reduction of complexity is considered a signature of reduced neuromuscular degrees of freedom. Potential causes for an increased complexity in the MCI population during the RDBT, such as a focus on the force-maintenance role or a reduction of crosstalk, remain to be explored. Further research should include various other measures of complexity to confirm these results and use neuroimaging methods to investigate the relationship between active inhibition and complexity in this age group.

Disclosures: **J. Rudisch:** None. **S. Fröhlich:** None. **C. Voelcker-Rehage:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.17/HH16

Topic: E.04. Voluntary Movements

Support: NSF Grant 2133879
NIH Grant UL1 TR001860
NIH Grant TL1 TR001860

Title: Children with Unilateral Congenital Below Elbow Limb Deficiency Can Proportionally Control the Residual Muscles of the Missing Hand

Authors: *J. J. FITZGERALD[1], M. A. BATTRAW[1], A. M. BAGLEY[2], M. A. JAMES[2], J. S. SCHOFIELD[1], W. M. JOINER[1];
[1]Univ. of California, Davis, Davis, CA; [2]Shriners Children's - Northern California, Sacramento, CA

Abstract: Children with a unilateral congenital below elbow deficiency (UCBED) have one typically developed limb and one that ends below the elbow, at the proximal level or mid forearm. Since these children were born with their limb difference, their affected muscles have never actuated an intact hand, and it has been assumed that their ability to control the muscles of their residuum is limited. Here, we examined the extent to which children with UCBED could use visual feedback of their muscle state to proportionally control their affected muscles. We used ultrasound imaging and machine learning to provide children control over a cursor with either their affected or unaffected limb. As children transitioned between two selected muscle states which corresponded to hand positions (e.g., an open hand: 0 % activation, and full power grasp: 100% activation), the cursor moved along one degree of freedom. We asked participants to move the cursor to targets which appeared at set intermediate levels (16.67, 33.33, 50, 66.67, 83.33% activation) and maintain that position for up to 10 seconds. We show that without prior

training, children with UCBED (N=8) can accurately reach and maintain multiple target cursor positions with both their affected and unaffected muscles. We examined the cursor position accuracy as a proxy of muscle state accuracy. We observed that across participants, for the affected limb, there was a significant main effect of goal location on average cursor position (One way ANOVA, $F = 117.5$ $p < 0.01$), suggesting children with UCBED can use visual feedback to control and perform online corrections to the state of their residual muscles. Post-hoc testing revealed that average cursor position was significantly different across all pairs of target goals within the affected limb (Tukey's HSD, $p < 0.01$). However, there were also idiosyncratic differences in the stability of cursor position across target goals and between limbs. These results suggest that despite never actuating a hand with their affected muscles, children with UCBED can both control their affected muscles, and can use sensory information to modulate this control. These results provide insight into how motor control develops in the absence of the typical effector, and contribute to the advancement of dexterous and intuitive prosthetic devices for this patient population.

Disclosures: **J.J. Fitzgerald:** None. **M.A. Battraw:** None. **A.M. Bagley:** None. **M.A. James:** None. **J.S. Schofield:** None. **W.M. Joiner:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.18/HH17

Topic: E.04. Voluntary Movements

Support: NIH R21NS114816A
University of Delaware Research Foundation 22A01471

Title: Age-related modulation of fMRI activity during upper and lower limb movements

Authors: *A. E. BOWER[1], J. CHUNG[2], S. CRISOMIA[1], R. G. BURCIU[1];
[1]Univ. of Delaware, Newark, DE; [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder that primarily affects the elderly population. As many of its motor symptoms overlap with aging-related symptoms, it is difficult to differentiate between the two. Importantly, PD research frequently employs older adults (OA) as control participants to enable the comparison of their results with those of individuals with PD. Despite separate studies in OA, the specific effects of healthy aging on brain function are not yet fully understood. Previous work revealed that OA exhibit higher levels of functional magnetic resonance imaging (fMRI) activity that is more widespread throughout the brain compared to younger adults (YA). However, these conclusions are largely based on studies of upper limb movements. Further research focusing specifically on OA without neurological disorders that explores different limbs is necessary to enhance our understanding of age-related changes in brain function. This research is crucial for differentiating age-related

changes from disease-related changes observed in PD. Here, we analyzed fMRI data obtained from 16 YA and 20 OA generating submaximal force using their hand and foot separately. The analysis of the hand data revealed that despite producing a similar level of force to that of YA, OA had lower levels of activity in the sensorimotor cortex (M1S1) and middle cingulate cortex, and higher levels of activity in the premotor cortex, pre-supplementary motor area (pre-SMA), and lobules VI and Crus I of the cerebellum. Although the mean force produced during the foot task was similar for both groups, OA had lower activity than YA in the M1S1, SMA, and putamen. Similar to the hand task, there was greater activity in OA in the frontoparietal cortices and lateral cerebellum. In summary, we found distinct activation patterns in OA for the hand and foot tasks. The production of lower levels of force using the foot is inherently more challenging than doing so with the hand, and this increased difficulty may impose greater challenges on the basal ganglia in OA. Furthermore, the stronger activation of frontoparietal and cerebellar regions in OA may reflect a compensatory response that could aid OA in maintaining task performance levels similar to those of YA.

Disclosures: **A.E. Bower:** None. **J. Chung:** None. **S. Crisomia:** None. **R.G. Burciu:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.19/HH18

Topic: E.04. Voluntary Movements

Support: Northeastern TIER 1 Grant

Title: Motor adaptation to self-assistance with a passive exoskeleton in a functional reach-to-grasp task

Authors: J. MANCZUROWSKY, H. MAYNE, D. NGUYEN, M. KHAN, R. AHMED, P. WHITNEY, ***C. HASSON**;
Northeastern Univ., Boston, MA

Abstract: For people after stroke, restoring arm and hand function is critical in rehabilitation to counteract significant long-term difficulties from hemiparesis in reaching, grasping, and manipulating objects. To complete a task like reaching for an object, a physical therapist may assist a patient's arm and hand by providing somatosensory feedback, facilitating proper motor action planning, and reducing possible spasticity. Patients should repeat such motions over hundreds of trials to promote motor adaptation and transfer to functional tasks. The constrained time of therapy sessions means practice must continue at home, however, without the benefits of physical assistance. Therefore, the therapeutic dose of whole-task practice could be significantly increased if patients had a device that allowed them to assist themselves. We developed an exoskeleton device that transfers forces and motion of one hand to the other hand through a self-contained pair of right- and left-hand exoskeletons coupled by a hydrostatic transmission. The

exoskeleton is passive and has no electronics; the user's motion powers the device. Whether humans can effectively assist themselves in a functional reach-to-grasp task with such a device is unknown. To test device feasibility, we hypothesized that practice while using the exoskeleton would improve the functional reach-to-grasp of healthy participants subjected to an artificial motor impairment. Two participants (one female) seated at a table were asked to perform a reach-to-grasp to lift a solid wood cylinder 5 cm off the table as fast as possible. As they reached for the object, closed-loop electrical stimulation activated their left wrist and hand flexor muscles. This perturbed task performance by causing their left hand to close prior to grasp, requiring significant effort to open the hand. Participants were then asked to don our passive exoskeleton system on both hands and practice the task while assisting the left hand to open with their unaffected right hand. Task performance was quantified by the task completion time (TCT). Early results showed participants had impaired performance with electrical stimulation (TCT increased by 65%) that was partially counteracted when they practiced assisting themselves using the passive exoskeleton (TCT decreased by 16% but was still 45% higher compared to no-stimulation). They continued to improve their TCT as they practiced. These preliminary findings suggest that using a passive exoskeleton with a hydrostatic transmission for self-assistance during a reach-to-grasp task is feasible, yet additional data and hypothesis testing is needed to reach a robust conclusion.

Disclosures: J. Manczurowsky: None. **H. Mayne:** None. **D. Nguyen:** None. **M. Khan:** None. **R. Ahmed:** None. **P. Whitney:** None. **C. Hasson:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.20/HH19

Topic: E.04. Voluntary Movements

Support: Edward and Barbara Bell Family Chair (JLA)

Title: The effect of age on grip force release

Authors: *S. DAVIDSON[1], K. LEARMAN[4], E. ZIMMERMAN[2], A. ROSENFELDT[5], J. L. ALBERTS[3];
[1]Cleveland Clin., Brook Park, OH; [3]Cleveland Clin., [2]Cleveland Clin., Cleveland, OH; [4]Youngstown State Univ., Youngstown, OH; [5]Cleveland Clin. Fndn., Cleveland, OH

Abstract: Previous research on the effects of aging on grip force modulation have focused on assessing overall modulation capabilities or the ability to precisely generate force. There is less research on the effects of aging on grip release. The aim of this study was to investigate the effects of age on precision grip release during force tracking tasks. Young adults (N=10, 18-28 years) and older adults (N=10, 64-77 years) completed a ramp-hold-release (0-35% of maximum grip) force tracking task with precision grip of their dominant hand. Data were analyzed by

group and phase (development, maintenance, release). Young adults outperformed older adults for all phases and variables. Repeated measures ANOVA showed a main effect of group ($F_{1,18}$=9.48, p=.006) and phase ($F_{2,36}$=3.54, p=.04) for percentage of time spent within 5% of the target force (%TWR), but not an interaction effect ($F_{2,36}$=0.68, p=.51). There was a main effect of group ($F_{1,18}$=11.44, p=.003) for relative root mean squared error (RRMSE), but not phase ($F_{2,36}$=2.87, p=.07) and no interaction between group and phase ($F_{2,36}$=0.60, p=.55). Post-hoc comparisons for %TWR revealed no significant differences between phases. Results show that young adults generally are more precise with a force tracking task than older adults, and this effect is not specific to force release. This study was limited by small sample size. The effect of age on grip release warrants further investigation.

Disclosures: S. Davidson: None. **K. Learman:** None. **E. Zimmerman:** None. **A. Rosenfeldt:** None. **J.L. Alberts:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.21/HH20

Topic: E.04. Voluntary Movements

Support: UH Health Research Institute

Title: Hand motor function in heart failure.

Authors: *H. HIBINO[1,3], S. YAZDEKHASTI[3], M. YAROSSI[1,2], E. TUNIK[1], S. L. GORNIAK[3];
[1]Dept. of Physical Therapy, [2]Dept. of Electrical & Computer Engin., Northeastern Univ., Boston, MA; [3]Dept. of Hlth. and Human Performance, Univ. of Houston, Houston, TX

Abstract: Introduction: Heart failure (HF) is a complex syndrome characterized by structural or functional defects of ventricular filling or ejection fractions of the heart that do not meet metabolic requirements. While dyspnea, fatigue, fluid retention are symptoms commonly reported by persons with HF (PwHF), indispensable reports demonstrating declined independence in activities of daily living (ADLs) are also mounting. As functional independence is associated with fine motor skills, the reduced independence in PwHF may be attributed to decline in hand motor function. To test this hypothesis, a battery of hand motor function assessments was delivered to PwHF and age- and sex-matched control subjects. Method: A total of 10 right-handed PwHF (age: 57.6 ± 12.5 years old, four females) and a total of 10 right-handed CO (age: 58.2 ± 12.2 years old, four females) were recruited. The assessments of fine motor skills included 9-hole pegboard test (9HPT), a timed test of fine motor skills, and two pinch force tracking tests (constant pinch force tracking test and sinusoidal pinch force tracking test), in which subjects produced and matched pinch force to a given target force. The constant pinch force tracking test required subjects to produce and maintain pinch force corresponding to

their 5% and 20% of their maximum pinch force. The sinusoidal pinch force tracking test involved tracing a target force oscillated between 5% and 20% of their maximum pinch force at 0.5 Hz. Results: The PwHF took longer to complete the 9HPT compared to CO ($p < 0.05$). In the constant pinch force tracking test, force steadiness (coefficient of variation) and target-force error (root mean square error) were nearly significantly different ($p = 0.06$) and significantly different ($p < 0.001$), respectively. However, the regularity of pinch force, represented by using approximate entropy, was comparable between groups. Contrary to the constant pinch force tracking test, the target-force error and target-force coupling (maximum cross-correlation) were comparable between groups in the sinusoidal pinch force tracking test. Our study demonstrated that HF affects fine motor skills and the ability to produce instructed pinch force, but not the force steadiness, in a constant manner. On the other hand, no difference was found between PwHF and CO regarding the ability to modulate the pinch force. These two different outcomes suggest that HF-related impairment in the hand motor function may be task-dependent, rather than a general impairment. In conclusion, HF affects the motor function of the hand; however, the underlying mechanism of HF-related impairment in the hand motor function requires further investigation.

Disclosures: H. Hibino: None. **S. Yazdekhasti:** None. **M. Yarossi:** None. **E. Tunik:** None. **S.L. Gorniak:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.22/HH21

Topic: E.04. Voluntary Movements

Support: German Research Foundation - Project-ID 431549029 - SFB1451
Institute for Collaborative Biotechnologies under Cooperative Agreement W911NF-19-2-0026 with the Army Research Office

Title: Corticospinal fibers from premotor areas are essential for complex motor control after stroke

Authors: *T. PAUL[1,2], M. CIESLAK[3], L. HENSEL[2], V. WIEMER[1,2], C. TSCHERPEL[2,4], C. GREFKES[4], S. T. GRAFTON[5], G. R. FINK[1,2], L. J. VOLZ[2];
[1]INM-3, Res. Ctr. Juelich, Juelich, Germany; [2]Neurol., Univ. Hosp. Cologne, Cologne, Germany; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Univ. Hosp. Frankfurt, Frankfurt am Main, Germany; [5]Dept. of Psychological & Brain Sci., Univ. of California, Santa Barbara, CA

Abstract: Damage to the corticospinal tract (CST) is a common reason for motor impairment after stroke. The CST consists of fibers descending from the primary motor cortex (M1) as well as different premotor areas including the supplementary motor area (SMA), ventral premotor cortex (PMv), and dorsal premotor cortex (PMd). Even though premotor areas are thought to

play an important role for motor recovery after stroke, previous studies have vastly neglected the role of their corticospinal output. Thus, the functional role of CST fibers descending from premotor areas in post-stroke motor control remains unclear.
We therefore assessed the differential impact of CST fibers originating from premotor areas and M1 on the control of basal and complex motor skills in 25 chronic stroke patients (20 male, 5 female, mean age=66.68, std=11.25). A novel diffusion imaging approach was applied to quantify microstructural integrity of different corticospinal subtracts via tractwise anisotropy by means of a compartmentwise approach that classifies voxels according to their number of trackable fiber directions.[1,2]
Our results indicate a dissociation between the execution of basal and complex motor control. While tractwise anisotropy derived from the M1 subtract was positively associated with basal and complex motor control, anisotropy derived from premotor subtracts was only correlated with complex motor skills. Thus, while descending signals from M1 seem to be essential for any form of upper limb motor control, the successful execution of complex motor skills relies on output from premotor areas. Of note, patients with persisting motor deficits showed an additional positive association between premotor subtract integrity and basal motor control. Descending motor output signals from premotor areas may hence aid to flexibly compensate for the impairment of basal motor functions in severely affected patients. In summary, our results imply that descending corticospinal output from premotor areas is crucial for motor control post-stroke, thereby identifying premotor areas as possible targets for therapeutic interventions aimed at enhancing motor recovery.
1. Volz LJ, Cieslak M, Grafton ST. A probabilistic atlas of fiber crossings for variability reduction of anisotropy measures. *Brain Struct Funct*. 2018;223(2):635-651. doi:10.1007/s00429-017-1508-x
2. Paul T, Cieslak M, Hensel L, et al. The role of corticospinal and extrapyramidal pathways in motor impairment after stroke. *Brain Commun*. 2023;5(1):fcac301. doi:10.1093/braincomms/fcac301

Disclosures: **T. Paul:** None. **M. Cieslak:** None. **L. Hensel:** None. **V. Wiemer:** None. **C. Tscherpel:** None. **C. Grefkes:** None. **S.T. Grafton:** None. **G.R. Fink:** None. **L.J. Volz:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.23/Web Only

Topic: E.04. Voluntary Movements

Support: NIGMS Grant P20-GM-109098
NIGMS Grant P30-GM-103503
NIGMS Training Grant T32AG052375

Title: Analysis of muscle activity patterns in degenerative cervical myelopathy and insights into the role of the spinal cord in neuromechanical tuning: a case study

Authors: *R. I. TAITANO[1], S. ABBAS[2], V. GRITSENKO[3];
[1]Neurosci., [2]Chem. and Biomed. Engin., [3]Human Performance - Physical Therapy, West Virginia Univ., Morgantown, WV

Abstract: Recent studies have demonstrated that restoration of functional movements following spinal cord injury (SCI) can be accomplished through electrical stimulation of spinal circuitry. In a healthy individual, the neural motor system embeds muscle anatomy and complex mechanics of the body and its external environment to provide adequate control, a concept termed neuromechanical tuning. However, it is unknown if or how the spinal cord contributes to neuromechanical tuning. Degenerative cervical myelopathy (DCM) is a type of nontraumatic SCI associated with chronic spinal cord compression that causes upper limb dysfunction, among other sensory and motor deficits. The limb dysfunction in DCM is caused by changes in muscle activity that are driven by the reorganization of spinal circuitry as it gets progressively disconnected from the descending input. The goal of this study is to quantify these changes in muscular activation to elucidate the capacity of the spinal cord to embed neuromechanical properties of the limb. Five healthy controls and one patient with a diagnosis of DCM were recruited to participate in our study. All participants were evaluated for grip and pinch strength using dynamometers, in addition to sensation and prehension performance using a modified Graded Redefined Assessment of Strength, Sensation, and Prehension (GRASSP, Kalsi-Ryan, et al., 2012). Twelve Delsys Trigno sensors were used to record upper limb electromyography and accelerometer data during the performance of GRASSP tasks by each participant. Our preliminary data demonstrates that DCM patients have unique muscle activation patterns compared to healthy controls when performing reaching and grasping tasks. For example, the DCM patient shows higher baseline muscle activation levels across tasks, with a marked decrease in discrete task-specific bursting compared to controls. Additionally, the patterns of muscle coactivation are altered in the participant with DCM. For example, the positive correlation between the activation of the biceps and triceps in control participants is changed in the DCM participant to a negative correlation between these muscle groups, indicative of their reciprocal activation. These quantitative changes in DCM provide fundamental insight into the role of the spinal cord in shaping muscle activation patterns. Furthermore, characterization of these changes may aid in earlier diagnosis for DCM patients, and objective metrics of disease progression useful for medical decisions in prescribing spinal decompression surgery.

Disclosures: R.I. Taitano: None. **S. Abbas:** None. **V. Gritsenko:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.24/HH22

Topic: E.04. Voluntary Movements

Support: NIH R01HD062744
NIDILRR 90REGE0010

NIH/NIBIB P41-EB018783
Stratton VA Medical Center
SCIRB- DOH-C37714GG
SCIRB- DOH-C38338GG

Title: Behavioral and EEG Features of Finger Proprioception and Passive Movement: Effect of Error Feedback on Proprioception

Authors: *A. FARRENS[1], M. TORRECILLA[1], L. GARCIA FERNANDEZ[1], C. JOHNSON[1], E. T. WOLBRECHT[6], D. J. REINKENSMEYER[2,3,4,5], D. GUPTA[7,8];
[2]Mechanical and Aerospace Engin., [3]Biomed. Engin., [4]Anat. and Neurobio., [5]Physical Med. and Rehabil., [1]Univ. of California, Irvine, Irvine, CA; [6]Mechanical Engin., Univ. of Idaho, Moscow, ID; [7]Natl. Ctr. for Adaptive Neurotechnologies, Stratton VA Med. Center, Albany, NY, ALBANY, NY; [8]Electrical and Computer Engin., State Univ. of New York, Albany, Albany, NY

Abstract: Purpose: Proprioception, the sense of body movement, is critical to motor learning and is predictive of responsiveness to motor rehabilitation after stroke, which often damages proprioception [1-5]. It is of interest to understand how to enhance proprioception. Here, we used the proprioceptive "Crisscross" task on Finger Individuating Grasp Exercise Robot (FINGER) [4-8], to determine if error feedback improves passive proprioceptive acuity, and identify neural markers of proprioceptive processing using EEG.
Methods: 19 healthy right-handed adults (age: 22-34 yrs, 12 male) participated with informed consent. In Crisscross, FINGER passively crossed the right index and middle fingers in symmetric, alternating flexion/extension trajectories (0-36 deg), at random speeds (8,11,16 deg/s) and inter-trial intervals (2-3.5 s), for 120 trials (6 runs, 20 trls/run), with vision of the hand occluded. Participants were tasked to press a button with the left hand at the instance of perceived finger crossing. Feedback group (FB, n=9) received visual feedback as a numerical error (absolute finger separation at press), while the no-Feedback group (nFB, n=10) received a visual cue of 'button press'. 19 channel EEG was acquired (DSI-24, Wearable Sensing, CA). EEG data was filtered (bandpass [0.1, 30] Hz), ICA denoised, baseline corrected (-200 to 0 ms) and epoched (-200 to 4000 ms) with respect to movement onset; noisy epochs were removed (+/- 100μV, <5% trials). Event Related Potentials (ERPs) were calculated as the mean across trials.
Results: Proprioceptive errors were smaller in the FB group compared to the nFB group (t-test, $p < 0.01$). 400-600 ms post button press, the FB group showed a lateralized ERP response in frontoparietal regions contralateral to the propriocepting hand, which increased with error magnitude (kw-test, $p < 0.01$), that was absent in the nFB group. EEG also showed a Contingent Negative Variation (CNV) at Cz, initiated at movement onset (initial stimulus), followed by a negative peak at perceived finger crossing (imperative stimulus). A mixed model (fixed factors: feedback, speed) performed on CNV magnitude (600-800ms post movement onset, 200-1200ms prior to cross over) showed an interaction between feedback and speed ($p < 0.03$). In the FB group, CNV magnitude increased with finger speed but not in the nFB group.
Conclusions: These results demonstrate that error feedback can improve proprioceptive acuity in healthy adults and is accompanied by an error-dependent ERP response in the sensorimotor cortex. The crisscross task also elicits a CNV response; we are the first to show modulation of CNV by proprioceptively-sensed speed and performance feedback.

Disclosures: A. Farrens: None. **M. Torrecilla:** None. **L. Garcia Fernandez:** None. **C. Johnson:** None. **E.T. Wolbrecht:** None. **D.J. Reinkensmeyer:** None. **D. Gupta:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.25/HH23

Topic: E.04. Voluntary Movements

Support: 1R35NS122333

Title: Neural basis of finger coordination in the human motor cortex

Authors: *A. R. SOBINOV[1], D. E. SHEETS[1], B. M. DEKLEVA[2], J. L. COLLINGER[2], S. J. BENSMAIA[1];
[1]Univ. of Chicago, Chicago, IL; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Manual interactions with objects involve the coordinated movement of many joints, an ability that critically depends on the primary motor cortex (M1) as evidenced by the selective deficits incurred after M1 lesions. While non-human primates (NHPs) have yielded many critical insights into the neural mechanisms underlying manual dexterity, some aspects of manual behavior remain understudied because NHPs cannot be instructed to produce well-controlled and repeated individual finger movements. Here, we leverage a clinical brain-computer interface trial in which 5 human participants are implanted with arrays of electrodes in M1 to investigate the neural basis of manual behaviors, ranging from digit individuation, to posture matching, and preshaping for grasp. In brief, we asked the participants to attempt a variety of tasks requiring coordinated finger movements in a virtual environment. In the first task, participants attempted flexion and extension of individual digits in random order. We found that digit movement in opposite directions (flexion vs extension) was encoded along different neural axes, likely associated with the different actuating muscles. Next, we investigated the coordinated control of multiple digits by having the participants attempt to shape their hands into postures involving several digits. We found that the neural responses in M1 for complex movements differed across postures, as expected, but did not reflect a superimposition of the M1 responses evoked during the constituent individuated digit movements. We combined these findings with prior work in non-human primates to develop a continuous decoder of hand posture that can accommodate manual behaviors ranging from individuated finger movements to complex, whole hand postures. Together, these results provide the most detailed characterization of the neural basis of coordinated finger movements in humans to date and provide a blueprint on how to decode manual behavior from M1.

Disclosures: A.R. Sobinov: None. **D.E. Sheets:** None. **B.M. Dekleva:** None. **J.L. Collinger:** None. **S.J. Bensmaia:** None.

Poster

PSTR485. Finger and Grasp Control in Health and Disease

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR485.26/HH24

Topic: E.04. Voluntary Movements

Title: The effect of transcutaneous spinal cord stimulation (tSCS) in improving the hand motor functions of spinal cord injury (SCI) patients

Authors: *S. PARK[1], N. VERMA[2], E. H. BEDOY[4], N. CHETTY[2], J. OH[5], A. G. STEELE[5], A. FARAJI[5], D. SAYENKO[5], D. J. WEBER[3];
[2]Dept. of Mechanical Engin., [3]Mechanical Engin. and Neurosci. Inst., [1]Carnegie Mellon Univ., Pittsburgh, PA; [4]Dept. of Neurobio. and Systems Neurosci. Inst., Univ. of Pittsburgh, Pittsburgh, PA; [5]Dept. of Neurosurg., Houston Methodist Res. Inst., Houston, TX

Abstract: Previous studies have shown that transcutaneous spinal cord stimulation (tSCS) enhances lower limb function in people with motor complete and incomplete spinal cord injury (SCI). However, few studies have investigated the effects of tSCS on upper limb functions and utilized high-density EMG (HD-EMG) to quantify the effects of tSCS on muscle recruitment. Here we explored the feasibility of HD-EMG in demonstrating the effects of tSCS on hand motor function in people with tetraplegia after SCI. We recorded HD-EMG without tSCS from the forearm muscles of five participants with tetraplegia while they performed 9 to 10 hand gestures such as individual finger movements and various types of hand grips, with 5 to 8 repetitions each. The subjects repeated the experiment while they received tonic tSCS over their C6-C7 vertebrae to facilitate the hand and forearm muscles. For each movement epoch, we evaluated the maximum activity for 6 muscles including extensor carpi ulnaris (ECU), extensor digitorum communis (EDC), extensor carpi radialis (ECR), flexor carpi ulnaris (FCU), flexor digitorum superficialis (FDS) and flexor carpi radialis (FCR). The EDC, which is primarily responsible for finger extension, showed an average increase in the peak activation of 23%, 18%, 18% and 13% during pinky, ring, middle and index finger extension movements with tSCS. Moreover, the EDC activation increased by 10% during hand opening. The ECR activation increased by 44% during wrist extension. During the flexion movements of index, middle, ring and pinky, the FDS had 18%, -1%, 12%, and 5% increase in muscle activation with stimulation. The activity of FDS was also higher during hand grasp, tripod grip and lateral grip by 20%, 13% and 35%, respectively. The analysis revealed that tSCS increased activity in the muscles primarily responsible for hand movements. These findings show that tSCS reorganizes muscle activation pattern to facilitate agonist hand muscles, which enhances hand function in people with tetraplegia post-SCI.

Disclosures: S. Park: None. **N. Verma:** None. **E.H. Bedoy:** None. **N. Chetty:** None. **J. Oh:** None. **A.G. Steele:** None. **A. Faraji:** None. **D. Sayenko:** None. **D.J. Weber:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.01/Web Only

Topic: E.04. Voluntary Movements

Support: DARPA Grant N66001-17-2-4018
Chilean National Agency for Research and Development/BIO-Fulbright Scholarship
Arizona State University GPSA JumpStart Research Grant

Title: Comparison of the effects of offline transcutaneous trigeminal and occipital nerve stimulation on motor learning

Authors: D. E. ARIAS, *C. A. BUNEO;
Arizona State Univ., Tempe, AZ

Abstract: Trigeminal (TNS) and occipital (ONS) nerve stimulation are novel neuromodulation approaches that are applied transcutaneously to address the symptoms of several neurological disorders. Stimulation of the vagus nerve, which has similar anatomical projections as the trigeminal and occipital nerves, has also shown the ability to enhance plasticity in motor areas. However, it is unknown whether TNS and ONS can exert similar effects. This study explored the effects of TNS and ONS on visuomotor learning using frequencies in the kilohertz range (3 kHz). Eighty-four right-handed healthy subjects (27 F, 57 M; 18-34 y/a) were randomly assigned to one of 4 groups: TNS, ONS, TNS+ONS, and sham. Participants performed a visuomotor rotation task that involved goal-directed arm movements to 8 targets. Three blocks of trials were performed as follows: one baseline block with veridical hand visual feedback, one stimulation/sham block where no task was performed, one adaptation block involving a 30° CCW rotation of the visual feedback, and one washout block where the rotation was removed. Regarding the stimulation groups, TNS was delivered via two surface electrodes placed on the forehead, and ONS was applied via two rubber electrodes/soaked saline sponges to the back of the head. Stimulation was provided for 20 min, with the intensity self-selected by each subject. For the sham group, currents of 6 mA (TNS-sham) and 9 mA (ONS-sham) were applied for 60 s, then quickly reduced to 0 mA (active sham). Directional errors (DE), defined as the angular difference between the hand position and visual feedback at peak velocity, were averaged across groups and fitted to double exponential models to quantify motor learning. DEs decayed exponentially during the adaptation block for all groups, but preliminary analyses showed that the ONS group demonstrated faster learning rates among groups. On the other hand, the TNS group did not appear to be substantially faster than the sham group, in contrast to a previous study using a passive sham. Also, subjects who received concurrent stimulation learned faster than sham and TNS but slower than ONS, which implies there were no synergistic effects. These results suggest that ONS may have more beneficial effects on motor learning than TNS.

Disclosures: D.E. Arias: None. **C.A. Buneo:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.02/HH25

Topic: E.04. Voluntary Movements

Title: Cerebellum compensates to promote Visuomotor Learning in Adults with Parkinson's Disease

Authors: *P. C. IYER[1], G. SHIN[1], M. WALLACE[2], M. YAMAMOTO[1], B. E. FISHER[1]; [1]Physical therapy and Biokinesiology, USC, Los Angeles, CA; [2]Biochem., Loyola Marymount Univ., Los Angeles, CA

Abstract: Despite basal ganglia (BG) dysfunction in Parkinson's disease (PD), people with PD demonstrate intact learning capability. Therefore, we need to study the contribution of the neural substrates known to be involved in motor learning, such as the cerebellum (CB) and BG. Previous neuroimaging studies in people with PD showed increased cerebellar activity compared to age-matched control while performing motor tasks proposing a compensatory role of the CB. However, the idea that CB compensates to preserve motor learning in people with PD is yet to be tested. *Hence, in this pilot study, we assessed motor learning in a BG-driven reinforcement task (RT) and measured cerebellar activity using transcranial magnetic stimulation (TMS), i.e., cerebellar inhibitory output (CBI) to the motor cortex (M1).* We tested BG contributions in RT (which is an upper extremity reaching task) in which the goal was to successfully move through a target. Feedback was provided as success or failure for each trial. We tested a subject with PD (SwPD: 65years), and two young adults (YA: 29±2years) by applying visual perturbation of 25^0 during task performance (baseline: 40 trials| perturbation: 320 trials| retention: 40 trials). For successful learning, reach angles (RA; the angle between the target and the cursor) in the first 15 retention trials must differ from the last 15 baseline trials. We measured CBI using TMS at baseline and compared that to early perturbation trials (start of asymptote) and late perturbation trials. The Test Stimulus (TS) applied to flexor dorsal interossei hotspot in M1 at 65±7% Machine Stimulator Output (MSO) produced a motor evoked potential (MEP) of 1mV. The Conditioning Stimulus (CS) to the ipsilateral cerebellum (75%MSO) 5ms prior to the TS produced an MEP< 1mV and was indexed as CBI-ratio [CBI-ratio= $MEP_{amplitude}$ (CS-TS)/(TS_{only})]. As expected, *RA differed between retention and baseline trials* in the YA subjects (B:0.53^0±0.06| Ret: -12.67^0±2.13) and the SwPD (B:0.98^0|Ret: -10^0), indicating successful learning. The *CBI-ratio increased in the SwPD compared to the YA* in the early timepoint (SwPD: 0.89 mV| YA: 0.69±0.3 mV) and at the end (SwPD: 0.55 mV| YA: 0.55±0.09 mV). Together, these results provide preliminary evidence demonstrating cerebellar compensation in PwPD.

Disclosures: P.C. Iyer: None. **G. Shin:** None. **M. Wallace:** None. **M. Yamamoto:** None. **B.E. Fisher:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.03/HH26

Topic: E.04. Voluntary Movements

Support: NIH Grant R01 AG041878

Title: Cerebellar degeneration blocks long-term yet spares short-term sensorimotor memory

Authors: *A. M. HADJIOSIF, M. A. SMITH;
John A. Paulson Sch. of Engin. and Applied Sci., Harvard Univ., Cambridge, MA

Abstract: The cerebellum is critical for the formation of sensorimotor memories, with several studies showing that cerebellar damage impairs motor adaptation across a wide variety of tasks including force-field, visuomotor, locomotor, saccade, and speech adaptation. However, it is not clear whether this impairment is due to reduced ability to form long-term memories, short-term memories, or both. A clue to this question may lie in differences between the severity of impairment reported in previous adaptation studies - even when using the same task. Three force-field (FF) adaptation studies in reaching arm movements have observed impairment as low as 30% and as high as 80%. Interestingly, the two studies with the lowest impairment (30-40%, Gibo et al, 2013; Criscimagna-Hemminger et al, 2010) displayed short inter-trial time intervals (ITIs) of 5-7s on average, allowing short-term sensorimotor memories to contribute to overall performance. In contrast, the study with the highest impairment (80%, Smith and Shadmehr, 2005) displayed much longer ITIs of 20-25s on average. These long ITIs would drastically reduce the contribution of short-term sensorimotor memories, as these memories were previously found to decay rapidly ($\tau<20$ sec) with time. We thus hypothesize that severe cerebellar damage specifically impairs formation of longer term, temporally-persistent motor memories, yet spares short-term, temporally-volatile memories. This would lead to increased reliance upon short-term memories, and thus increasing impairment for longer ITIs which let these memories decay. To test this, we reanalyzed the data from Criscimagna-Hemminger et al (2010) and Gibo et al (2013). We first dissected memories of motor learning into temporally-persistent and temporally-volatile components based on prolonged ITIs due to rest breaks between blocks. Memory decay over these prolonged ITIs increased with cerebellar damage, in line with a difficulty to form temporally-persistent memories. Both temporally-persistent learning and the difference between temporally-persistent and temporally-volatile learning were significantly lower in patients vs. controls (both $p<0.005$). We then examined the effects of frequent but small variations in ITIs within experiment blocks finding that, in line with our hypothesis, the rate of memory decline with ITI was significantly ($p<0.01$) steeper for cerebellar patients. The finding that cerebellar degeneration impairs long-term sensorimotor memory but spares short-term memory suggests that the cerebellum may be a gateway for forming long-term procedural memories, analogous to the role of the medial temporal lobe for declarative memories.

Disclosures: A.M. Hadjiosif: None. **M.A. Smith:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.04/HH27

Topic: E.04. Voluntary Movements

Support: CIHR PJT 165987

Title: Newly learned movements are encoded in somatosensory cortex

Authors: *S. EBRAHIMI[1], D. J. OSTRY[2];
[2]McGill Univ., [1]McGill Univ., Montreal, QC, Canada

Abstract: Previous research has provided evidence for the involvement of the motor and somatosensory cortices in human motor learning and memory consolidation. However, their specific role in the retrieval of newly acquired movements remains unclear and requires further investigation. In the present study, we tested the hypothesis that newly learned movements are encoded in primary somatosensory cortex. Participants were trained using gradually introduced rotated visual feedback, where the visual feedback of their movements was rotated by 30 degrees. Continuous Theta Burst Stimulation (cTBS) was applied to different brain regions following learning, and this, in turn, was followed by tests of retention. The primary motor cortex (M1) and primary somatosensory cortex (S1) were targeted for stimulation, while the occipital cortex served as a control region. In retention testing, participants were assessed using active movements, and also recognition memory for learned movements, which involved passive movements produced by a robot arm. The findings of the study revealed that disruption of somatosensory cortex resulted in impaired memory retrieval for both active movement (analogous to recall in verbal memory) and for recognition memory as well. In contrast, the suppression of the motor cortex had minimal impact on memory retrieval as indicated by comparable retention levels in both the control and motor cortex conditions. Based on the results, it can be inferred that primary somatosensory cortex plays a crucial role in the encoding of newly acquired movements. The findings suggest that the somatosensory cortex is involved in the formation and storage of motor memories, enabling the retrieval of these memories during both recall and recognition memory tasks. On the other hand, the primary motor cortex appears to have little involvement in the initial encoding process of newly learned movements.

Disclosures: S. Ebrahimi: None. **D.J. Ostry:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.05/HH28

Topic: E.04. Voluntary Movements

Support: CIHR grant PJT-165987

Title: Contribution of Somatosensory Cortex to Motor Learning and Retention

Authors: *M. DARAINY[1], D. J. OSTRY[2,3];
[2]McGill Univ., [1]McGill Univ., Montreal, QC, Canada; [3]Child Study Ctr., Yale Sch. of Med., New Haven, CT

Abstract: This study tests for a function of somatosensory cortex, that, in addition to its role in processing somatic afferent information, somatosensory cortex plasticity underlies both motor learning and the stabilization of motor memory. Prior to force-field training, continuous theta-burst magnetic stimulation (cTBS) is applied, in order to disrupt activity in either primary somatosensory cortex, primary motor cortex or to a control zone over the occipital lobe. It was observed that for participants that received cTBS to somatosensory cortex, there was significantly less compensation for the applied force field during initial training, in comparison to subjects tested following cTBS to motor cortex or to a control zone over the occipital cortex. This progressive impairment in performance was present for much of the somatosensory condition and thus provides evidence that plasticity in somatosensory cortex contributes directly to motor learning. The kinematic differences between conditions serve to dissociate the roles of somatosensory and motor cortex in learning, and they likewise argue against the possibility that somatosensory cortex stimulation indirectly effects motor cortex. Retention testing was conducted at the start of the second session and was based upon forces applied during force-channel trials, without any other force application. Retention tests revealed a substantial loss of information when cTBS was applied to somatosensory cortex and a substantial though smaller loss of information following cTBS to motor cortex. Following cTBS to somatosensory cortex, retention was about 50% of that in the control condition, whereas following cTBS to motor cortex retention averaged 65% relative to controls. The drop in retention for somatosensory cortex presumably reflects both the initial impairment in learning and possibly an additional loss of information related to motor memory stabilization. In the case of motor cortex, the effects are wholly related to retention which indicates motor cortex participation in retention of motor memory.Taken together, the findings are consistent with the idea that motor learning is dependent on plasticity in somatosensory cortex and that newly learned movements are jointly encoded in somatosensory and motor cortex.

Disclosures: M. Darainy: None. **D.J. Ostry:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.06/II1

Topic: I.08. Methods to Modulate Neural Activity

Support: FWO SB fellowship 1S32421N
FWO-Project G0B4520N
NIH 1R01MH123508-01

Title: Transcranial direct current stimulation over primary motor cortex does not improve motor sequence learning in multi-session serial reaction time task

Authors: *S. KERSTENS[1], L. VAN BOEKHOLDT[1], H. VANDERHEYDEN[1], L. DE SMEDT[1], G. ALBOUY[2], B. R. KING[3], J.-J. ORBAN DE XIVRY[4], M. MC LAUGHLIN[4]; [1]Univ. of Leuven, Leuven, Belgium; [3]Univ. of Utah, [2]Univ. of Utah, Salt Lake City, UT; [4]KU Leuven, KU Leuven, Leuven, Belgium

Abstract: Transcranial direct current stimulation (tDCS) is a noninvasive neuromodulation method that applies a small DC current through stimulation electrodes on the scalp aiming to modulate neural activity. Until recently, it was assumed that tDCS elicits its neuromodulatory effects by increasing cortical excitability due to direct polarization of cortical neurons. However, recent studies have shown that the resulting electric field that reaches the cortex is relatively weak and suggested that a stronger electric field in the scalp may stimulate peripheral nerves that give input to the reticular activating system in the brain stem. Activation of this system in the brain stem via stimulation of peripheral nerves can also indirectly affect cortical excitability and plasticity in tDCS.
Many studies have shown that tDCS can improve motor sequence learning, also indicated as the most reliable tDCS effect in many tDCS reviews. Therefore, we aimed to investigate if tDCS motor sequence learning effects are caused by direct polarization of cortical neurons or by indirect peripheral nerve stimulation (or a combination of both) in a motor sequence learning paradigm. To separate the two potential tDCS mechanisms, we developed a novel tDCS control condition, referred to as transcranial-only tDCS, in which the peripheral input is blocked using topical anesthetics. In a double-blinded preregistered study with 99 healthy volunteers, we compared the effect of 1mA anodal tDCS over the left primary motor cortex (M1) on motor sequence learning in a multiple-sessions right-handed serial reaction time task (SRTT) to sham stimulation and transcranial-only tDCS. Our results showed that tDCS did not improve motor sequence learning in the SRTTs compared to sham and to-tDCS: although all stimulation groups significantly improved in motor sequence learning each session compared to the previous sessions and the baseline performance, no significant differences were observed between the stimulation groups.
In conclusion, we were unable to replicate the effect of tDCS on motor sequence learning as described in earlier tDCS studies, despite our strong experimental design and substantial sample size. Consequently, we could not test if the tDCS effects were caused by the electric field in the brain or by indirect stimulation of peripheral nerves in the scalp. Although other studies had already highlighted issues concerning reproducibility of tDCS results, our findings are still highly important for the tDCS field as they highlight again the importance of experimental design, substantial sample sizes, preregistration of studies, and standardization in tDCS research.

Disclosures: S. Kerstens: None. **L. van Boekholdt:** None. **H. Vanderheyden:** None. **L. De Smedt:** None. **G. Albouy:** None. **B.R. King:** None. **J. Orban de Xivry:** None. **M. Mc Laughlin:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.07/II2

Topic: E.04. Voluntary Movements

Support: Penn State Dorothy Foehr Huck and J. Lloyd Huck Endowment for Kinesiology and Neurology (Prof. Sainburg)
NIH Grant R01HD059783
AHA Grant 16GRNT31010001

Title: Concurrent anodal HD-tdcs to the left and the right posterior-parietal cortex (PPC) have opposite effects in the early phase of learning a direction dependent skill task with the dominant right arm.

Authors: *J. YUK[1], R. SAINBURG[1,2];
[1]Kinesiology, Penn State Univ., University Park, PA; [2]Neurol., Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: Previous evidence suggests that direction learning in a motor task is specialized to the dominant hemisphere/arm. Following visuomotor rotation (VMR) adaptation, direction learning transfers asymmetrically between the arms. Emerging evidence from lesion studies has indicated that the left posterior parietal cortex (LPPC) plays a critical role in direction learning with either dominant right and non-dominant left arm. Also, when HD-tdcs was applied to the LPPC, the stimulation disrupted late phase of learning in dominant right arm and modulated the intermanual transfer. However, these findings have been limited to adaptation paradigms, in which the task requires learning to move in the presence of a perturbation. We now examine whether LPPC also mediates motor direction learning and intermanual transfer in a non-perturbation skill-task. We present a virtual air hockey task in which the player controls a cursor to strike a puck toward a target. The puck direction was determined as the velocity component transferred to the puck at impact that passes through the center of the puck. The puck distance was determined by the amplitude of the vector component of cursor velocity toward that direction with a virtual friction coefficient. Participants were instructed to initiate the movement from starting locations located at 45° and 135° relative to the initial puck location. However, the design of the task rendered puck distance irrelevant to task success, as the target was determined completely by direction but not the distance of the puck. A total of 60 participants were randomly assigned to three groups: Left PPC (LPPCS), Right PPC (RPPCS), and sham stimulation groups. Participants practiced the task initially using the right arm concurrent with 20 minutes of 2 milliamp HD-tDCS. Following this, participants used the left arm to assess transfer. Based on our hypothesis, we predicted better task learning and inter-manual transfer in the LPPCS group compared to other groups. Our data support this prediction for the early phase of learning. In contrast, the RPPCS group showed impaired performance during the early stage of learning. We found no difference between the groups in inter-manual transfer. We conclude that the LPPC plays a critical role in contralateral learning of a skill task that is dependent on direction control. Interestingly, in the skill task LPPC stimulation affected the early phase of learning, while previous studies of VMR have indicated

an effect of LPPC on the later phase of learning, or slow component of learning. Further research is necessary to determine why contralateral PPC differentially effects these two phases of learning in skill vs adaptation tasks.

Disclosures: J. Yuk: None. **R. Sainburg:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.08/II3

Topic: E.04. Voluntary Movements

Support: NIH Grant NS112367

Title: Frontal and parietal contributions to proprioception and motor skill learning

Authors: *M. WALI, H. J. BLOCK;
Indiana Univ., Bloomington, IN

Abstract: Proprioception, our sense of our limbs in space, is crucial for making accurate movements. Deficits in proprioception reduce movement accuracy and increase variability which diminishes motor functions. The neural basis of these interactions is not fully understood, but likely involves numerous brain regions. One possibility is Dorsolateral Prefrontal cortex (DLPFC). Modulating DLPFC using anodal tDCS increased the functional connectivity between the somatosensory cortex and DLPFC, which was linked to improved proprioception and motor performance (Stagg et al., 2013). Posterior parietal cortex (PPC), an interface between the sensory and motor cortex, is involved in processing higher-level proprioceptive information. Decreased PPC function, specifically supramarginal gyrus (SMG), might be associated with decreased position sense (Shabat et al., 2015; Findlater et al., 2016). Here we compare the role of activity in SMG and DLPFC to modulate proprioception in the context of affect motor skill learning. We used continuous theta burst transcranial magnetic stimulation (cTBS) to modulate activity in SMG, DLPFC, or sham, before participants perform an upper limb motor skill learning task using the Kinarm Endpoint robotic manipulandum. After a familiarization session, subjects are randomly assigned to one of three experimental groups: DLPFC, SMG and Sham. Planned enrollment is 24 per group. Anatomical brain scans are taken to locate subject-specific target positions for the participants in the DLPFC and SMG groups. During the main experimental session, participants first perform assessments of proprioception and motor skill (speed-accuracy tradeoff) to get baseline measures (Pre), followed by cTBS over the target region and then another set of behavioral assessments post intervention (Post 1). Participants then train on the motor skill, a maze-tracing task, for 120 trials, with another motor skill assessment after 40 trials. The experiment ends with another set of behavioral measures (Post 2 for Proprioception and Post 3 for motor skill). In a similar task (Mirdamadi and Block, 2020), subjects who did not receive cTBS improved in-track accuracy by ~5% after 120 training trials.

In comparison, one pilot subject who received cTBS over DLPFC improved in-track accuracy by 5% after 120 training trials, similar to subjects who did not receive cTBS. One pilot subject who received cTBS over SMG reduced in-track accuracy by 3% after 120 training trials. These preliminary results are consistent with a role for SMG in this form of motor skill learning, potentially by way of SMG's importance for proprioception.

Disclosures: M. Wali: None. **H.J. Block:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.09/II4

Topic: E.04. Voluntary Movements

Support: NIH Grant R01HD059783
AHA Grant 16GRNT31010001
Dorothy Foehr Huck and J. Lloyd Huck Distinguished Chair of Kinesiology and Neurology (Prof. Sainburg)

Title: Posterior parietal cortex stimulation inhibits transfer of visuomotor learning from the non-dominant to dominant upper limb: An anodal HD-tDCS study

Authors: *N. M. KITCHEN[1,2], B. DEXHEIMER[3,2], C. W. SANDBERG[2], R. SAINBURG[2,1]; [1]Penn State Univ., Hershey, PA; [2]Penn State Univ., State College, PA; [3]Virginia Commonwealth Univ., Richmond, VA

Abstract: The posterior parietal cortex (PPC) has been shown as a critical neural region for integrating sensory feedback necessary for both adjusting ongoing movements and adapting movements to novel task conditions. We previously reported that focal lesions to left PPC disrupts visuomotor learning in both arms, demonstrating a lateralized role of this cortical region, suggesting that targeting left PPC with non-invasive brain stimulation may be a beneficial means of supplementing rehabilitation from neurological disease. To explore this, we recently conducted a study in which we targeted PPC with excitatory (anodal) high-definition transcranial direct current stimulation (HD-tDCS) in young healthy individuals during a visuomotor rotation task. We found that, despite stimulation having no effect on initial learning, left PPC stimulation facilitated transfer of learning from the dominant, right arm (DA) to the non-dominant, left arm (NDA). We now replicate this study under reversed conditions of interlimb transfer (training the NDA and transfer to the DA) to examine whether left PPC stimulation has a robust lateralized effect on interlimb transfer of visuomotor learning.
We recruited 60 young, right-handed, neurotypical adults who received 20min of stimulation (either anodal left PPC, anodal right PPC or sham stimulation; all n = 20) while making targeted reaches with their NDA, first under veridical (baseline) and then 30deg rotated (adaptation) visual feedback conditions. This was followed immediately by adaptation with the DA to assess

interlimb transfer of learning. Similar to our prior experiment, we found no effect of stimulation on the initial learning of the NDA. However, we did find a mediating effect of stimulation on interlimb transfer, such that both left and right PPC stimulation *reduced* transfer of learning to the DA, compared with sham stimulation - a stark contrast from our previous finding of left PPC stimulation facilitated transfer of learning from the DA to the NDA. We suggest these results indicate an asymmetric role of posterior parietal cortices for interlimb transfer of visuomotor learning, potentially due to differences in the recruitment of ipsilateral and contralateral cortices during visuomotor learning with either the DA or NDA.

Disclosures: N.M. Kitchen: None. **B. Dexheimer:** None. **C.W. Sandberg:** None. **R. Sainburg:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.10/II5

Topic: E.04. Voluntary Movements

Support: Florence Blondiaux is a FRIA grantee of the Fonds de la Recherche Scientifique – FNRS (Be)
Lise Colmant is a research Fellow of the Fonds de la Recherche Scientifique - FNRS (Be)
The FNRS provided salary and research support for Bernard Hanseeuw under grant number CCL40010417
The FNRS provided salary and research support for Bernard Hanseeuw under grant number FRFS-WELBIO40010035
Frédéric Crevecoeur is supported by a grant from the FNRS under grant number 1.C.033.18

Title: Preserved long-latency feedback responses in Essential Tremor implies internal stabilisation deficits

Authors: *F. BLONDIAUX[1], L. COLMANT[2], L. LEBRUN[2], G. DUCHÊNE[3], L. DRICOT[2], B. J. HANSEEUW[2], F. CREVECOEUR[1];
[1]UCLouvain, Louvain-la-Neuve, Belgium; [2]UCLouvain, Brussels, Belgium; [3]MR applications, Gen. Electric Healthcare, Diegem, Belgium

Abstract: Essential tremor (ET), one of the most common movement disorder, is often characterized by involuntary oscillations of the limbs during movement. The pathophysiology of this disorder remains not fully understood. Several studies reported general deficits in motor adaptation and our previous work on upper limb and saccadic adaptation suggested that these impairments were linked to movement execution while the initial movement components related to anticipation were preserved. To better understand the role of feedback control, we investigated

feedback responses to mechanical loads in 24 ET patients and 28 age-matched healthy volunteers (HV). We measured hand kinematics, applied forces to the handle (KINARM) and surface muscular activity (EMG). After maintaining the hand in a target, participants experienced step perturbations of unpredictable direction and amplitude and were instructed to counter the perturbation and steer their hand back to the starting position. ET patients experienced deficits when stopping and stabilizing their hand in the final target. ET patients oscillated around the target resulting in longer path length and increased stopping time. The deficits were dependent on perturbation magnitude and therefore linked to feedback control. The early muscular responses (Long Latency Responses - LLR, 45-105ms after perturbation onset) of ET patients were preserved following the perturbation, suggesting intact sensory feedback and initial transcortical pathways. We showed that these end-of-movement difficulties with intact LLRs could be explained by a model of upper limb control with erroneous compensation for the sensorimotor delay. Because delay-compensation has been linked with cerebellum, we segmented the Dentate Nucleus using Quantitative Susceptibility Mapping (QSM) and measured its resting state functional connectivity. We expect to provide preliminary results linking dentate nucleus connectivity and deficits in limb control.

Disclosures: **F. Blondiaux:** None. **L. Colmant:** None. **L. Lebrun:** None. **G. Duchêne:** A. Employment/Salary (full or part-time):; GD was employed by GE Healtcare at the moment of the study. He participated to the latter as scientific support and had no direct financial interest in it.. **L. Dricot:** None. **B.J. Hanseeuw:** None. **F. Crevecoeur:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.11/II6

Topic: E.04. Voluntary Movements

Support: DBT IISc Partnership Program (awarded to Aditya Murthy)
Prime Minister's Research Fellowship (awarded to Niranjan Chakrabhavi)

Title: Trajectory control of goal directed reaching movements and its implications in Ataxia and Parkinson’s Disease

Authors: *N. CHAKRABHAVI[1], P. SINGH[1], A. LENKA[2], A. STEZIN[2], K. JHUNJHUNWALA[2], P. K. PAL[2], A. GHOSAL[1], A. MURTHY[1];
[1]Indian Inst. of Sci., Bengaluru, India; [2]Natl. Inst. of Mental Hlth. and Neurosciences, Bengaluru, India

Abstract: Goal directed reaching movements are attributed with straight-line trajectories and bell-shaped velocity profiles. There has been a debate in the field as to whether these invariant kinematic patterns are planned by the central nervous system (CNS) or are they derived from optimizing a cost function. The current work addresses this problem using trajectory

decorrelation during the movement and we showed that the CNS indeed cares about an average trajectory and that the control was significantly higher in goal movements as compared to non-goal movements. The differences in control between goal and non-goal movements emerged very early on, providing the first behavioral evidence of internal-feedback control in reaching movements. We also found two distinct phases of control - at the early and the late time points, demonstrating that the trajectory control was discreet in nature. Further, we performed decorrelation analysis on different task conditions and did not find significant differences in trajectory control among goal-directed movements of dominant and non-dominant arms or across different speeds of movement (fast and slow). We went on to test this on clinical patients with autosomal dominant cerebellar ataxia and Parkinson's Disease and found that the trajectory control was impaired distinctly in both cohorts as compared to healthy age-matched subjects, suggesting deficits in the ability to utilize feedback control.

Disclosures: **N. Chakrabhavi:** None. **P. Singh:** None. **A. Lenka:** None. **A. Stezin:** None. **K. Jhunjhunwala:** None. **P.K. Pal:** None. **A. Ghosal:** None. **A. Murthy:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.12/II7

Topic: E.04. Voluntary Movements

Title: Whole-brain activity patterns underlying uninstructed behavioral switching in mice

Authors: *P. WANKEN[1,2], B. J. EDELMAN[1,3], A. ABLITIP[1], J. MARTINEZ DE PAZ[1,2], E. MACÉ[1,2];
[1]Brain Wide Circuits for Behavior, Max Planck Inst. for biological Intelligence, Munich, Germany; [2]Dept. of Ophthalmology, Univ. Med. Ctr., Goettingen, Germany; [3]Emotion research department, Max Planck Inst. for Psychiatry, Munich, Germany

Abstract: The ability to switch between different behaviors is essential to all animals' survival. Behavior selection is guided by multiple factors, such as sensory inputs, internal states, and memory, which suggests that many regions across the brain are involved in the decision to switch. While whole-brain information is necessary to investigate the neural basis of behavioral switching, brain-wide imaging in behaving mice has proved challenging. We employed functional ultrasound imaging (fUS) to record large-scale neural dynamics in head-fixed mice while simultaneously tracking their behavioral state. Our aim was to identify brain regions that predict self-initiated transitions of behavior occurring in the absence of external triggers. Accordingly, we utilized the virtual burrow assay in which head-fixed mice are placed in an air-floating tube, from which they can voluntarily egress. Leveraging the unsupervised behavior segmentation framework VAME, we found that mice (N = 11, 60 sessions) robustly exhibit distinct, uninstructed behavioral states in this assay, including egress, whisking, inactivity, and grooming. Utilizing brain-wide fUS, we subsequently observed activity patterns associated with

these distinct behavioral states and performed whole-brain time-resolved decoding around behavioral transitions. Remarkably, our results revealed that whole-brain activity can predict a spontaneous egress event seconds before its onset, indicating that a change in uninstructed behavior is preceded by a detectable change in brain state. Furthermore, region-wise decoding revealed specific brain areas driving the prediction of behavioral transitions. Through this unbiased approach, our work sheds light on the neural dynamics preceding uninstructed transitions of behavioral state.

Disclosures: P. Wanken: None. **B.J. Edelman:** None. **A. Ablitip:** None. **J. Martinez de Paz:** None. **E. Macé:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.13/II8

Topic: E.04. Voluntary Movements

Support: LR20C070002
32150015

Title: The morphological and functional analysis of vestibulospinal neurons

Authors: *J. XIE, L. GAO;
Westlake Univ., Hangzhou, China

Abstract: Vestibulospinal tract (VST) is one of dominant descending tract, which originate from vestibular nuclei (Ve). Previous studies had reported the Ve neurons were correspondence to head, eye movement and locomotion. Among them, the locomotion related results were concluded based on electrophysiology. There was lack of direct behavior evidence related VST's specific function on locomotion. Therefore, it is necessary to investigate the morphology and functional analysis of Ve neurons in the spinal cord comprehensively. In this study, we first used tissue clearing and tiling light sheet microscopy to investigate the projection pattern of vestibular neurons in the whole spinal cord from three- dimensional perspective, and found the vestibulospinal tract could project to both ipsilateral and contralateral of spinal cord. In order to know the branch pattern of the axons, we sparse labelled the axons and traced the labelled axons. Surprisingly, we found abundant branches in C5-T2 in bilateral spinal cord. Furthermore, we found the branches in ipsilateral accumulated at mid-ventral while branches in contralateral distributed in mid-ventral and lateral of spinal cord. To better understanding the functions of the two population neurons, we used chemogenetics experiment combing various behavior tests to test the functions on motor behaviors and motor skills. We measured the total path length and locomotion speed and found there were no obvious difference between the control group and ipsi-/contralateral CNO group. During the beam walking analysis, the cross duration had no significant change after silenced the neurons in three groups. Next, we analyzed the neurons'

function in skill locomotor tasks using accelerating rotarod and single pellets skill reaching task. The results showed the mice behaved impairment in skill performance due to silencing the ipsilateral neurons while as the silencing the contralateral neurons induced obstacle in skill learning. Finally, in order to know whether the Ve axons had synaptic connection with motor neurons, AAV-hSyn -tdTomato was inected in right Ve area of ChAT-EGFP mice. We performed the tissue with hydro-gel baes tissue expansion, and expanded the tissue 3-4 folds, then imaged the intact cervical region under tiling light sheet microscopy. we detected abundant synaptic connections between the Ve axons and motor neurons. This result hinted the Ve neurons connected with motor neuron and drive the forelimb muscles directly. To sum up, our study deeply investigated the morphology and function of the VST and further offered a new method to investigate the descending tract from three-dimensional perspective.

Disclosures: J. Xie: None. **L. Gao:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.14/II9

Topic: E.04. Voluntary Movements

Support: FWO Research Grant G097818N
GSKE/FMRE Queen Elizabeth Medical Foundation for Neuroscience, Young Investigator Grant to AT
ERA-NET NEURON

Title: Cell type-specific visual information routing via the Superior Colliculus regulates forelimb-reaching movements.

Authors: *G. VICENTE-ORTIZ[1,2,3], P.-A. SURAY[1,2,3], M. D'ANDOLA[1,2,3], A. TAKEOKA[1,2,4,3];
[1]Neuro Electronics Res. Flanders (NERF), Leuven, Belgium; [2]Dept. of Neurosciences, KU Leuven, Leuven, Belgium; [3]Leuven Brain Inst., Leuven, Belgium; [4]IMEC, Leuven, Belgium

Abstract: Optimizing motor performance relies on comparing feedforward predictive signals to sensory inputs to update motor commands. In this context, visual information is critical in providing spatial coordinates for goal-directed behaviors. However, the contribution of visual information to motor performance remains unclear, as individuals who are blind or have closed eyes can execute reaching behaviors without apparent motor deficits. To determine the visual circuit components required for executing goal-directed reaching behavior, we first quantify single-pellet reaching performance of intact mice, mice carrying a retinal degeneration 1 mutation (*Pde6b^{rd1}; RD1* mutants), and mice with lesioned primary visual cortex. We find that lack of visual inputs entering the brain in *RD1* mutants impaired limb and postural kinematics and deteriorated reaching performance. However, lesioning the primary visual cortex has

minimal effect. Muscimol silencing of three major retinorecipient areas reveals that suppressing Superior Colliculus uniquely disrupts the execution of the reaching behavior. Further cell type-specific ablation experiments highlight the critical role of narrow-field neurons in the superficial Superior Colliculus (sSC), which project to deeper layers of the SC for executing reaching movements. Using a combination of mouse genetics and viral tracing techniques, we demonstrate that narrow-field neurons preferentially contact Pontine Reticular Nucleus (PRN: PnO+PnC) projection neurons residing in the caudal intermediate Superior Colliculus (iSC). Selective silencing of iSC-PRN projection neurons using chemogenetics to assess the functionality of the iSC-PRN circuit reveals that acute silencing degraded endpoint precision during reaching. Collectively, our findings elucidate a visual pathway critical for performing reaching behaviors from the retina to the PRN through the Superior Colliculus. Additionally, cell type-specific viral tracing uncovers recurrent circuits between the PRN and the iSC, suggesting a potential reciprocal activity modulation between the two areas. Moreover, the PRN receives direct inputs from sensorimotor cortical regions, potentially carrying signals related to motor intention. Taken together, this circuit organization places the Superior Colliculus in a prime position to act as a comparator, integrating visual information from the retina and an efference copy of motor commands from the PRN. This integration enables the comparison between feedforward predictive signals and sensory inputs, where the superior colliculus serves as a critical contributor to optimize motor performance.

Disclosures: G. Vicente-Ortiz: None. **P. Suray:** None. **M. D'Andola:** None. **A. Takeoka:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.15/II10

Topic: E.04. Voluntary Movements

Support: Wu Tsai Human Performance Alliance
Joe and Clara Tsai Foundation
Knight Campus Undergraduate Scholars

Title: Neural representational models of reference frame transformation for skilled action

Authors: *A. A. M. HARRIS CACERES[1], J. SMITH[1], M. MARNEWECK[2];
[1]Univ. of Oregon, Eugene, OR; [2]Human Physiol., Univ. of Oregon, EUGENE, OR

Abstract: Every action, from picking up your phone to dribbling a basketball, requires the ability to transform positional information of the object with reference to one's eye, body, and hand into an actionable plan to interact with the object. This process of encoding reference frames occurs continuously and seamlessly in our everyday life; however, it is understudied in humans as many previous studies have only investigated the neural code by which reference

frames are represented in non-human primates. In this experiment, we address this gap by measuring fMRI activity in human subjects while they engage in a button-pressing task that dissociates reference frames between the eye and target, the hand and target, and the hand and eye. There are 8 conditions that isolate spatial activity pattern differences between small and large distances for each of the reference frames of interest. Preliminary results show an overlap in the spatial representation of reference frames within the posterior parietal cortex. Translating and understanding the neural encoding of sensory information into an actionable plan could improve neural prosthetics to better serve patients such as amputees.

Disclosures: A.A.M. Harris Caceres: None. **J. Smith:** None. **M. Marneweck:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.16/II11

Topic: E.04. Voluntary Movements

Support: NIH/NINDS R01NS131662
NTRAIN/NICHD K12HD093427
SNSF P2EZP3_191858

Title: Cortico-brainstem vs. corticospinal projections and their role in motor control and functional recovery after injury

Authors: *J. KAISER[1], A. LAMMERS[1], P. PATEL[1], E. ZITRIN[1], E. SUNG[1], A. IQBAL[1], V. V. SAHNI[1,2,3];
[1]Burke Neurolog. Inst., White Plains, NY; [2]Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [3]Weill Cornell Grad. Sch. of Med. Sci., New York, NY

Abstract: Skilled motor control requires precise connections between subcerebral projection neurons (SCPN) in the cerebral cortex and their appropriate subcerebral targets in the brainstem or spinal cord. The brainstem has been widely recognized as a key integration and processing hub between “upper” motor centers and spinal circuits involved in execution of movements. Cortical inputs to the brainstem may fine-tune movement execution and support motor planning and are established by two SCPN supopulations: 1) cortico-brainstem neurons (CBN) that limit their axon extension to supraspinal levels, directly innervating brainstem nuclei, and 2) corticospinal neurons (CSN) that extend their axons into the spinal cord but additionally collateralize within the brainstem. Classical anatomical tracing approaches are not able to distinguish CBN from CSN, since these distinct subpopulations reside interdigitated in cortex. To date, this limitation has precluded investigation of CBN function in skilled motor movement execution and planning.
We used a combination of transcriptomics, tracing, and imaging techniques to identify the molecular program that distinguishes developing CBN vs. CSN at the time point of initial axon

extension. We identified that Neuropeptide Y (Npy) is specifically enriched in CBN in lateral cortex, while CART prepropeptide (Cartpt) delineates cervical-projecting CSN. This now provides, for the first time in the field, molecular access to specifically investigate direct cortical input into the brainstem by molecularly distinct SCPN subpopulations using established Cre mouse lines. Our preliminary results suggest a topographic organization of axonal collaterals from these distinct subpopulations within the brainstem, suggesting a functional difference between them. Selective silencing of Npy+ CBN within the lateral cortex will allow us to investigate the contribution of CBN specifically to skilled motor movements. This will further enable investigation into the functional impact of CBN specifically on motor recovery after central nervous system damage such as spinal cord injury. Our results provide a foundation for future studies aiming to unravel the complexity of cortico-brainstem and cortico-spinal circuits, and their contribution to movement control in health and disease.

Disclosures: J. Kaiser: None. **A. Lammers:** None. **P. Patel:** None. **E. Zitrin:** None. **E. Sung:** None. **A. Iqbal:** None. **V.V. Sahni:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.17/II12

Topic: E.04. Voluntary Movements

Support: NIH Grant NS105697

Title: Neural oscillations in motor and dorsal premotor cortex in monkeys

Authors: *R. SEBASTIAN[1], N. CHEHADE[2], O. A. GHARBAWIE[2];
[1]Bioengineering, [2]Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The motor system has characteristic neural oscillations. It is well-established that in sensorimotor cortex, beta band (15-40 Hz) power is relatively high in the absence of movement and during isometric force. Movement onset is affiliated with beta power attenuation, which gives way to gamma (80-120 Hz) power increase. An intriguing feature of this dynamic is that beta attenuation spans sensorimotor areas and somatotopic zones (i.e., rostro-caudal and medio-lateral cortical axes), whereas gamma increase is considerably more focal. The spatiotemporal modulations in beta and gamma have animated many interpretations of the interplay between cortical oscillations and movement. Nevertheless, most of the supporting evidence has been acquired with neural recording tools that provide limited spatial resolution (e.g., MEG, EEG, ECoG) or limited spatial coverage (e.g., Utah array). This motivated us to investigate cortical oscillations with invasive, high-density recordings, during arm and hand movements. Two macaque monkeys performed an instructed task with 3 conditions: (1) reach-to-grasp, (2) reach only, and (3) no movement. In the contralateral hemisphere, we mapped the forelimb representations in motor (M1) and dorsal premotor (PMd) cortex with intracortical

microstimulation. We used the motor map to guide acute penetrations of 32-channel linear electrode arrays (100 μm between sites). We recorded neural signals from 215 penetrations (across monkeys) that were in arm and hand zones of M1 and PMd. Multi-taper spectrograms of the local field potentials (LFPs) were used to quantify changes in beta and gamma as a function of movement. Results from the movement conditions uncovered several unexpected findings. (1) There was no change in beta power in 49% of the penetrations. (2) Beta power attenuation was limited to only 29% of the penetrations. (3) Beta power even increased in a comparable number of penetrations (22%). In contrast, gamma power increased in 61% of penetrations, which was consistent with the proportion of sites with spike rate modulations. Our results do not support the prevailing perspective of spatially widespread beta power attenuation with movement onset. Instead, a more complex interplay likely exists between beta modulation, cortical architecture, and movement. We are now exploiting the same dataset to understand how somatotopic zones and cortical layers contribute to the spatio-temporal organization of beta and gamma modulation. Determining the principles that govern these oscillations would provide insight into neural coding and information flow in sensorimotor cortex.

Disclosures: **R. Sebastian:** None. **N. Chehade:** None. **O.A. Gharbawie:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.18/II13

Topic: E.04. Voluntary Movements

Support: NIH Grant 1DP2NS111817-01

Title: Neurons in primate premotor cortex display mixed selectivity with respect to reach direction and grasp type

Authors: *M. MAKWANA, J. HYNES, N. TOLLEY, I. PENIDO, J. P. DONOGHUE, C. E. VARGAS-IRWIN;
Dept. of Neuroscience, and Carney Inst. for Brain Sci., Brown Univ., PROVIDENCE, RI

Abstract: Skilled, voluntary reaching and grasping behaviors in primates emerge from multi-scale cortical network activity patterns that combine sensory and internal information to generate an integrated action, but how groups of neurons work together to compute their output is not well understood. The ‘dual path’ view suggests that reach direction and grasp types are separately planned in dorsal (PMd) and ventral (PMv) areas, however neurons with reach and grasp properties have been reported at both cortical sites. In this study, we employed a novel approach to identify related groups of premotor cortex neurons (PMv and PMd) from monkeys recorded using multichannel arrays in a reach-to-grasp task that varied grasp type (power vs. key), orientation (horizontal vs. vertical), and position in space. We quantified the relationships between neuron firing patterns using SIMNETS[1], a framework that compares the

computational properties of individual neurons by assessing the congruence between their individual latent spaces, which are generated using spike train similarity metrics. Our results show that although individual neurons display heterogeneous encoding properties, they can be organized into functional clusters with distinct combinations of features. In the one monkey analyzed so far, we found grip related clusters both invariant to, as well as sensitive to position. Clusters have members from both PM areas, potentially explaining the intermingling of reach and grasp related neurons previously encountered in both PMv and PMd. This initial observation reveals context-related mixed selectivity that suggest the existence of combinatorial subnetworks spanning PM for organizing coordinated reach and grasp behavior. Further, SIMNETS appears to be a tool useful to assess subnetwork organization independent of coded variables and it is useful to develop decoding approaches to improve neuroprosthetic devices and rehabilitation strategies aimed at restoring dexterous hand function.
1. Hynes, J. B., et al. (2023). bioRxiv (p. 463364). https://doi.org/10.1101/463364

Disclosures: M. Makwana: None. **J. Hynes:** None. **N. Tolley:** None. **I. Penido:** None. **J.P. Donoghue:** None. **C.E. Vargas-Irwin:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.19/II14

Topic: E.05. Brain-Machine Interface

Support: NIH R01NS123663
Chen Neuroscience Institute
HHMI

Title: Neural code for hand action in intraparietal cortex of non-human primate

Authors: *L. SHE, S. LEE, M. SHAPIRO, D. A. WAGENAAR, R. A. ANDERSEN; Caltech, Pasadena, CA

Abstract: The intraparietal area (IPS) in non-human primates (NHPs) is a brain region involved in planning hand actions. It encodes the hand shape, arm, and objects involved in the actions, as well as abstract concepts such as observed actions. Recent studies have shown that the homologue of anterior IPS in humans also encodes the action of other body parts, observed actions and action verbs, and objects in allocentric coordinates. These findings suggest that the IPS is part of a world model represented in our brain. To study the neural code in IPS of NHPs in detail, we developed a visually guided reach and grasp task and a 3D hand tracking system using stereo cameras. Using functional ultrasound (fUS) imaging, we mapped in detail the parietal reach region (PRR) located in the medial bank of the intraparietal sulcus, consistent with the existing literature using single neuron recordings. With fUS imaging we found that a larger area of PRR was active during precise reaches than less precise reaches. After mapping functional

subregions in this area, we will further study the neural code by recording the activity of large populations of neurons using a neuropixels probe. This study will provide new insights into the neural basis of hand action, improve our understanding of how the brain represents the world, and develop better brain-machine interfaces and intelligent systems that can learn and generalize effectively.

Disclosures: **L. She:** None. **S. Lee:** None. **M. Shapiro:** None. **D.A. Wagenaar:** None. **R.A. Andersen:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.20/II15

Topic: E.04. Voluntary Movements

Support: 2019M3E5D2A01060293

Title: Neural dynamics of primary motor cortex and posterior parietal cortex during updating of unexpected changes in visual target location

Authors: ***M.-K. KIM**[1], S. KIM[3], H. PARK[1], J.-W. SOHN[2];
[1]Catholic Kwandong Univ., Suwon, Korea, Republic of; [2]Catholic Kwandong Univ., Gangneung-si, Korea, Republic of; [3]Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Posterior parietal cortex (PPC) is involved in sensory-motor transformations with a high degree of plasticity in planning and controlling arm movements in response to changes in visual target location. However, the reciprocal switching between primary motor cortex (M1) and PPC during updating plans with dynamic motor errors in response to unexpected changes in visual target location remains unclear. In this study, we investigate the neural population dynamics of M1 and PPC and the functional connectivity between them during unexpected changes in visual target location. To this end, we collected neural responses from M1 and PPC of two primates performing a five radial target center-out reaching task in which an initial (fake) target is followed by a randomly changed (true) target. The preliminary results show that PPC exhibits an immediate and distinct neural response to the degree of target change compared to M1. Specifically, M1 neurons faithfully engaged in all movements, while PPC was more selectively excited by the first perceived target directions rather than subsequent targets. We also found that M1 and PPC interact with each other in terms of neural subspace, depending on the sequential appearance of the target directions. These results suggest that dynamic changes in the interface between visual targets and ballistic arm movements may provide useful clues for the development of state-of-the-art decoding algorithms for brain-machine interfaces that respond quickly to unexpected target changes.

Disclosures: **M. Kim:** None. **S. Kim:** None. **H. Park:** None. **J. Sohn:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.21/II16

Topic: I.08. Methods to Modulate Neural Activity

Support: CBN Pilot Project

Title: Examination of neurophysical alterations associated with tDCS in a rodent model used to study PTSD

Authors: *A. BOLEY[1], S. M. PEREZ[1], K. H. BUCCELLATO[2], C. L. STRAUD[3], D. J. LODGE[1], D. MORILAK[1], F. R. CARRENO[1];
[1]Pharmacol., [3]Psychiatry & Beh Sci., [2]UT Hlth. San Antonio, San Antonio, TX

Abstract: In the U.S. ~16 million people experience a traumatic event and will develop posttraumatic stress disorder (PTSD). Symptoms of PTSD can be debilitating and include fear, anxiety, and uncontrollable negative thoughts. Unfortunately, current interventions for PTSD have limited efficacy. The structures involved in processing of fear related information are well known and include the prefrontal cortex (PFC), amygdala, and the hippocampus. The PFC is central to cognitive flexibility, extinction of conditioned fear, and volitional regulation of negative emotion - all of which are altered in PTSD. Importantly, hypoactivity of the PFC is a consistent observation in functional imaging studies and in rodent models used to study PTSD. Advances in non-invasive brain stimulation allow for a novel approach to modulate PFC activity. Specifically, transcranial direct current stimulation (tDCS) is an approach to neuromodulation that targets superficial areas of the brain. Anodal stimulation causes depolarization and increases in neural excitability, which can increase prefrontal cortical function. We posit that anodal tDCS will reverse PFC hypoactivity and may serve as a novel therapeutic approach for the treatment of PTSD. Here, we apply tDCS to the PFC of rats to investigate the neurophysiological alterations in brain activity. We observed an increase in c-fos expression in rats given tDCS demonstrating activation of the PFC. Further, we investigated a downstream, stress sensitive brain region and found that tDCS increased dopamine neuron activity in the ventral tegmental area (VTA), where decreases are thought to underlie anhedonia. In a rodent model of PTSD, previous studies have shown chronic unpredictable stress (CUS) induces a dramatic decrease in PFC responsivity, as measured by *in vivo* electrophysiology. In the CUS rodent model, tDCS was applied as an adjunct treatment during sub-effective extinction and enhanced efficacy in a cognitive flexibility task, attentional set shifting (AST). This indicates that we can model PTSD in a rodent and target the PFC to ameliorate symptoms and target a key site of pathophysiology in PTSD. In collaboration, a phase II, two-arm, randomized clinical trial is being conducted to examine tDCS vs. placebo delivered in combination with written exposure therapy (WET). Both arms will complete five sessions of WET which is a first line exposure-based therapy known to be effective as a treatment for PTSD. The treatment arm will receive 30 minutes tDCS stimulation

targeted to the dorsolateral PFC over five weekly sessions. Findings from this study may support a novel adjunct treatment that can be used to treat PTSD.

Disclosures: A. Boley: None. **S.M. Perez:** None. **K.H. Buccellato:** None. **C.L. Straud:** None. **D.J. Lodge:** None. **D. Morilak:** None. **F.R. Carreno:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.22/II17

Topic: E.04. Voluntary Movements

Support: NIH NINDS K99/R00-NS101127
Frank & Evangeline Thompson Opportunity Fund
Steve Palermo Foundation

Title: Changes in directional tuning and modulation within M1 of initial and corrective submovements during precision reaching

Authors: *K. SCHWARTZE[1], W. LEE[1], A. G. ROUSE[2];
[1]Univ. of Kansas Med. Ctr., Kansas City, KS; [2]Neurosurg., U of Kansas Med. Ctr., Kansas City, KS

Abstract: Precision reaching tasks often require corrective submovements for successful completion. Most studies of reaching have focused on single initial movements, and the cortical encoding model was implied to be the same for all submovements. However, corrective submovements may show different encoding patterns from the initial submovement with distinct patterns of activation across the population. Two rhesus macaques performed a precision reaching center-out-task with small targets. Neural activity from single units in primary motor cortex and associated behavioral data were recorded to evaluate movement characteristics. Neural population data and individual neuronal firing rates identified with a peak finding algorithm to identify peaks in hand speed were examined for encoding differences between initial and corrective submovements. Individual neurons were fitted with a regression model that included the reach vector, position, and speed to predict firing rate. For both initial and corrective submovements, the largest effect on neural firing rate remained movement direction despite increased importance of position and speed for corrective submovements. Approximately 40% of all significantly tuned neurons changed their preferred direction 45-180° between initial and corrective submovements. Neuronal depth of modulation followed the expected trend of larger modulation for the initial submovements; however, when the submovements were adjusted for movement speed, considerable variation was observed, with some submovements firing almost exclusively for either initial or corrective submovements. By utilizing principal components analysis, simulated neural trajectories of initial and corrective submovements were generated to evaluate neural subspace occupancy. Initial and corrective submovements showed trajectories

utilizing mostly different neural subspaces with only 30.1% overlap. These findings all suggest that different neural encoding patterns exist for initial and corrective submovements within the cortex. We hypothesize that this variation in how neurons change to encode small, corrective submovements might allow for a larger portion of the neural space being used to encode a greater range of movements with varying amplitudes and levels of precision.

Disclosures: K. Schwartze: None. **W. Lee:** None. **A.G. Rouse:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.23/II18

Topic: E.04. Voluntary Movements

Support: NIH/NINDS R01NS131662
NIH/NINDS R21NS127622

Title: Investigating the role of subcerebral connectivity in motor function using Fezf2 null mice

Authors: *A. KUNWAR[1], N. ALAM[1], G. T. PRUSKY[1], V. V. SAHNI[1,2,3];
[1]Burke Neurolog. Inst., White Plains, NY; [2]Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [3]Weill Cornell Grad. Sch. of Med. Sci., New York, NY

Abstract: Multiple genes that control the specification and differentiation of distinct cortical projection subtypes have been identified. Of note, the transcription factor Fezf2 is necessary for specification of subcerebral projection neurons. Loss of Fezf2 function in Fezf2 null mice results in a complete loss of connectivity between the cerebral cortex and its subcerebral targets in the brainstem and spinal cord (Molyneaux et. al., 2005; Chen et. al., 2005a). Fezf2 null mice lack both, a corticospinal tract (CST) as well as projections from visual cortex to superior colliculus. Molecular analyses have revealed downstream mechanisms by which Fezf2 specifies subcerebral projection neuron fates (McKenna et. al., 2011; Lodato et. al., 2014). However, the functional consequence of congenital absence of subcerebral connectivity in Fezf2 null mice has not been investigated to date. In this study, we investigated potential differences in motor ability between Fezf2 WT and Fezf2 null (congenitally CST deficient) mice using behavioral paradigms for testing skilled motor function. Our hypothesis is that cortical inputs to subcerebral targets in the brainstem and spinal cord via the CST will be required for execution of fine motor skills, but not for gross overground locomotion. We therefore investigated challenged forward locomotion on a rectangular balance beam using the pose estimation algorithm DeepLabCut (DLC) to assess balance, coordination, and fine motor skills. With user-defined points of interest, DLC enables scoring of ethologically relevant behaviors- reach, successful step, failed step, stationary slip, hop, and provides datapoints to measure stride length, cadence, paw placement consistency, etc. We analyzed regular forward overground locomotion using the automated Noldus CatWalk XT system to assess gross motor performance. Consistent with our hypothesis, preliminary data finds

no difference between Fezf2 WT and null mice in the regular forward locomotion task. Ongoing work will investigate whether Fezf2 null mice exhibit any deficits in challenging tasks that require fine motor ability. We also quantified spatial vision in Fezf2 null mice using a virtual opto-kinetic system (Prusky et. al., IOVS 2004). Interestingly, we find that Fezf2 null mice are capable of opto-kinetic tracking and have normal visual thresholds, suggesting that the function of accessory optic system is not debilitated by the absence of cortical projections to superior colliculus. Collectively, these results begin to establish some of the fundamental organizational principles regarding the role of subcerebral projections in the development of motor functions.

Disclosures: A. Kunwar: None. **N. Alam:** None. **G.T. Prusky:** None. **V.V. Sahni:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.24/II19

Topic: E.04. Voluntary Movements

Support: R01NS108424
UF1NS115821

Title: A Pattern Generation Circuit for the Top-down Control of Birdsong

Authors: *M. TRUSEL[1], D. ALAM[1], H. PANCHOLI[1], B. G. COOPER[2], T. F. ROBERTS[1];
[1]UT SouthWestern Med. Ctr., Dallas, TX; [2]Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Networks of interconnected circuits spanning the thalamus, basal ganglia, and cortex are involved in learning skilled motor behaviors. However, once a behavior is learned it appears that only subsets of these brain networks continue to be necessary for controlling it. Whether similar network-level consolidation occurs in the control of natural behaviors is not known. Here we address this question by systematically defining the neuronal network necessary for controlling birdsong. Adult zebra finch song involves the serial and stereotyped production of ~5-7 complex song syllables. Learning this song relies on a large network of forebrain circuits that are connected with a region analogous to the mammalian premotor cortex, named HVC. Using opsin-assisted circuit manipulations we constructed the first cellular resolution map of HVC's synaptic input and output pathways. We then methodically tested the role of neuronal ensembles in HVC and HVC's various input pathways in song motor control. We find that at any moment during song, even brief optogenetic perturbation of neurons in HVC (10 ms) can immediately halt and reset the song back to the first syllable in the sequence, suggestive of a song-pattern generation circuit residing in HVC. Using lesions and optogenetic stimulation approaches we demonstrate that HVC can generate song autonomously of any of its major input pathways. These findings indicates that song learning culminates with massive network-level consolidation. The result of which is neuronal ensembles within HVC that function like a song

central pattern generator, exerting top-down control over this complex and ethologically relevant behavior.

Disclosures: M. Trusel: None. **D. Alam:** None. **H. Pancholi:** None. **B.G. Cooper:** None. **T.F. Roberts:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.25/II20

Topic: E.04. Voluntary Movements

Support: NIH Grant RO1 NS108424
UF1 NS115821

Title: Testing the Functional Encoding of Birdsong with Two-Photon Holographic Manipulation of Premotor Neuronal Ensembles

Authors: *H. PANCHOLI[1], M. TRUSEL[2], T. F. ROBERTS[2];
[2]Neurosci., [1]Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: Precise sequential activity in neuronal ensembles underlies the production of skilled motor behaviors. How individual classes of neurons within these ensembles uniquely contribute to network function and behavior is poorly understood. We have begun to tackle this by examining the role of two different types of excitatory neurons embedded within the zebra finch song nucleus HVC, a premotor region necessary for the control of birdsong. HVC contains two principal classes of projection neurons that form sequences of activity associated with song production. It is unclear how these non-overlapping yet synaptically interconnected populations of neurons may uniquely contribute to the vocal-motor actions in song. Here we describe our progress in using two-photon optogenetics (2PO) to dissect the function of these neurons in song production. We show that 2PO permits manipulation of either class of projection neurons with cellular resolution. In other work from the lab, we have found that optogenetic manipulations in HVC while birds are singing causes immediate halting and resetting of song. Therefore, we conducted our 2PO manipulations during offline states (i.e. when birds are not singing). With the goal of editing connectivity and activity across neuronal ensembles, we repeatedly played-in a sequence of activity into a population of HVC neurons (~70-100 neurons/bird, sequence repeated ~500-700 times). We then examined how these manipulations impacted various aspects of song production. Our current findings indicate that 2PO manipulations to a single class of HVC projection neurons during offline states can result in consistent changes in song across birds. Neuronal sequences in HVC have been suggested to encode either timing or gestural aspects of song. Our work suggests that our cell-type selective 2PO approach can provide insights into these models of HVC function. This work also supports the use of two-photon holographic

optogenetics in the songbird and as a powerful method for cellular resolution manipulation of neuronal ensembles.

Disclosures: H. Pancholi: None. **M. Trusel:** None. **T.F. Roberts:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.26/II21

Topic: E.04. Voluntary Movements

Support: NIH DP2 NS111817-01
NIH NS25074
Israel Brain Prize

Title: Universals for coding action in motor cortex: insights from latent space geometry

Authors: *I. PENIDO[1,2], J. B. HYNES[1,2], J. DONOGHUE[1,2], C. VARGAS-IRWIN[1,2,3]; [1]Brown Univ., Providence, RI; [2]Robert J. and Nancy D. Carney Inst. for Brain Science, Brown Uni., Providence, RI; [3]Dept. of Veterans Affairs Med. Ctr., Ctr. for Neurorestoration and Neurotechnology, Rehabil. Res. and Develop. Service, Providence, RI

Abstract: Computations performed by the cerebral neocortex arise from a collective network architecture that can generate rich cognitive, and behavioral flexibility, but the principles of cortical information processing fundamental to these abilities are unclear. Progress towards uncovering universal mechanisms of neural ensemble computation is complicated by the fact that no two brains are exactly alike, as complex synaptic arrangements vary, and it is not possible to establish a one-to-one correspondence between sets of neurons across individuals. Neural computations occur in a subject-specific, high-dimensional system, but ensemble activity patterns during a behavior are often confined to a low dimensional neural latent space. Here we present a technique to project neural data from multiple subjects to a single unified latent space. We find that neural ensemble activity converges to a common geometry in different non-human primates performing similar reaching or object grasping behaviors. To gain a better understanding of the potential minimal size of computational ensembles, we performed alignment and decoding between neural latent spaces calculated from a variable subset of recorded neurons. As neurons are added to latent space calculations, the rate of convergence is exponential when using a spike train similarity embedding. By contrast, convergence requires more neurons when using binned firing rates. On average, activity patterns across just a few select, local neurons contain enough meaningful variance to reliably generate a low-dimensional representation for each task. Both within-subject and across-subject latent space geometries converge above chance levels with less than 20 neurons on average for both the reaching and grasping tasks (Wilcoxon rank sum, one-tailed, $p < 0.05$). These results support the hypothesis that cortical networks engaged by similar behavioral challenges produce activity patterns that

converge onto a common latent space geometry across subjects and sessions and highlight the importance of spike timing in determining the intrinsic structure of these latent spaces.

Disclosures: I. Penido: None. **J.B. Hynes:** None. **J. Donoghue:** None. **C. Vargas-Irwin:** None.

Poster

PSTR486. Neural Recordings and Manipulations to Understand Skilled Behaviors

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR486.27/II22

Topic: E.04. Voluntary Movements

Support: Simons Collaboration on the Global Brain

Title: Using interpretable, generative models to probe interactions across cortical and subcortical motor system regions.

Authors: *M. AGRIOS[1], S. HSU[1], N. T. KOH[1], S. P. SAVYA[2], A. C. KRISTL[1], S. A. SOLLA[3], A. A. MIRI[1];
[1]Northwestern Univ., Evanston, IL; [2]Neurobio., [3]Neurosci., Northwestern Univ., Chicago, IL

Abstract: As technology enables us to record from more and more neurons simultaneously, analyses of such large datasets must strike a balance between computational tractability and interpretability. Here, we demonstrate that generalized linear models (GLMs) can be a powerful tool for building generative models that can recapitulate observed neural dynamics across large populations, while maintaining fidelity to underlying biological mechanisms. Using four simultaneously implanted neuropixels in a head-fixed mouse, we record the activity of hundreds of neurons in premotor cortex, primary motor cortex, primary sensory cortex, striatum, and thalamus, while mice perform a naturalistic self-initiated climbing task. The spiking activity of each neuron is used as input to a GLM that learns pairwise statistical relationships between the firing patterns of individual neurons. These relationships are realized as temporal filters that describe how the activity of one neuron excites or inhibits the activity of the other across varying time lags. These temporal filters can then be used to simulate network activity by creating a Poisson point process out of the incoming filters associated with each neuron, producing spike trains that mimic the statistics of the experimental data. The resulting simulated spike trains exhibit mean firing rates similar to those of the observed spike trains, and the Kullback-Liebler divergence of interspike interval distributions of the simulated relative to the actual data is low. Additionally, the eigenvalue distribution of the covariance matrix for simulated population spike trains is strikingly similar to that of the observed spike trains. Our GLMs shed light on individual neuron-to-neuron interactions, but the neural dynamics of interregional communication occurs at the scale of neural populations. Thus, we are extending our methodology to construct coarse-grained GLMs based on latent variables that provide a low-dimensional description of the population dynamics in each of the recorded brain areas. In this formulation, the GLM units

represent latent variables, and the temporal filters describe interactions between latent variables, i.e., between coordinated patterns of activity within and between brain regions. Our approach offers interpretable analyses at multiple spatial and temporal scales; these will be useful for understanding the mechanisms that underlie neural system function in the motor system and beyond.

Disclosures: **M. Agrios:** None. **S. Hsu:** None. **N.T. Koh:** None. **S.P. Savya:** None. **A.C. Kristl:** None. **S.A. Solla:** None. **A.A. Miri:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.01/II23

Topic: E.04. Voluntary Movements

Support: NIH Grant NS058487

Title: Purdue pegboard test and functional activity in the basal ganglia are impaired in Idiopathic rapid eye movement behavior disorder and Parkinson's disease

Authors: *E. R. TOBIN[1], D. J. ARPIN[1], M. B. SCHAUDER[1], M. L. HIGGINBOTHAM[1], R. B. BERRY[2], E. A. CHRISTOU[3], M. S. JAFFEE[4], D. E. VAILLANCOURT[5];
[1]Applied Physiol. and Kinesiology, [2]Med., [3]Applied Physiol. and Kinesiology and Fixel Inst. for Neurolog. Dis., [4]Fixel Inst. for Neurolog. Dis., [5]Applied Physiol. and Kinesiology, Fixel Inst. for Neurolog. Disease, Biomed. Engin., Univ. of Florida, Gainesville, FL

Abstract: Introduction: Idiopathic rapid eye movement behavior disorder (iRBD) is a disruptive sleep-related disorder that has a high risk of developing Parkinson's disease (PD). About 80-90% of individuals with iRBD will develop a neurodegenerative alpha-synuclein disorder in the following decades. It is unclear how early and in which structures we observe functional changes in the brain in patients with iRBD that do not have another diagnosed neurodegenerative disorder. **Objective:** 1) To evaluate upper limb motor control, we use the Purdue Pegboard Test (PPT) in PD, iRBD, and healthy controls (HCs). 2) To evaluate neuronal function during an upper limb motor task, we use task-based functional magnetic resonance imaging to observe blood oxygen level-dependent (BOLD) signal changes in the primary motor cortex (M1), thalamus, basal ganglia (BG), and cerebellum. **Methods:** We studied a total of 82 subjects (22 HCs, 21 iRBD, 39 PD). All subjects performed 4 PPT tasks and a MANOVA was performed covarying for age and sex. All subjects were scanned in a 3T Philips MRI scanner, while they performed a unimanual grip force task. We performed a three-factor (3X3) repeated measures ANCOVA for regions in the M1, BG, thalamus, and cerebellum, covarying for age and sex. Scan (3 scans) was a within-subjects factor, and diagnosis (PD, iRBD, HCs) was a between-subject factor. **Results:** The PPT both hands task was the most sensitive task to distinguish HCs, PD, and iRBD [$F(2,79)= 29.3$, $p<.001$]. In all PPT tasks, PD had significantly lower scores

compared to HCs (p's<.001). iRBD subjects had significantly lower scores in the dominant hand, both hands and assembly tasks (p's<0.05) compared to HCs. There was a main effect of diagnosis in the left M1 [F(2,77)= 7.01, p=.002], caudate [F(2,77)= 4.72, p=.012], putamen [F(2,77)= 7.10, p=.001], thalamus [F(2,77)= 4.53, p=.014], and right cerebellum [F(2,77)= 5.86, p=.004]. In post hoc analyses for M1 and cerebellum, PD subjects have significantly lower BOLD signal compared to HCs (p's<.01). In post hoc analysis for the BG (thalamus, putamen, caudate), PD and iRBD had a significantly lower BOLD signal compared to HCs (p's<.05). **Conclusion:** We replicate prior work that during a force control task, M1, putamen, thalamus, and cerebellum have a reduced BOLD signal in early-stage PD. The novel finding here is that there are functional changes in the BOLD signal in the BG and thalamus in iRBD compared to HCs. In addition, there are no significant changes in the BOLD signal of M1 or cerebellum in iRBD compared to HCs. These findings suggest that in iRBD functional changes occur in the BG and thalamus before changes occur in the M1 and cerebellum.

Disclosures: E.R. Tobin: None. **D.J. Arpin:** None. **M.B. Schauder:** None. **M.L. Higginbotham:** None. **R.B. Berry:** None. **E.A. Christou:** None. **M.S. Jaffee:** None. **D.E. Vaillancourt:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.02/II24

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI Grant Numbers 20K11493
JSPS KAKENHI Grant Numbers 23K10782

Title: Resting-state brain activity is related to the ability of motor skill learning

Authors: *H. KADOTA[1], H. SEKIGUCHI[2];
[1]Kochi Univ. of Technol., Kami, Japan; [2]Univ. of Yamanashi, Kofu, Japan

Abstract: Humans learn various motor skills in daily life or during sports. However, there are individual differences in the ability of motor skill learning; some people can learn a new motor skill quickly, while others take longer time. In this study, we investigated the relationship between resting-state brain activity and motor skill learning ability using a juggling task as a novel motor skill. Sixty-eight healthy right-handed participants (forty-four men and twenty-four women) with no juggling experience were recruited. First, participants were required to stare at a fixation cross in a magnetic resonance imaging (MRI) scanner. They were monitored to keep their eyes open using EyeLink 1000. Their resting-state brain activity was measured using functional MRI (fMRI) for about 10 min. Next, participants moved to another room and were required to train juggling. Training consisted of moving two balls with the right hand in a rolling-out pattern for 250 trials. A trial ended when participants dropped the ball or stopped

moving. After resting-state fMRI data were preprocessed using SPM12, we calculated the fractional amplitude of low-frequency fluctuations (fALFF) and the mean fALFF (mfALFF), and divided the fALFF by the global mean using SPM8 and toolbox called Data Processing Assistant for Resting-State fMRI (DPARSF). As an index of motor skill learning ability, we counted the total number of catches at juggling training. The total number of catches ranged from 96 to 1,417, indicating differences in the ability of juggling skill acquisition among participants. Subsequently, we conducted a regression analysis with the mfALFF and the total number of catches, which showed that the left sensorimotor cortex was positively correlated with the total number of catches ($p<0.001$ uncorrected at voxel level and $p<0.05$ with family-wise error correction at cluster level). Namely, the more activated the left sensorimotor cortex before juggling training, the better the juggling skill. These results suggest that greater activation of the left sensorimotor cortex in resting state improves motor skill acquisition. Furthermore, resting-state activation of the left sensorimotor cortex may predict the ability of motor skill learning, such as juggling with the right hand.

Disclosures: H. Kadota: None. **H. Sekiguchi:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.03/II25

Topic: E.04. Voluntary Movements

Support: Startup fund

Title: Dynamic causal roles of lateral prefrontal cortex and primary motor cortex in the expression of novice and expert motor skills

Authors: *Q. NGUYEN, K. MICHON, J. BRISSENDEN, T. LEE;
Univ. of Michigan, Ann Arbor, MI

Abstract: While there is growing interest in mapping the neural reorganization due to motor skill development, commonly used neuroimaging techniques such as functional magnetic resonance imaging (fMRI) limit the strength of inferences that can be made about the causal role brain regions commonly implicated in skill learning. Dorsolateral prefrontal cortex (DLFPC) is thought to facilitate top-down or ‘explicit’ control during early phases of learning, while primary motor cortex (M1) supports automatized motor memories that are operative in later phases of learning. However, the dynamics of how of goal-directed control evolves during the transition from novice to expert are currently unclear: Does the scaffolding provided by prefrontal networks completely yield to the automated execution enabled by a core motor network, or do both networks continue to run in parallel to support performance, cooperating and competing to varying extents? While many neuroimaging studies have found a decrease in brain activity of DLPFC and M1 over the course of learning, it is unclear if this decrease reflects reduced

importance or increased metabolic efficiency. Here, we address these questions by combining transcranial magnetic stimulation (TMS) with fMRI analysis of cross-expertise patterns of behavioral performance after six weeks of training on a discrete sequence production task. Our design allowed us to assess the expression of motor skills at three different levels of expertise in a single session. While disruption of both the DLPFC and M1 produce deficits at all levels of expertise, these regions play dissociable roles as a function of expertise. Using a representational similarity analysis (RSA) on the neuroimaging data, we found that sequence-specific patterns reorganized to become more distinct from one another as expertise developed, but that the nature of this reorganization differed along the rostral-caudal axis of frontal cortex. Additionally, we found that DLPFC and M1 stimulation differentially altered neural distinctiveness in several task-related cortical areas. These results provide causal evidence for the differential roles of DLPFC and M1 in motor skill expertise.

Disclosures: Q. Nguyen: None. **K. Michon:** None. **J. Brissenden:** None. **T. Lee:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.04/II26

Topic: E.04. Voluntary Movements

Title: Changes in cortical activation during passive and active robot-assisted upper-limb training in healthy participants: a functional near-infrared spectroscopy (fNIRS) study

Authors: *M. N. LAFITTE[1], C. S. ANDREA[2], H. KADONE[3], M. YAMAZAKI[4], K. SUZUKI[3];
[1]Sch. of Integrative and Global Majors, Univ. of Tsukuba, Tsukuba, Ibaraki, Japan; [2]Artificial Intelligence Lab., [3]Ctr. for Cybernics Res., [4]Dept. of Orthopaedic Surgery, Fac. of Med., Univ. of Tsukuba, Tsukuba, Japan

Abstract: Upper-limb motor disabilities have a very detrimental impact on independence and quality of life and are expected to increase with aging society. Incidentally, robotic devices have been increasingly used for motor training as they allow highly repetitive and consistent training, which has been shown to be very appropriate for motor recovery. Several studies suggest that active rehabilitation, especially, is all the more pertinent and prone to induce cortical reorganization and functional recovery. Yet, little literature compares the effects of active and passive robot-assisted training on brain activity. We aimed here to compare cortical activation during unassisted, active robot-assisted and passive robot-assisted shoulder elevation training. We used the shoulder type Hybrid Assistive Limb, an intention-based arm-raising active exoskeleton triggered by the wearer's deltoid activity. Using a 51-channel continuous functional near infrared spectroscopy system, we monitored the hemodynamic response in bilateral frontal and parietal lobes of 17 right-handed healthy participants (9 females, 8 males, mean age 27.4 ± 4 years). Regions of interest (ROI) included dorsolateral prefrontal cortex (DLPFC), premotor

cortex (PMC), supplementary motor area (SMA), primary motor cortex (M1) and primary somatosensory motor cortex (S1). The task was a 15s block design of self-paced shoulder elevations in the scapular plane performed in the 3 conditions. We report a significant general bilateralized activation (Benjamini-Hochberg correction with 5% false discovery rate) in all ROI during the task. Between-conditions comparisons showed that normalized oxyhemoglobin concentration during unassisted motion was consistently higher than both active and passive assistance in the ipsilateral hemisphere (paired t-test, $P<0.05$). Passive mode induced significantly lower activation than active assistance in the distal contralateral DLPFC and PMC ($P<0.05$).These results suggest that unassisted, passive and active robot-assistive motion activate the same areas but at different levels. The reduced ipsilateral activation with robot assistance could suggest a more targeted action, while passive mode triggers less activation than active assistance in contralateral anterior distal regions. Consistently with the literature, the results indicate that brain activation correlates with muscle force. The present results highlight the differences in cortical activation between unassisted, and passive or active robot-assisted shoulder training. A better understanding of their effect on brain activity could help optimize rehabilitation strategies for patients.

Disclosures: **M.N. Lafitte:** None. **C.S. Andrea:** None. **H. Kadone:** None. **M. Yamazaki:** None. **K. Suzuki:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.05/II27

Topic: E.04. Voluntary Movements

Support: NIH Grant NS085122
NIH Grant HD058301

Title: Using exergaming to examine the impact of aging on cognitive-motor interactions

Authors: S. SALEH[1], M. GLASSEN[1], Q. QIU[1], G. FLUET[1], *S. ADAMOVICH[2,1];
[1]Dept. of Rehabil. and Movement Sci., Rutgers, The State Univ. of New Jersey, Newark, NJ;
[2]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ

Abstract: The objective of this preliminary EEG study is to identify brain regions associated with higher cognitive effort in dual-task activities. Our custom-developed exergames involve both cognitive and sensorimotor components and are controlled by finger/wrist/elbow/shoulder isolated or coordinated movements. Aging individuals with a lower cognitive reserve are expected to perform less efficiently in these exercises. By manipulating the motor and cognitive complexity of the exergames and thus affecting cognitive reserve, we identify and analyze brain regions and networks that modulate their activity during these manipulations. We acquired EEG data while participants played a custom-developed car racing exergame. At the start of the game,

participants were asked to memorize one or three objects presented on the computer screen. They then practiced controlling a racing car on a two- or three-lane road using forearm supination and pronation movement to switch lanes and collect the memorized objects or avoid other objects. For EEG data collection, we used the actiCHamp-Plus amplifier and 64-channel gel-based electrode cap. Data was recorded at 500Hz and was processed using custom MATLAB scripts using the EEGLAB and Fieldtrip toolboxes. Processing steps included filtering (1-50Hz bandpass), Artifact Subspace Reconstruction, ICA, and source reconstruction using regions from the Brainnetome atlas. Kinematic data were collected from the LEAP motion controller, and movement onsets were identified. Movements were labeled offline as correctly or incorrectly collected or avoided objects. EEG data were epoched to these movement onsets after processing, and Event Related Spectral Perturbations (ERSPs) were calculated. Preliminary results show higher desynchronization in ERSP of sensorimotor regions (parietal and premotor cortices included) in the three objects condition versus one object condition, showing a higher neuromodulatory effect of exergaming with increased cognitive effort.

Disclosures: **S. Saleh:** None. **M. Glassen:** None. **Q. Qiu:** None. **G. Fluet:** None. **S. Adamovich:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.06/II28

Topic: E.04. Voluntary Movements

Support: NSF NCS 2024923
NSF GRFP DGE 1746045

Title: Temporal Dynamics of Brain Activity Predicting Illusory Agency over Involuntary Movements

Authors: ***J. P. VEILLETTE**[1], P. LOPES[2], H. C. NUSBAUM[1];
[1]Psychology, [2]Computer Sci., Univ. of Chicago, Chicago, IL

Abstract: Our muscles are the primary means through which we affect the external world, and the sense of agency (SoA) over action through those muscles is fundamental to self-awareness. However, SoA research to date has focused almost exclusively on agency over action outcomes rather than over the musculature itself, as it was believed that SoA over the musculature could not be manipulated directly. Drawing on methods from human-computer interaction and adaptive experimentation, we use human-in-the-loop Bayesian optimization to tune the timing of electrical muscle stimulation so as to robustly elicit an illusion of agency over electrically-actuated muscle movements. We use time resolved decoding of subjects' EEG to estimate the time course of neural activity which predicts illusory agency on a trial-by-trial basis. Like paradigms which assess SoA over action consequences, we find the late (post-conscious) neural

activity predicts SoA. Unlike typical paradigms, however, we also find patterns of early (sensorimotor) activity with distinct temporal dynamics predicts agency over muscle movements, suggesting that the "neural correlates of agency" may depend on the level of abstraction (i.e. direct sensorimotor feedback vs. downstream consequences) most relevant to a given agency judgement.

Disclosures: J.P. Veillette: None. **P. Lopes:** None. **H.C. Nusbaum:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.07/JJ1

Topic: E.04. Voluntary Movements

Support: NSF Grant EEC-2127509

Title: Does repetition in a motor task result in weaker movement-related EEG modulations?

Authors: *A. PAEK, S. PRASHAD;
Washington State Univ., Pullman, WA

Abstract: Neuroimaging studies often utilize very repetitive motor tasks to help characterize their neural correlates. Consistent tasks ensure that similar cortical areas are engaged and aim to yield similar neural signatures. However, movement-related neural signals of interest can also change over time due to the participant's habituation with the task, especially if it is very repetitive and predictable. Sensory adaptation is a known phenomenon where the individual can become desensitized to a prolonged sensory stimulus. In this study, we explored if a similar phenomenon could occur where the repetitiveness of a motor task causes motor-related neural modulations to weaken. Twenty human participants performed a task where they used a computer mouse to pursue a target that moved along a horizontal track. The target moved in two conditions: 1) the target moved in a repetitive and predictable pattern, and 2) the target randomly changed directions as it moved from left to right. Both conditions were alternated in four blocks of 30 trials. To assess the conditions' effect on the participants' attention to the task, participants were also instructed to monitor the moving target's shape while they pursued it. They were instructed to press the space bar on the keyboard as quickly as possible when the target's shape changed from a circle to a triangle and omit key presses when it changed into a square. During this task, 64-channel scalp electroencephalography (EEG) was recorded, and we estimated spectral power modulations from the rest to movement phases. From these phases, spectral power in the alpha (8-13 Hz) and beta (20-30 Hz) bands was extracted in 3-second time windows. The event-related desynchronization (ERD) was estimated as the ratio of spectral power from rest to movement. ERDs were compared between the predictable vs. random conditions with the paired sample t-test. We found that the random condition yielded significantly stronger alpha band ERDs in posterior areas (O1: $p = 0.044$, PO4: $p = 0.032$) and

stronger beta band ERDs in central and parietal areas (CP5: $p = 0.0042$, C5: $p = 0.041$, P7: $p = 0.0074$, P2: $p = 0.027$). We also found that the reaction times to the target shape changes were significantly slower in the random condition ($p < 0.001$), which confirms that the randomly moving target induces attentional demands on the participant. These results suggest that implementing random elements in a behavioral task can engage more attention from the participant and strengthen movement-related neural modulations. This addition can enhance EEG-based applications that monitor movement-related neural features such as brain machine interfaces and neurofeedback-based rehabilitation.

Disclosures: **A. Paek:** None. **S. Prashad:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.08/JJ2

Topic: E.04. Voluntary Movements

Support: the Korea Health Industry Development Institute (KHIDI) HI22C1927
KAIST-funding Global Singularity Research Program for 2023 (N10230057)

Title: Evaluating the Efficacy of an Immersive Virtual Reality-Based Videogame with Vibrotactile Feedback for Hand Rehabilitation in Chronic Stroke Survivors

Authors: *S. BAE, H.-S. PARK;
Mechanical engineering, Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: The loss of hand function due to neurological disorders such as stroke significantly impacts the quality of life and the ability to perform daily activities. High-intensity, repetitive, goal-directed rehabilitation training has been reported as an effective way to recover hand function, but it requires high participant engagement. Virtual reality (VR) and video games have been proven to effectively induce engagement and attention, crucial factors for functional recovery after rehabilitation. In our previous study, we developed a VR-based hand rehabilitation system using a video game and vibration feedback. The system was designed to immerse stroke survivors in a virtual environment where they mimic gestures of an approaching target, customized based on their hand condition. When participants successfully mimic the gesture, the virtual target "explodes." This is paired with a vibration on the participant's wrist and matching visual and auditory signals. This system was validated by observing significant cortical activation in the hand area of the primary sensorimotor cortex of both hemispheres, the prefrontal cortex, and the premotor cortex in a stroke survivor. The current study aims to further evaluate the effectiveness of our VR-based hand rehabilitation system through clinical trials for stroke survivor. We recruited a stroke survivor with mild left-sided hemiplegia and conducted a

30-minute training session. Pre- and post-training assessments were conducted using the Modified Ashworth Scale (MAS), Manual Muscle Testing (MMT), Box and Block Test, grip/pinch strength test, and the 9-hole peg test. Our preliminary results indicate that the VR-based hand rehabilitation system has potential benefits for stroke survivors. The participant showed an increase in MMT in the finger and grip/pinch strength, indicating improved muscle strength. Additionally, the Box and Block Test score improved, suggesting enhanced manual dexterity and the time for the 9-Hole Peg Test decreased, indicating increased hand dexterity. These findings suggest that our VR-based hand rehabilitation system, which combines immersive VR, video games, and vibration feedback, can effectively engage stroke survivors in their rehabilitation process and potentially improve their hand function. Future studies with larger sample sizes are needed to confirm these findings and further explore the impact of our system on sensorimotor function and attention. This innovative approach to hand rehabilitation is worthy of further clinical study and may provide a promising tool for stroke rehabilitation.

Disclosures: S. Bae: None. **H. Park:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.09/JJ3

Topic: E.04. Voluntary Movements

Support: NIH Grant NS112367

Title: Resting state functional connectivity changes among multisensory and motor regions associated with visuo-proprioceptive recalibration

Authors: *K. FOLCO[1], H. CHENG[1], S. D. NEWMAN[2], A. PILACINSKI[3], M. WALI[1], R. BABU[1], H. J. BLOCK[1];
[1]Indiana Univ., Bloomington, IN; [2]Univ. of Alabama, Tuscaloosa, AL; [3]Ruhr-University Bochum, Bochum, Germany

Abstract: The brain estimates hand position using visual and proprioceptive (position sense) cues. When a spatial mismatch is introduced between visual and proprioceptive cues, e.g. by shifting a visual cursor away from the unseen hand, the brain recalibrates visual and proprioceptive estimates of hand position to compensate. Visuo-proprioceptive recalibration occurs in the context of visuomotor adaptation paradigms such as cursor rotation, but it is a distinct process. Visuomotor adaptation is known to involve activity in the cerebellum and primary motor cortex (M1), but little is known about the neural basis of visuo-proprioceptive recalibration itself. Under a traditional framework, M1, ventral premotor cortex (PMv), and cerebellum (CB) would be considered motor structures while primary somatosensory cortex (S1) and anterior superior parietal lobule (aSPL) would be considered sensory, but today, most would agree that sensory and motor functions emerge from distributed processing among these and

other regions. M1 and S1 have both been linked to proprioceptive activity, and aSPL, PMv, and CB have been linked to multisensory (visual and proprioceptive) activity. Here we ask whether resting state functional connectivity among these regions undergoes any change following a task involving visuo-proprioceptive recalibration, but not motor adaptation. Participants completed a resting state scan, a hand position estimation task using veridical cues (baseline task), a second resting state scan, a hand position estimation task where the visual cue gradually shifted 70 mm away from the proprioceptive cue (recalibration task), and finally a third resting state scan. Using a seed-based network analysis, preliminary data (N=6) indicates that connectivity between S1, a traditionally sensory region, and PMv, a traditionally motor region, may be altered after the recalibration task relative to baseline. In addition, we found that connectivity between CB and aSPL may be related to the magnitude of proprioceptive recalibration measured behaviorally. In addition, visuo-proprioceptive recalibration in a pre-defined sensorimotor network consisting of the right anterior superior parietal lobule, M1, S1PMv, and left cerebellum lobule VI was related to changes in network measures. Taken together, these results support the idea that visuo-proprioceptive recalibration is associated with changes in resting state functional connectivity among both motor and multisensory regions.

Disclosures: K. Folco: None. **H. Cheng:** None. **S.D. Newman:** None. **A. Pilacinski:** None. **M. Wali:** None. **R. Babu:** None. **H.J. Block:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.10/JJ4

Topic: E.04. Voluntary Movements

Support: Réseau provincial de recherche en adaptation-réadaptation (REPAR)
University Laval Research Chair in Cerebral Palsy
Réseau de Bio-Imagerie du Québec (RBIQ)
Fonds de recherche Québec Santé (FRQS)
Fonds de recherche Québec Nature et technologies (FRQNT)
NSERC Discovery Grant
USherbrooke institutional research chair in neuroinformatics

Title: A proposed method to identify motor planning corticocerebellar pathways in children with cerebral palsy.

Authors: *O. MARTINIE[1], P. KARAN[2], C. MERCIER[3], M. DESCOTEAUX[4], M. T. ROBERT[1];
[1]Univ. Laval, Quebec, QC, Canada; [2]Sherbrooke Univ., Sherbrooke, QC, Canada; [3]CIRRIS - Laval Univ., Quebec, QC, Canada; [4]Computer Sci., Univ. De Sherbrooke, Sherbrooke, QC, Canada

Abstract: Cerebral palsy (CP) is a neuromotor disorder characterized by various brain lesions occurring in fetuses or around birth. These lesions alter the microstructure of the white matter within the brain of children living with CP. Therefore, different neurological pathways are impacted and associated to their sensorimotor deficits. However, most of the studies focused on motor functions and their related neurological pathways whereas children with CP also showed motor planning deficits. Hence, the corticocerebellar pathways, which are involved in motor planning, remain largely unknown in children with CP due to the methodological challenges to identify subtle pathways in brains with large lesions. Moreover, the current literature in CP lack of rigorous and consensual methodology for pre-process and analyze the data obtained from diffusion neuroimaging acquisition. This study aims to apply a rigorous method to pre-process and extract corticocerebellar pathways such as fronto-ponto-cerebellar and cerebello-thalamo-frontal tracts. Eight children with CP (4 girls, 4 boys) between 8 and 12 years old, with light to moderate upper limb deficits participated into a single diffusion magnetic resonance imaging (MRI) session without sedation. Inclusion criteria were: 1) a diagnostic of hemiplegic cerebral palsy, 2) aged between 8 and 17 years old. Exclusion criteria were: 1) contraindication to MRI (e.g., implants), 2) claustrophobia. MRI scans were performed on a 3-Tesla scanner (Philips). Anatomical (T1) and diffusion images (HARDI sequence) were acquired in 21 minutes. Data were corrected and analyzed using Tractoflow, an open-source, robust and automatic tool. The tracts were extracted with customized procedure based on existing atlases and standardized and free-accessed libraries such as ANTs and Scilpy. DTI, CSD and NODDI diffusion metrics were computed for each tract. Our results showed the feasibility to reconstruct the frontal part of the corticocerebellar pathways for most of our participants, despite the heterogeneity and the significance of the lesions. However, this method was not possible for the participant with bigger lesion volume because registration of atlases failed. The diffusion metrics showed some consistency according to the lesion side while some suggested discrepancies. Our results highlight the importance to choose appropriate metrics and the feasibility to identify subtle motor planning white matter pathways. Ultimately, this will increase our understanding of the underlying mechanisms of clinical symptoms to provide precision medicine and improve current intervention which have limited efficiency.

Disclosures: O. Martinie: None. **P. Karan:** None. **C. Mercier:** None. **M. Descoteaux:** None. **M.T. Robert:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.11/JJ5

Topic: E.04. Voluntary Movements

Support: Honda R&D Co., Ltd.
NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc
JSPS KAKENHI Grant Number 21H00967

Title: Neural substrates of the motor enhancement induced by emotional imagery

Authors: A. SHIINA[1,2], S. K. SUGAWARA[2], Y. HOSHI[1,2], N. USUDA[2], M. NISHIJIMA[1,2], Y. UEDA[1,2], J. OKUMA[1,2], M. MOMOKI[3,2], T. IBARAKI[3,2], *M. HOSHINO[1,2], Y. NISHIMURA[2]; [1]Honda R&D Co., Ltd., Wako, Japan; [2]Neural Prosthetics Project, Tokyo Metropolitan Inst. of Med. Sci., Setagaya, Japan; [3]Neuro Innovation Unit, NTT DATA Inst. of Mgmt. Consulting, Inc., Chiyoda, Japan

Abstract: During practices and before matches, athletes usually imagine not only physical movements but also emotional outcomes (e.g., winning the match or a new record). Thus, we believe voluntary control of own mental state plays a critical role in producing the best performance. However, it is unknown whether the self-generated emotional state enhances following motor performances and how the brain converts the self-generated state into the vigor for motor outputs. Fifteen healthy adults participated in the behavioral experiment to test whether the self-generated emotional state enhances the outcome of motor outputs. The behavioral results showed that compared to non-emotional imagery or not imaging, the emotional imagery about a goal achievement increased subjective emotional arousal and improved following reaction time and peak grip force. Thus, we concluded that the self-generated emotional state enhances following motor performance without any incentives. Next, we conducted a functional MRI experiment on forty-one healthy adults to investigate the neural substrates of such motor enhancement induced by emotional imagery. Because it was difficult to rate the subjective arousal immediately after the mental imagery in the MRI scanner, we measured electro-cardiogram across the scan session to evaluate the change in heart rate induced by mental imagery. The mental imagery of a goal achievement significantly increased heart rates compared to not imaging, confirming that the achievement imagery was emotional. Even in an MRI scanner, the mental imagery of a goal achievement significantly improved following reaction time and peak grip force compared to not imaging. These results confirmed that the self-generated emotional state enhances following motor performances. Emotional imagery significantly activated the ventral midbrain (VM) as well as the dorsal premotor area, supplementary motor area, dorsomedial prefrontal cortex, putamen, and cerebellar cortex. The VM activity significantly correlated with peak heart rate change during emotional imagery. Furthermore, the VM activity also correlated with the imagery-related reaction time improvement but not peak grip force. These findings suggest that the VM might convert the self-generated emotional state into the vigor for motor outputs.

Disclosures: **A. Shiina:** A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **S.K. Sugawara:** 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.; NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc.. F. Consulting Fees (e.g., advisory boards); NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc. **Y. Hoshi:** A. Employment/Salary (full or part-time):; Honda R&D Co. **N. Usuda:** None. **M. Nishijima:** A. Employment/Salary (full or part-time):; Honda R&D. **Y. Ueda:** A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **J. Okuma:** A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **M. Momoki:** A. Employment/Salary (full or part-time):; NTT DATA Institute of Management Consulting, Inc.. 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.; Honda R&D Co.,Ltd. **T. Ibaraki:** A. Employment/Salary (full or part-time):; NTT DATA Institute of Management Consulting, Inc.. 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.; Honda R&D Co.,Ltd. **M. Hoshino:** A. Employment/Salary (full or part-time):; Honda R&D Co., Ltd. **Y. Nishimura:** F. Consulting Fees (e.g., advisory boards); NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc..

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.12/JJ6

Topic: E.04. Voluntary Movements

Support: International Postdoc Grant, Swedish Research Council Grant no: 2021-00238

Title: Dual colour widefield calcium imaging across isocortex in two distinct cell types during behaviour

Authors: *S. MYSORE SURYANARAYANA[1,2], H. MOHAN[1], X. AN[1], S. ZHAO[1], Z. HUANG[1];
[1]Neurobio., Duke Univ., Durham, NC; [2]Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Animals exhibit distinct behavioural states, which are reflective of different undelying brain activity patterns and cognitive processing. During uninstructed naturalistic behaviour, animals can cycle through these multiple states, reflecting different degrees of attention, which can be quantified by sets of motor variables including whisker movements, facial movements, pupil dilation and locomotion. Growing evidence indicates that variability of these metrics is reflected in changes in brain wide network activity. Moreover, widefield calcium imaging has revealed that navigating through these distinct behavioral states produces distinct changes in local and inter-areal network dynamics in the isocortex. However, how these changes are reflected in the activities of distinct cell types remains poorly understood. Taking advantage of our comprehensive set of orthogonal Cre and Flp driver lines, which allow us to label specific excitatory (PT, IT and CT) and inhibitory (Sst, Vip and PV) neuronal subtypes across isocortex, we used genetic and viral approaches to express GCaMP8f and jRGECO1a in two distinct pyramidal neuron subtypes or excitatory/Inhibitory subtype combinations in the same mouse. We built a dual color widefield imaging system and used it to simultaneously measure calcium dynamics from two cell types in head-fixed mice during different behavioural states. Our results indicate the presence of cell type specific parallel information processing streams across the isocortex. Furthermore, we are revealing distinct activity patterns in different behavioural states

allowing us to define and reconstruct cortical areal networks mediating these states in an unprecedented resolution of interactive dynamics between distinct cell types.

Disclosures: S. Mysore Suryanarayana: None. **H. Mohan:** None. **X. An:** None. **S. Zhao:** None. **Z. Huang:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.13/JJ7

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI Grant Number 21H00967
JSPS KAKENHI Grant Number 21K18570

Title: Ventral midbrain activity links to short- and long-term fluctuations in motor performance

Authors: *S. K. SUGAWARA[1,2,3], N. USUDA[1], Y. H. HAMANO[2,4,5], Y. NAKAYAMA[1], H. FUKUYAMA[6], K. AMEMIYA[6], M. FUKUNAGA[2,3], N. SADATO[2,3,7], Y. NISHIMURA[1]; [1]Neural Prosthetics Project, Tokyo Metropolitan Inst. of Med. Sci., Setagaya, Japan; [2]Section of Brain Function Information, Natl. Inst. for Physiological Sci., Okazaki, Japan; [3]Dept. of Physiological Sci., SOKENDAI, Hayama, Japan; [4]Fac. of Sci. and Engin., Waseda Univ., Shinjuku, Japan; [5]JSPS Res. Fellow, Chiyoda, Japan; [6]Dept. of Radiology, Tokyo Metropolitan Matsuzawa Hosp., Setagaya, Japan; [7]Res. Organization of Sci. and Technol., Ritsumeikan Univ., Kusatsu, Japan

Abstract: The performance of even simple motor responses varies over time within a day and even from day to day. Such performance fluctuations are divided into short-term and long-term fluctuations. We previously found that pre-movement activity in the ventral midbrain (VM) correlated with the trial-by-trial fluctuation in the strength of force generation, either with or without expected rewards. However, due to the difficulty of repetitive neuroimaging in the same person for months, the neural substrates of long-term performance fluctuation are largely unknown. Here, to address this issue, we repeatedly measured the task-related activity in a single human across eight months using fMRI. One male volunteer participated in thirty fMRI sessions. In each session, he underwent the ready-set-go task, which asked them to prepare to respond at the ready cue and to grip the force device as quickly as possible at the go cue in the MRI scanner. Reaction time (RT) and peak grip force (PF) varied across trials but did not correlate. Even in the single human fMRI data, we replicated that the VM pre-movement activity correlated only with subsequent PF, while pre-movement activities in cortical motor-related areas correlated with following RT and PF. Thus, the VM is related to the short-term fluctuation in the strength of force generation. Unlike the trial-by-trial performances, the long-term performance indices were significantly correlated with each other: median RT and PF, variability in RT and PF, omission rate, and false-start rate. To avoid the multicollinearity among these

indices, we conducted a principal component analysis and found two components explaining more than 80 % of the variance. The first component (PC1) was related to shorter median RT, less variable RT, and fewer omissions, reflecting task engagement. The second component (PC2) was mainly contributed by greater median PF and less false-start, reflecting the controllability. Although we did not find the brain region related to higher controllability, more significant pre-movement activity in VM and cortical motor-related areas, including contralateral M1, supplementary motor area, and dorsal premotor area, correlated with higher task engagement. Taken together, we conclude that the VM contributes to both short- and long-term fluctuations in motor performance.

Disclosures: **S.K. Sugawara:** F. Consulting Fees (e.g., advisory boards); NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc.. **N. Usuda:** None. **Y.H. Hamano:** None. **Y. Nakayama:** None. **H. Fukuyama:** None. **K. Amemiya:** None. **M. Fukunaga:** None. **N. Sadato:** None. **Y. Nishimura:** F. Consulting Fees (e.g., advisory boards); NTT DATA INSTITUTE OF MANAGEMENT CONSULTING, Inc..

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.14/JJ8

Topic: E.04. Voluntary Movements

Support: NCATS Grant TL1TR002493
NCATS Grant UL1TR002494
VA Grant I01RX000622
NIDA T32DA037183

Title: Motor Cortical Dynamics of Impaired Cognitive Control in PTSD: Revealing the Role of Beta Bursts in Adaptive Control Using Drift-Diffusion Modeling

Authors: *E. RAWLS[1], O. L. CALVIN[2], C. A. MARQUARDT[3], S. R. SPONHEIM[3];
[1]Psychiatry and Behavioral Sci., [2]Neurosci., Univ. of Minnesota, Minneapolis, MN;
[3]Minneapolis VA Hlth. Care Syst., Minneapolis, MN

Abstract: Cognitive control deficits are common in PTSD and impact the daily lives of people who experienced trauma. Lateralized somatomotor beta desynchronization, an electrophysiological marker of controlled movement, is a product of cognitive control processes. Somatomotor beta activity is best characterized as sporadic burst-like events, rather than rhythmic oscillations. We measured somatomotor beta bursts (BBs) during a cognitive control test in 131 U.S military veterans from Operations Iraqi and Enduring Freedom. BBs displayed a two-phase response. Pre-response BBs (0-200 ms post-stimulus) were depressed preceding errors within subjects and were associated with slowed reaction times across subjects. Peri-response BBs (200-600 ms) reflected controlled responding within subjects and correlated negatively with

accuracy rates across subjects. This implies pre-response BBs produce indiscriminate inhibition that delays responding to prevent impulsive responses, whereas peri-response BBs govern response control processes influenced by cognitive control. We observed correlations between impaired behavioral performance, increased peri-response BBs, and heightened PTSD severity. This suggests cognitive control deficits in PTSD are tied to response control processes, which are reflected in motor cortical circuits. Yet, the cognitive mechanisms subserved by BBs are not well understood. We thus employed drift-diffusion modeling, breaking the decision process into four parameters: drift rate, response threshold, bias, and non-decision time. We permitted single-trial BBs to modulate decision parameters, revealing that pre-response BBs modulated non-decision time (i.e., perceptual factors delaying the start of evidence integration), whereas peri-response BBs modulated drift rate (i.e., the rate of evidence integration). Cognitive control deficits in PTSD were thus linked to impaired target evidence integration within motor cortical circuits, rather than perceptual factors or impaired distractor inhibition. By uniquely combining an innovative quantification of motor cortical beta activity with a single-trial computational behavior model, we unveiled the cognitive mechanisms reflected in a two-phase BB response and shed light on the computational mechanism contributing to control deficits in PTSD. Our approach holds substantial promise for illuminating computational mechanisms of cognitive control, and their neurophysiological substrates, across other disorders marked by cognitive control impairments.

Disclosures: E. Rawls: None. **O.L. Calvin:** None. **C.A. Marquardt:** None. **S.R. Sponheim:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.15/JJ9

Topic: E.04. Voluntary Movements

Support: Michal and Renata Hornstein Chair in Cardiovascular Imaging (CJG)
Natural Sciences and Engineering Research Council of Canada (CJS, CJG)
Heart and Stroke Foundation of Canada (CJS)
Quebec Bioimaging Network (CJS)
Canadian Institutes for Health Research (CJS)
CIHR Doctoral Award (ST)

Title: Multivariate Assessment of White Matter Microstructure and its Link to Multidomain Behavioural Functioning

Authors: Z. ALASMAR[1], S. TREMBLAY[2,4], T. BAUMEISTER[6], F. CARBONELL[7], C. J. GAUTHIER[3,5], Y. ITURRIA-MEDINA[6], ***C. J. STEELE**[1,8];
[1]Psychology, Concordia Univ., Montréal, QC, Canada; [2]Concordia Univ., Montreal, QC,

Canada; [3]Physics, Concordia Univ., Montréal, QC, Canada; [4]Montreal Heart Inst., Montréal, QC, Canada; [5]Montreal Heart Inst., Montreal, QC, Canada; [6]Neurol. & Neurosurg., Montreal Neurolog. Inst., Montreal, QC, Canada; [7]Biospective Inc., Montreal, QC, Canada; [8]Neurol., Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany

Abstract: White matter (WM) supports communication between brain regions, and there is strong evidence that individual differences in WM microstructure are related to behaviour. One promising approach to assess the link between WM and behaviour is to integrate several non-invasive imaging metrics while accounting for their covariance, which can provide composite assessments of voxel-wise microstructural differences for relating to behaviour.
MRI scans from the Human Connectome Project were used to compute 10 voxel-wise microstructural metrics in WM, and the Mahalanobis Distance (D2) between each subject and the group average was computed at every voxel (D2 accounts for covariance between metrics). We used 4 tasks from the NIH toolbox (2 cognitive, 2 motor) and ran partial least square singular value decomposition to examine the D2-behaviour relationship.
The first latent variable (LV1) explained 39% of the covariance (widespread negative weights in WM and positive weights in the cognition, Fig1). The connectivity profiles of 3 clusters were dominated by frontal/higher order motor cortices, putatively reflecting cognitive and higher-order planning-related motor behavior. LV2 explained 25%, and showed both negative and positive weights in WM with strong positive weighting on grip strength linked to the corticospinal tract and supported by its connectivity profile.
Our multivariate statistical framework integrated multimodal imaging metrics to account for their shared covariance and revealed a multivariate mapping between WM and behavioural function. We show that there are distinct and partially overlapping WM representations of behavioral domains, which indicates the crucial role of microstructure (and the associated brain connectivity) in supporting behaviour. Our approach provides a holistic mapping between WM microstructure and behaviour in health, which may be further used to assess microstructural deviations from normality in disease.

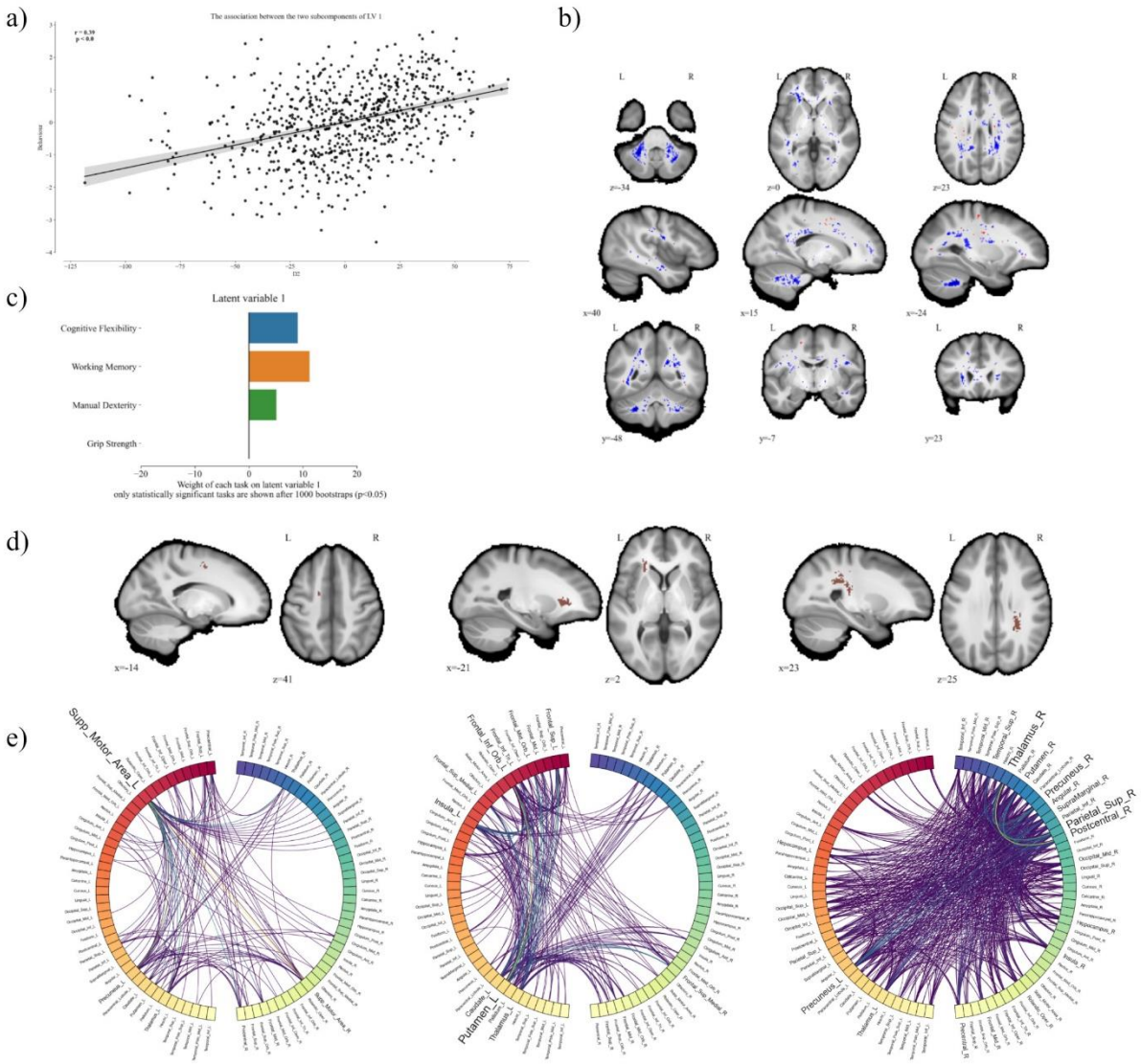


Figure 1: (a) PLS scores correlation, (b) weights of voxels, and (c) tasks after 1000 bootstrap ($p < 0.05$), (d) identified clusters of interest, (e) connectivity profile of clusters based on AAL of latent variable 1.

Disclosures: Z. Alasmar: None. **S. Tremblay:** None. **T. Baumeister:** None. **F. Carbonell:** None. **C.J. Gauthier:** None. **Y. Iturria-Medina:** None. **C.J. Steele:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.16/JJ10

Topic: E.09. Motor Neurons and Muscle

Support: R21NS111310

Title: Functionally-specific identification of brainstem activation during stretch-evoked responses

Authors: *R. C. NIKONOWICZ, F. SERGI;
Biomed. Engin., Univ. of Delaware, Newark, DE

Abstract: The reticulospinal tract, located in the brainstem, plays a crucial role in motor recovery from corticospinal lesions, yet methodological constraints have hindered direct in-vivo measurement of its function. We developed StretchfMRI, a non-invasive imaging method combining electromyography, robotics, and functional magnetic resonance imaging (fMRI) to map motor function in the reticular formation (RF) for the first time. StretchfMRI utilizes precisely evoked long latency responses (LLRs) as a reliable means to stimulate the brainstem and measure motor-related activity via fMRI. This work focuses on refining the technique to understand whether brainstem activity measured via StretchfMRI is functionally specific to LLRs or results from the required muscle preconditioning activation, and on increasing the signal-to-noise ratio (SNR) of the stimulus via a paradigm where participants are perturbed under different task instructions. We performed a Fast-Slow fMRI protocol during which 17 participants' wrists were perturbed in extension at one of two velocities (150 deg/s, 35 deg/s) such that LLRs were elicited at only high velocity perturbations. We also performed a Yield-Resist fMRI protocol during which 10 participants were perturbed when asked to yield or resist to the perturbation. fMRI images for both experiments were collected using a whole-brain sequence (Multi-Band Accelerated EPI with 2 mm^3 voxel resolution, TR=1000 ms). For both protocols, we used a general linear model to identify neural regions significantly associated with stretch-evoked responses under both task instructions while controlling for background contraction. Brainstem-specific Fast-Slow analysis revealed LLR-specific bilateral activation in the posterior pontomedullary interface, posterior pons, medulla, and midbrain. Yield-Resist analysis showed greater activation extent and intensity for the resist condition compared to yield, confirming the brainstem's role in processing the task-dependent component of LLRs. Activation for Resist localized in the right pons, bilaterally in the posterior medulla and midbrain. Yield activation was less widespread, localized in the right pons, right medulla, and left midbrain. Given the small number of individuals in the two protocols, the threshold for statistical significance was identified as t=3, smaller than the threshold of t=5.74 (Fast-Slow) or t=7.54 (Yield-Resist) determined using small volume correction. Future work will aim to establish the effect of different data processing strategies to improve the SNR of fMRI of the brainstem, and to isolate the contributions of the brainstem to voluntary responses and LLRs.

Disclosures: R.C. Nikonowicz: None. **F. Sergi:** None.

Poster

PSTR487. Cortical Planning and Execution: Neuroimaging

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR487.17/JJ11

Topic: E.09. Motor Neurons and Muscle

Support: NIH/NIBIB Grant P41-EB018783

Title: Cortical activity during gait motor imagery combined with action observation

Authors: *A. VATO[1,2,3], H. MOJTABAVI[1], J. BRANGACCIO[1], S. INTERLICCHIO[1], J. SHUBHADA[1], T. M. VAUGHAN[1], J. R. WOLPAW[1,2];
[1]Natl. Ctr. for Adaptive Neurotechnologies, Albany Stratton VA Med. Ctr., Albany, NY; [2] Electrical and Computer Engin., University at Albany, State Univ. of New York, Albany, NY; [3]Dept. of Biomed. Engin., Catholic Univ. of America, Washington, DC

Abstract: Brain-computer interfaces (BCIs) technology use brain signals rather than muscles for communication and control applications. A BCI user may imagine movements in order to produce brain signals that serve as motor output. Preparing for movement or imagining movement (motor imagery (MI)) can generate oscillations in sensorimotor cortices that standard electroencephalographic (EEG) techniques may record. Understanding the brain networks involved and appropriately measuring and enhancing MI is important for both basic science and translational research. We seek to develop a new subject-specific BCI that trains individuals' ability to control MI-related brain activity. Here we measured the impact on the brain activity generated during a kinesthetic MI walking task of combining action observation with motor imagery. We recorded 64-channel EEG signals from participants watching a 20-sec video of a person walking away down a hallway. They were asked to imagine themselves following the person in the video and imitating their gait in speed and step sequence. We extracted time-frequency features in alpha (8-12 Hz) and beta (13-24 Hz) frequency bands from the recorded EEG signals in order to identify the gait phases of the observed/imagined movement. Most participants showed a correlation between the observed/imagined stance and swing gait phases and the power of the brain signals recorded over the sensorimotor cortex during the task. The ability to decode stance and swing phases of locomotion from EEG signals may provide new insight into their neural mechanisms and into the impact of action observation combined with the motor imagery practice of walking. Our goal is to use this knowledge to enable people with severe motor deficits to use motor imagery as a surrogate for skill-specific practice, thereby improving their sensorimotor function recovery. Motor imagery might provide a new therapeutic method to enhance functional recovery for people with stroke, spinal cord injury, or other disorders with sensorimotor impairments that prevent effective skill-specific practice.

Disclosures: A. Vato: None. **H. Mojtabavi:** None. **J. Brangaccio:** None. **S. Interlicchio:** None. **J. Shubhada:** None. **T.M. Vaughan:** None. **J.R. Wolpaw:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.01/JJ12

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R, A3803R)
NIH NIDCD (U01DC017844)
NIH NIDCD (R01DC014034)
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Toward assistive communication using a discrete ten-digit single hemisphere intracortical brain computer interface

Authors: *S. E. LÜTSCHG ESPINOSA[1], T. HOSMAN[1,2,4], C. NICOLAS[5], S. ALLCROFT[1,4], N. HERRICK[1,4], L. R. HOCHBERG[4,1,5,6,2], C. E. VARGAS-IRWIN[3,2,4], J. D. SIMERAL[4,1,2]; [1]Sch. of Engin., [2]Carney Inst. for Brain Sci., [3]Dept. of Neurosci., Brown Univ., Providence, RI; [4]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; [5]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [6]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain computer interfaces (iBCIs) have the potential to improve assistive communication options for people with paralysis or other severe motor impairments. Current assistive communication technologies for people with tetraplegia typically rely on head or eye movements that can be physically strenuous and exhausting. Although speech-to-text applications are also available, their use is limited for people who have difficulty speaking (for example, in advanced stages of ALS). Building on a novel ten-digit discrete decoder previously demonstrated in a freely-paced on-screen piano playing task (Society for Neuroscience, Program No.475.02, 2022), we present a novel assistive typing paradigm involving a virtual keyboard. The virtual keyboard has four rows each with 10 keys, where only one row is active at a time and each key in that row is mapped to a different finger or thumb. In this preliminary investigational study as part of the BrainGate2 pilot clinical trial, we decoded left-hand and right-hand finger presses attempted by participant T11, a 38-year-old-man with tetraplegia (C4 AIS-B) with two 96-channel microelectrode arrays recording neural activity in the left precentral gyrus. In initial sessions, we explored three different tasks to measure T11's ability to type using this ten-digit paradigm. The first task asked the participant to attempt individual finger presses corresponding to one of ten keys randomly cued on a single, unchanging keyboard row. The next task also randomly cued one of ten keys, however the keyboard row would change with each trial. The final task asked T11 to copy text shown on screen, highlighting the corresponding key and keyboard row for the upcoming letter. These fixed-pace tasks featured a two second cue-attempt period followed by a one second inter-trial interval, enforcing a typing speed of 20 characters per minute. We are integrating decoding additional hand gestures (e.g., wrist up or wrist down) to enable the participant to quickly switch between rows of the virtual keyboard providing a rich typing character set for uncued, free-paced typing. While this work to date relies on decoding neural signals from a single hemisphere, we anticipate that single and simultaneous bilateral digit decoding would be improved when recording motor activity from both hemispheres. Ultimately, the goal of this study is to enable ten-digit typing rates approaching that of able-bodied

individuals, providing a fluent and efficient means of text-based communication for individuals with severe motor or communication impairments.

Disclosures: S.E. Lütschg Espinosa: None. **T. Hosman:** None. **C. Nicolas:** None. **S. Allcroft:** None. **N. Herrick:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input;, MGH is a subcontractor on an NIH SBIR with Paradromics. **C.E. Vargas-Irwin:** None. **J.D. Simeral:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.02/JJ13

Topic: E.05. Brain-Machine Interface

Support: U01NS123101
Wu Tsai Neuroscience Institute, Stanford University
Pamela and Larry Garlick
NSF GRFP
McKnight foundation
NIH grant EB028171

Title: Beyond orofacial motor cortex: ventral precentral gyrus represents hand motor gesture sequences.

Authors: *B. MESCHEDE-KRASA[1], F. WILLETT[2], D. R. DEO[3], F. KAMDAR[4], N. HAHN[5], L. R. HOCHBERG[6], K. V. SHENOY[7], J. M. HENDERSON[3], S. DRUCKMANN[3];
[1]Stanford Univ. Neurosci. Phd Program, Palo Alto, CA; [2]Neurosurg., Stanford Univ. / HHMI, Stanford, CA; [3]Stanford Univ., Stanford Univ., Stanford, CA; [4]Stanford Univ., Mountain View, CA; [5]Neurosurg., Stanford Univ., Stanford, CA; [6]Brown Univ., Brown Univ., Providence, RI; [7]HHMI & Stanford Univ., Stanford Univ. & HHMI, Stanford, CA

Abstract: The neural activity underlying the preparation and execution of sequences of motor gestures in humans has not been well described at a high spatial resolution. In non-human primates, it has been shown that M1 and PMd do not encode whole sequences. Instead, individual arm reaches are independently prepared and executed [1]. Unexpectedly, we found distinct regions in human ventral premotor cortex that are tuned differently to individual motor gestures vs. sequences of gestures.
A BrainGate2 participant (T12) with bulbar-onset ALS underwent placement of two microelectrode arrays (inferior and superior) in ventral precentral gyrus targeting orofacial areas of motor cortex (localized to area 6v using the human connectome project pipeline). T12 retains partial movement of all limbs. In an instructed delay task, individual or sequences of wrist

gestures were cued and executed using a physical joystick while simultaneously recording neural activity and the joystick position.
We found distinct patterns of activity between the inferior and superior areas of 6v during the preparation and execution of individual vs sequence contexts of wrist movements. Only the superior area was tuned to preparation and execution of individual joystick movements; individual joystick gestures were not decodable from inferior 6v. When the same movements were performed in sequence, however, neural activity in the inferior array was tuned to the preparation of whole sequences. Similar results were found for tongue gesture sequences. This suggests inferior 6v is specifically activated during planning of sequential motor gestures.This demonstration of sequence specific encoding in inferior 6v during motor sequences adds to the growing body of evidence for functionally independent regions along PCG that do not obey a strict homuncular organization [2,3,4].
[1] Zimnik, Andrew J., and Mark M. Churchland. "Independent generation of sequence elements by motor cortex." Nature neuroscience 24.3 (2021): 412-424.[2] Willett, Francis R., et al. "Hand knob area of premotor cortex represents the whole body in a compositional way." Cell 181.2 (2020): 396-409.[3] Glasser, Matthew F., et al. "A multi-modal parcellation of human cerebral cortex." Nature 536.7615 (2016): 171-178.[4] Gordon, Evan M., et al. "A somato-cognitive action network alternates with effector regions in motor cortex." Nature (2023): 1-9.

Disclosures: B. Meschede-Krasa: None. **F. Willett:** None. **D.R. Deo:** None. **F. Kamdar:** None. **N. Hahn:** None. **L.R. Hochberg:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); MGH is a subcontractor on an NIH SBIR with Paradromics.. F. Consulting Fees (e.g., advisory boards); The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); KVS consults for Neuralink Corp. and CTRL-Labs Inc. (part of Facebook Reality Labs) and is on the scientific advisory boards of MIND-X Inc., Inscopix Inc., and Heal Inc. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock options in Maplight Therapeutics. F. Consulting Fees (e.g., advisory boards); advisor for Neuralink and Enspire DBS. **S. Druckmann:** F. Consulting Fees (e.g., advisory boards); CTRL labs.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.03/JJ14

Topic: E.05. Brain-Machine Interface

Support: AHA (19CSLOI34780000)
Office of Research and Development, Rehabilitation R&D Service
Dept of Veterans Affairs (N2864C, A2295R)
NIH NINDS (U01NS123101)
The content is solely the responsibility of the authors and does not

necessarily represent the o"cial views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government. CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Using intracortical BCI decoding in human motor cortex for restoring natural control of sustained hand grips with a soft robotic glove

Authors: *J. T. GUSMAN[1,2,4], D. S. DE LUCENA[5], T. HOSMAN[1,2,4], C. NICOLAS[6], A. KAPITONAVA[6], D. A. WAGNER[5], N. HAHN[7], J. M. HENDERSON[7,8,9], J. D. SIMERAL[4,1,2], C. E. VARGAS-IRWIN[3,2,4], C. J. WALSH[5], L. R. HOCHBERG[4,1,6,10,2];
[1]Sch. of Engin., [2]Carney Inst. for Brain Sci., [3]Dept. of Neurosci., Brown Univ., Providence, RI; [4]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; [5]John A. Paulson Sch. of Engin. and Applied Sci., Harvard Univ., Cambridge, MA; [6]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [7]Neurosurg., [8]Wu Tsai Neuro. Inst., [9]Bio-X Program, Stanford Univ., Stanford, CA; [10]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain-computer interfaces (iBCIs) record neural signals to give people with tetraplegia the ability to control external devices such as computer cursors and robotic arms. The opportunity to record and decode signals directly from the motor cortex with iBCIs allows for highly precise predictions of motor intent. Therefore, iBCIs can provide users with an option to employ highly familiar motor imageries when controlling iBCI effectors - a quality we feel is especially important when iBCIs are combined with functional electrical stimulation or soft robotics to control one's *own* limbs. However, some movements such as sustained grasps (i.e. "grasp and hold") may not be well represented by cortical signals. In this study, we investigated the representations of sustained grasps in the hand knob areas of two BrainGate clinical trial participants and assessed the performance of three iBCI control schemes in maintaining hand postures using a soft robotic glove (SRG). Experimental sessions were performed by participant T11, a 38-year-old man with C4 AIS-B spinal cord injury, and participant T5, a 69-year-old man with C4 AIS-C spinal cord injury. Both participants had two 96-electrode arrays implanted in the hand knob area of their left precentral gyri as part of the BrainGate Clinical Trial. A fabric-based, pneumatically actuated glove and control system were manufactured and programmed to provide 4 functionally relevant grip states: power, pinch, open hand, and relax. The participants were instructed to attempt and hold hand postures for 30s at a time, first in an "open-loop" setting without neural decoding and then in "closed-loop" using neural decoders (LDA-HMM) that were optimized based on the open-loop results. We directly compared two robotic control strategies: a "Continuous" controller where SRG postures reflect continuously decoded estimates of intended grip type, and a "Toggle" controller where transient gesture attempts are used to *toggle* between SRG end postures. For both participants, the Toggle controller provided far more consistent hand posture maintenance than the Continuous controller, however T5 still preferred the Continuous controller because of its more naturalistic control strategy. We also tested a third control scheme, the "Latch and Lock" controller, which allows for a more naturalistic user experience while matching the functional performance of the Toggle controller. From understanding motor representations in the brain to engineering user-friendly control schemes, this work highlights the various facets necessary to consider when attempting to deliver naturalistic iBCI control of novel effectors.

Disclosures: **J.T. Gusman:** None. **D.S. de Lucena:** None. **T. Hosman:** None. **C. Nicolas:** None. **A. Kapitonava:** None. **D.A. Wagner:** None. **N. Hahn:** None. **J.M. Henderson:** Other; JMH is a consultant for Neuralink Corp and Proteus Biomedical, and serves on the Medical Advisory Board of Enspire DBS. **J.D. Simeral:** None. **C.E. Vargas-Irwin:** None. **C.J. Walsh:** Other; CJW is an inventor on patent and patent applications filed by Harvard University that describes the inflatable soft robotic components in this research. **L.R. Hochberg:** Other; The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input., MGH is a subcontractor on an NIH SBIR with Paradromics.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.04/JJ15

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R, B6453R, A6779I)
NIH NIDCD (U01DC017844)
NIH NIDCD (R01DC014034)
NIH NINDS (UH2NS095548)
NIH NINDS R25NS065743
The American Academy of Neurology Clinical Research Training Scholarship
Harvard Catalyst KL2/CMeRIT Award
NIH NIDCD (R01DC009899)
AHA (19CSLOI34780000)
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Spatiotemporal transformers accommodate future neural nonstationarities for iBCIs with minimal training data through contrastive learning

Authors: ***J. JUDE**[1,2], T. PUN[3,4], T. HOSMAN[3,4,5], C. NICOLAS[2], A. KAPITONAVA[2], J. N. KELEMEN[2], L. R. HOCHBERG[5,3,2,6,4], D. RUBIN[2];
[1]Ctr. for Neurotechnology and NeuroRecovery, [2]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [3]Sch. of Engin., [4]Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [5]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; [6]Harvard Med. Sch., Dept. of Neurol., Boston, MA

Abstract: Stable neural activity is critical for accurate decoding of intended movement for cursor control in implanted intracortical brain-computer interfaces (iBCIs) for people with tetraplegia. However, recordings of neural activity underlying intended arm and hand movement are prone to nonstationarities that accrue over time, resulting in the decline in performance of iBCIs over the course of hours to days. Reliable decoding of consistent behaviour thus requires occasional recalibration on newly observed neural activity in order to maintain adequate decoding performance. This calibration process usually occurs under supervision and can be time-consuming and inconvenient to the users. However, recent work has shown that recurrent non-linear modelling approaches can generalise to unseen neural data for months into the future when trained on a wealth of historic recording session data. While drift in spiking activity cannot be predicted accurately at the level of individual neurons, population level variations over several days may be learnable as the underlying low-dimensional manifold corresponding to such neural activity has been shown to be stable over time in non-human primates. In this work, we use a contrastive learning approach in which conceivable nonstationarities that occur over time for a given population of neurons are simulated and a transformer decoder model is trained to recapitulate the original neural activity from these simulated nonstationarities. To this end, we first train a spatiotemporal transformer in a self-supervised fashion to predict the original neural patterns from highly perturbed neural data and use the activations of this network to inform our downstream transformer decoder. We show that this training procedure produces an ensemble decoder capable of days-long high-performance real-time cursor control from just a few minutes of calibration data from a single recording session, with no further retraining. Transformers allow for the rapid training of a large quantity of non-stationary simulated neural data, which would be infeasible when using autoregressive models such as recurrent neural networks.

Disclosures: **J. Jude:** None. **T. Pun:** None. **T. Hosman:** None. **C. Nicolas:** None. **A. Kapitonava:** None. **J.N. Kelemen:** None. **L.R. Hochberg:** Other; The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input., MGH is a subcontractor on an NIH SBIR with Paradromics.. **D. Rubin:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.05/JJ16

Topic: E.05. Brain-Machine Interface

Support: R01DC014034
Wu Tsai Neurosciences Institute at Stanford
Larry and Pamela Garlick
Howard Hughes Medical Institute
Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R)
Content is solely the responsibility of the authors and does not necessarily

represent the official views of the NIH, or the Department of VA or the US Government. CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Decoding Dexterous Finger Movements for Intracortical Neuroprosthetic Control

Authors: *M. WILLSEY[1,2], N. SHAH[2,3], D. AVANSINO[2,4,3,5,6], N. HAHN[2], R. JAMIOLKOWSKI[2], L. R. HOCHBERG[11,12,13,14,15], K. V. SHENOY[3,5,6,7,4], J. M. HENDERSON[2,8,9,10];
[2]Neurosurg., [3]Electrical Engin., [4]Howard Hughes Med. Inst., [5]Bioengineering, [6]Neurobio., [7]Wu Tsai Neurosciences Inst., [8]Stanford Univ. Sch. of Med., [9]Wu Tsai Neurosci. Inst., [10]Stanford Bio-X, [1]Stanford Univ., Palo Alto, CA; [11]Carney Inst. for Brain Sci., [12]Sch. of Engin., Brown Univ., Providence, RI; [13]Dept. of VA, Med. Ctr., RR&D Ctr. for Neurorestoration and Neurotechnology, Providence, RI; [14]Neurol., Harvard Med. Sch., Boston, MA; [15]Neurol., Massachusetts Gen. Hospital, Ctr. for Neurotechnology and Neurorecovery, Boston, MA

Abstract: Restoration of hand function with dexterous finger movements is a high priority for people with quadriplegia. Intracortical brain-computer interfaces (iBCIs) could eventually restore this lost function. Herein a research participant with paralysis and microelectrode arrays in motor cortex demonstrates real-time, closed-loop decoding of three independent virtual finger groups that includes 2D thumb movement. A 69-yo man with C4 AIS C spinal cord injury ('T5') had two 96-channel silicon microelectrode arrays placed in hand 'knob' area of left precentral gyrus in 2016. A virtual finger task on a computer display was developed for online, closed-loop control of three finger groups (thumb, index/middle, ring/small) that included 2D thumb movements. On paired trials, the participant was cued to move two simultaneous finger groups from a center position to random targets within the active range-of-motion of fingers. On the subsequent trial, targets are placed back at the center (trial timeout time of 10s and hold time of 500ms). A neural network decoding algorithm, adapted from Willsey et al. (2022), mapped electrode spike-band power to a control signal for finger velocities. A metric for signal-to-noise ratio (SNR) was defined and then SNR was empirically calculated during the time interval 200-700ms after the start of a trial. On 8 days and 1046 trials of online, closed-loop control, T5 completed 99.2 % of trials with a mean acquisition time (excluding hold time) of 1980±40ms (mean±S.E.M.). In the final two days, mean acquisition time had improved to 1560±40ms on average. Decoder stability was tested on two of the test days by fixing the parameters and running trials until the participant could not acquire the targets, which required 228 trials on one day and 482 trials on a second day. The dependency between SNR and channel count revealed that SNR increased with channel count raised to the fractional power of 0.26 ($R^2 = 0.96$), meaning a doubling of the SNR requires a 14-fold increase in channel count. Finally, to test finger decoding in a real-world environment, the finger control was mapped to a control paradigm for a virtual drone, which demonstrates the feasibility of using this control for a real-world application. Herein, we demonstrate smooth, continuous decoding of 3 independent finger groups with a 2D thumb movements that can be potentially used for fine motor control, digital interfaces, video game play, or other neuroprostheses.

Disclosures: M. Willsey: None. **N. Shah:** None. **D. Avansino:** None. **N. Hahn:** None. **R. Jamiolkowski:** None. **L.R. Hochberg:** 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.; MGH is a subcontractor on an NIH SBIR with Paradromics.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); KVS consulted for Neuralink Corp. and CTRL-Labs Inc. (part of Facebook Reality Labs) and is on the scientific advisory boards of MIND-X Inc., Inscopix Inc., and Heal Inc. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Recipient of stock options in MapLight Therapeutics. F. Consulting Fees (e.g., advisory boards); Neuralink, Enspire DBS.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.06/JJ17

Topic: E.05. Brain-Machine Interface

Support: NIH-NINDS/OD DP2NS127291
Emory Neuromodulation and Technology Innovation Center (ENTICe)
NIH F32HD112173
NIH T32EB025816
NIH-NIDCD U01DC017844
Department of Veterans Affairs Rehabilitation Research and Development Service A2295R

Title: An intermittent movement intention decoding paradigm for BCI control

Authors: *M. RIGOTTI-THOMPSON[1], S. R. NASON-TOMASZEWSKI[1], Y. H. ALI[1], D. MIFSUD[1], C. NICOLAS[2], S. ALLCROFT[3,5], L. R. HOCHBERG[5,3,2,6,4], N. AU YONG[7,1], C. PANDARINATH[1,7];
[1]Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlanta, GA; [2]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [3]Sch. of Engin., [4]Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [5]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of Veterans Affairs Med. Ctr., Providence, RI; [6]Dept. of Neurol., Harvard Med. Sch., Boston, MA; [7]Dept. of Neurosurg., Emory Univ., Atlanta, GA

Abstract: Brain-computer interfaces (BCIs) are a promising avenue that could allow people with tetraplegia to control assistive devices. Standard intracortical BCIs employ continuous control paradigms, which infer moment-by-moment movement commands from incoming neural activity to update the position of an effector (e.g., on-screen computer cursor or robotic arm). However, since instantaneous neural activity is noisy, this results in a continuous human-in-the-loop process by the user to correct for instantaneous decoding errors, limiting control performance.

One approach to overcome this issue is to leverage pre-movement neural activity to decode the intended movement target. This has resulted in increased performance for tasks with discrete end-points and fixed timing. In this work, we propose a decoding paradigm which expands on this approach, by (1) not being limited to a discrete number of possible target positions, and (2) decoding the intermittent intention to initiate movement from neural activity, allowing control without a fixed task timing. To evaluate the effectiveness of this decoding paradigm, we designed a 2D cursor following task where the participant is instructed to prepare a movement to a randomly cued peripheral target during a known delay indicated by a timer (random between 0.4s and 1.0s). When the timer expires ("go cue"), the participant is instructed to attempt a quick movement (0.4s) to the cued target. This task was performed by participant T11, a 38-year-old man with C4 AIS-B spinal cord injury who had two 96-channel microelectrode arrays (Blackrock Microsystems) placed in his left precentral gyrus as part of the BrainGate2 clinical trial. Consistent with previous studies, offline analyses of neural activity reveal a target-independent movement onset signal aligned to the go cue, that can be reliably predicted across various anticipated delay conditions (within 200ms of go cue for >80% of trials). Additionally, movement end-point could be accurately decoded from a single 200ms window of neural activity preceding the go cue ($R2 > 0.65$). As a proof of concept for BCI use, T11 performed a variation of the task where the direction of his intended movement was decoded online right after the go cue. An on-screen cursor then moved quickly (0.4s) to the position at a fixed distance in the decoded direction (with 50% aim assistance), providing working closed-loop control (accuracy > 75%, 8 targets). Future research sessions will involve online movement onset decoding for self-paced control. Overall, these results show the potential for intermittent movement intention decoding as a paradigm for fast and accurate BCI control.

Disclosures: M. Rigotti-Thompson: None. **S.R. Nason-Tomaszewski:** None. **Y.H. Ali:** None. **D. Mifsud:** None. **C. Nicolas:** None. **S. Allcroft:** None. **L.R. Hochberg:** 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.; Paradromics. F. Consulting Fees (e.g., advisory boards); Neuralink, Synchron, Reach Neuro, Axoft, Precision Neuro. **N. Au Yong:** None. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Synchron, Meta (Reality Labs).

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.07/JJ18

Topic: E.05. Brain-Machine Interface

Support: NIH-NINDS/OD DP2NS127291

Title: Hybrid neural dynamics models enable high performing and stable iBCI decoding from local field potentials

Authors: *B. M. KARPOWICZ[1], B. BHADURI[1], J. D. MCCART[2,1], Y. H. ALI[1], R. D. FLINT, III[3], M. W. SLUTZKY[3,4,5,6,7], C. PANDARINATH[1,8,2];
[1]Wallace H. Coulter Dept. of Biomed. Engin., Georgia Tech. & Emory Univ., Atlanta, GA; [2]Ctr. for Machine Learning, Georgia Inst. of Technol., Atlanta, GA; [3]Dept. of Neurol., [4]Dept. of Neurosci., [5]Dept. of Biomed. Engin., [6]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [7]Shirley Ryan AbilityLab, Chicago, IL; [8]Dept. of Neurosurg., Emory Univ., Atlanta, GA

Abstract: Population-level analyses of neural recordings via latent variable models (LVMs) are a promising approach for improving intracortical brain-computer interfaces. LVMs have been shown to infer robust denoised representations of neural data, to yield high-accuracy decoding from many brain areas and behaviors at the single-trial level, and often to require less training data than decoding from single neurons. Typically LVMs are trained on spiking activity, which tends to yield higher decoding accuracy and precision in subsequent analyses than alternative signals. However, extracting spikes from neural recordings can be unreliable as nonstationarities may occur due to tissue immune responses and shifts in electrode position relative to surrounding neurons.

Supplementing spiking data with more consistent signals like local field potentials (LFP) can improve decoding performance when spiking data alone is unreliable. In addition, LFP often yields more consistent decoding performance over longer timescales than spiking data in light of nonstationarities. Here we apply latent factor analysis via dynamical systems (LFADS; Pandarinath et al., 2018) to estimate denoised spiking activity (i.e., firing rates (FRs)) from simultaneously collected LFP. We hypothesized that this approach would combine the stability of LFP with the high accuracy behavioral predictions enabled by spikes to improve BCI stability. We trained LFADS to use only LFP to compute FR estimates that explain the spiking data under a Poisson observation model. To mitigate the effects of empirical LFP nonstationarities, we applied data augmentation (e.g., channel dropping, temporal shift, magnitude scaling) to the LFP and trained LFADS to estimate the denoised spiking data from the augmented LFP. After training, the model requires only unaugmented LFP to produce FR estimates.

We tested this model using data from one monkey during a radial-8 reaching task. When given high-frequency LFP (150-450 Hz; HF-LFP) from one session, the model produced single trial FRs that accurately represented the spiking data (median PSTH $R^2 = 0.41$) and were highly predictive of behavior (median velocity $R^2 = 0.84$), comparable to spiking-only LFADS (med. vel. $R^2 = 0.85$) and exceeding smoothed HF-LFP (med. vel. $R^2 = 0.72$). When tested with only HF-LFP data from unseen sessions separated by up to 40 days, velocity decoding remained more stable (med. $R^2 = 0.71$; half life = 160 days) than spiking-only LFADS (med. $R^2 = -0.11$; half life = 2 days) or smoothed HF-LFP (med. $R^2 = 0.48$; half life = 96 days). Future work will test this model with different tasks (e.g. random target reaching), species (e.g. human), and LFP inputs (e.g. 0-1000 Hz).

Disclosures: B.M. Karpowicz: None. **B. Bhaduri:** None. **J.D. McCart:** None. **Y.H. Ali:** None. **R.D. Flint:** None. **M.W. Slutzky:** None. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Consultant to Synchron and Meta (Reality Labs)..

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.08/JJ19

Topic: E.05. Brain-Machine Interface

Support: NSF 1926576
NIH F31HD098804
NIH R01GM111293
NIH T32NS007222
NIH F32HD112173
NIH DP2NS127291
NIH T32EB025816
Craig H. Neilsen Foundation 315108
Simons Undergraduate Research Fellowship
MCubed 1482
A. Alfred Taubman Medical Research Institute
NIH-NIDCD U01DC017844
Department of Veterans Affairs Rehabilitation Research and Development Service A2295R
Emory Neuromodulation and Technology Innovation Center (ENTICe)

Title: Modeling neural population dynamics improves individuated finger movement decoding

Authors: *S. R. NASON-TOMASZEWSKI[1], N. HO[2], D. MIFSUD[1], M. RIGOTTI-THOMPSON[1], Y. H. ALI[1], C. NICOLAS[3], L. R. HOCHBERG[3,4,5,6], M. S. WILLSEY[7,9], P. G. PATIL[7,8,9,10], C. A. CHESTEK[9,11,10,12], N. AU YONG[13,1], C. PANDARINATH[1,13];
[1]Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlanta, GA; [2]Computer Sci., Georgia Inst. of Technol., Atlanta, GA; [3]Ctr. for Neurotechnology and Neurorecovery, Massachusetts Gen. Hosp., Boston, MA; [4]Carney Inst. for Brain Sci. and Sch. of Engin., Brown Univ., Providence, RI; [5]Harvard Med. Sch., Boston, MA; [6]Dept. of Veterans Affairs Med. Ctr., Providence, RI; [7]Neurosurg. Dept., [8]Dept. of Neurol., Univ. of Michigan Med. Sch., Ann Arbor, MI; [9]Biomed. Engin. Dept., [10]Neurosci. Grad. Program, [11]Robotics Inst., [12]Electrical Engin. and Computer Sci., Univ. of Michigan, Ann Arbor, MI; [13]Dept. of Neurosurg., Emory Univ., Atlanta, GA

Abstract: A critical challenge with improving intracortical brain-computer interfaces (iBCIs) lies in understanding how neural populations represent movement intention. For reaching movements, modeling neural populations as nonlinear dynamical systems has provided new insights into how the brain generates behavior, leading to dramatic open-loop decoding performance increases. Yet to date, these benefits have not held for hand movements: modeling neural populations as dynamical systems during grasping does not improve decoding performance (Suresh, Goodman et al., 2020). It remains unclear if this result generalizes to other hand behaviors or is exclusive to grasping. Here, we investigated whether dynamics are useful for modeling and decoding neural population activity during an individuated finger task performed by a nonhuman primate (NHP) and a human clinical trial participant. We recorded

neural activity from precentral gyrus using 96 microelectrodes (Blackrock Microsystems) in an able-bodied NHP (Monkey N) and 192 microelectrodes in participant T11, a 38-year-old man with C4 AIS-B spinal cord injury enrolled in the BrainGate2 clinical trial. Both subjects performed an individuated finger task in which they were instructed by a virtual hand to individuate physical (Monkey N) or imagined (T11) movements of their index finger from their grouped middle, ring, and small fingers. We trained two single timestep optimal linear estimator decoders to predict finger group velocities at 100Hz. The first (B-OLE) used exponentially-smoothed binned spike counts and the second (L-OLE) used firing rates inferred by Latent Factor Analysis via Dynamical Systems (LFADS), a nonlinear dynamical systems neural population model. With both subjects, L-OLE improved open-loop velocity prediction performance over B-OLE, from a coefficient of determination (R2) of 0.17 to 0.41 with Monkey N and from 0.24 to 0.34 with T11. To begin to explain this improvement, we visualized Monkey N's neural population activity structure during this task using principal component analysis decomposition of the LFADS-inferred firing rates. The structure revealed single trial neural state space trajectories that were more consistent than smoothed spikes across repetitions of the same finger movements. Importantly, during trials with atypical kinematics, the corresponding state space trajectories were also markedly atypical, suggesting the model captures neural variability on a single trial basis. Altogether, these results suggest that precentral gyrus activity during individuated finger movements can be modeled by a dynamical system, and doing so may improve iBCI decoding performance.

Disclosures: **S.R. Nason-Tomaszewski:** None. **N. Ho:** None. **D. Mifsud:** None. **M. Rigotti-Thompson:** None. **Y.H. Ali:** None. **C. Nicolas:** None. **L.R. Hochberg:** 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.; Paradromics. F. Consulting Fees (e.g., advisory boards); Neuralink, Synchron, Reach Neuro, Axoft, Precision Neuro. **M.S. Willsey:** None. **P.G. Patil:** None. **C.A. Chestek:** None. **N. Au Yong:** None. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Synchron, Meta (Reality Labs).

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.09/JJ20

Topic: E.05. Brain-Machine Interface

Support: NSF GRFP Grant No. DGE-1656518
NSF Grant No. 1828993
NIH NINDS Grant No. U01NS123101
Stanford Wu Tsai Neurosciences Institute
Pamela and Larry Garlick
Howard Hughes Medical Institute
Sloan Fellowship, Alfred P Sloan Foundation

Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R)
The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, or the Dept. of VA or the US Government. CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: Cross-participant transfer for intracortical handwriting BCIs

Authors: *A. D. LEVIN[1], F. WILLETT[6], F. KAMDAR[2], D. AVANSINO[3], L. R. HOCHBERG[7], K. V. SHENOY[8], S. LINDERMAN[4], J. M. HENDERSON[5];
[1]Computer Sci., Stanford Univ., Stanford, CA; [2]Stanford Univ., Mountain View, CA; [3]Stanford Univ., San Francisco, CA; [4]Statistics, Stanford Univ., Menlo Park, CA; [5]Neurosurg., Stanford Univ., Stanford, CA; [6]Stanford Univ. / HHMI, Stanford, CA; [7]Mass. Gen. Hosp./Brown/PVAMC, Brown Univ., Boston, MA; [8]Electrical Engin., Stanford Univ. & HHMI, Stanford, CA

Abstract: Intracortical brain-computer interfaces (BCIs) are a promising tool for restoring rapid communication to people with paralysis. However, intracortical BCIs that decode complex movements, such as handwriting and speech, require substantial training data to achieve high performance. Each new BCI user must therefore engage in extensive supervised data collection during the initial setup phase, which is impractical for real-world use. Here, we explored the feasibility of cross-participant transfer, aiming to leverage the neural activity recordings of one BCI user to reduce the data collection burden on another, new user. We used recurrent neural network-based handwriting BCI as a testbed, as they require a large amount of training data—over 200 sentences—to achieve the highest possible performance (Willett et al. 2021).
In this study, we replicated the handwriting BCI that was first demonstrated in a research participant (T5) who had two microelectrode arrays placed in the hand area of dorsal premotor cortex (area 6d). Our handwriting BCI was replicated in another research participant (T12), who had two microelectrode arrays placed in the ventral premotor cortex (area 6v). We achieved a character error rate (CER) of 10.1% compared to T5's originally reported CER of 5.9% (Willett et al. 2021). This is the first replication of handwriting decoding in another participant.
With handwriting data from two participants, we conducted a preliminary cross-participant BCI transfer test. We trained RNN decoders on a large T5 dataset (471 sentences across 10 days) supplemented with a subset of increasing amounts of T12 data (10-95 sentences from a single day), and then evaluated their performance on a held-out set of T12 data. We found that, for smaller T12 datasets, adding the large library of T5 data improved T12 decoding performance. For example, when training decoders on just 20 sentences of T12 data, the CER was 46.2%. After supplementing those same 20 sentences with the large T5 dataset, the CER dropped to 28.5%. Further work will explore different decoding algorithms, training and fine-tuning methods in order to achieve high-performance decoding with just a small amount of data from a new user.

Disclosures: A.D. Levin: None. **F. Willett:** None. **F. Kamdar:** None. **D. Avansino:** None. **L.R. Hochberg:** Other; Research agreements: Neuralink, Synchron, Axoft, Precision Neuro, Reach Neuro. **K.V. Shenoy:** F. Consulting Fees (e.g., advisory boards); Neuralink, CTRL-Labs, MIND-X, Inscopix and Heal. **S. Linderman:** None. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding

diversified mutual funds); Maplight Therapeutics. F. Consulting Fees (e.g., advisory boards); Neuralink, Enspire DBS.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.10/JJ21

Topic: E.05. Brain-Machine Interface

Support: Emory Neuromodulation and Technology Innovation Center (ENTICe)
NIH-NINDS/OD DP2NS127291
NIH NIBIB T32EB025816
NIH F32HD112173
NIH-NIDCD U01DC017844
Department of Veterans Affairs Rehabilitation Research and Development Service A2295R

Title: Closed-loop latent dynamics modeling for iBCI control

Authors: *Y. H. ALI[1], D. M. MIFSUD[1], S. R. NASON-TOMASZEWSKI[1], M. RIGOTTI-THOMPSON[1], B. BHADURI[1], C. NICOLAS[2], S. ALLCROFT[3,5], N. AU YONG[6,1], L. R. HOCHBERG[5,3,2,7,4], C. PANDARINATH[1,6];
[1]Wallace H. Coulter Dept. of Biomed. Engin., Emory Univ. and Georgia Inst. of Technol., Atlanta, GA; [2]Ctr. for Neurotechnology and Neurorecovery, Massachusetts Gen. Hosp., Boston, MA; [3]Sch. of Engin., [4]Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [5]Dept. of VA Med. Ctr., VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence, RI; [6]Dept. of Neurosurg., Emory Univ., Atlanta, GA; [7]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Intracortical brain-computer interfaces (iBCIs) have allowed individuals with tetraplegia to control assistive devices like computers by decoding their movement intention from neural recordings. These decoders rely on the incoming neural recordings to contain salient information about the behavior being predicted, but noise in the recordings makes it difficult for a decoder to extract this information. Latent Factor Analysis via Dynamical Systems (LFADS) is an artificial neural network (ANN) that denoises neural data by modeling the latent dynamics of a neural population. In offline analysis, LFADS has been shown to dramatically increase decoding accuracy, but it has not been evaluated in closed-loop iBCI control. In this study, we demonstrate the first use of LFADS for real-time neural denoising in a human closed-loop cursor control task. Neural activity was recorded from participant T11, a 38-year-old man with C4 AIS-B spinal cord injury who has two 96-channel microelectrode arrays (Blackrock Microsystems) placed in his left precentral gyrus as a part of the BrainGate2 clinical trial. T11 performed cursor control with and without LFADS-denoised neural firing rates in a radial-8 center-out-and-back task. In each trial, T11 was given 10 seconds from target onset to move the cursor over the target and hold it there for 0.5 seconds. We compared the performance of two optimal linear estimator

(OLE) decoders at controlling the cursor in this task: one using binned spikes and spike-band power (B-OLE) and the other using LFADS firing rates (L-OLE). The B-OLE decoder was trained on three minutes of open-loop cursor following with the radial-8 task. The LFADS model and L-OLE were trained on four minutes of radial-8 closed-loop cursor control that T11 had previously performed using the B-OLE decoder. Both decoders produced cursor velocity predictions at 100 Hz. T11 achieved a median target acquisition time of 1.715 ± 0.633 seconds (95.92% success, 98 trials) with LFADS and L-OLE and 1.760 ± 0.632 seconds (100% success, 124 trials) with B-OLE. These results provide initial evidence that ANN-based denoising models like LFADS can be effective for closed-loop iBCI control. While LFADS did not provide a measured improvement in target acquisition time in this simple task, future studies will investigate whether LFADS improves the control of more complex behaviors like cursor-based typing or individuated finger control. This initial proof-of-concept lays the groundwork for evaluating how ANN-based denoising models like LFADS can be used to improve iBCI control.

Disclosures: Y.H. Ali: None. **D.M. Mifsud:** None. **S.R. Nason-Tomaszewski:** None. **M. Rigotti-Thompson:** None. **B. Bhaduri:** None. **C. Nicolas:** None. **S. Allcroft:** None. **N. Au Yong:** None. **L.R. Hochberg:** Other; The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input., MGH is a subcontractor on an NIH SBIR with Paradromics., These entities did not support this work. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Synchron, Meta (Reality Labs).

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.11/JJ22

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (A2827R, A3803R , N2864C)
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use

Title: A second-generation battery-powered embedded processing system for large scale broadband intracortical BCI

Authors: *J. D. SIMERAL[1,2,3], T. HOSMAN[2,3,4], A. V. NURMIKKO[2,3], D. M. ROSLER[5,2,6,3], L. R. HOCHBERG[5,2,7,8,3];
[1]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. VA Med.Ctr., Providence, RI; [2]Sch. of Engin., [3]Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [4]VA RR&D Ctr. for

Neurorestoration and Neurotechnology, Dept. of VA Med.Ctr., Providence, RI; [5]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; [6]Dept. of Neurol., [7]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [8]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: Advances in neural signal decoding for human iBCI have demonstrated high-accuracy 2D cursor control via recursive neural networks (Hosman et al., NER 2019, 2023), brain-to-text communication via imagined handwriting (Willett et al., 2021), and real-time generation of spoken sentences assembled from phonemes decoded from imagined speech (Willett et al., BCI Annual Award, 2022). However, these and other promising iBCI demonstrations rely on networks of computers to meet the requisite real-time computational demands. Individuals with paralysis or the inability to speak would experience greater benefit from an iBCI that could enable such assistive capabilities in a turnkey device available on a wheelchair throughout daily life. Toward this goal, we previously demonstrated a prototype low-power mobile neural signal processing system that leveraged a System-on-Chip (SoC) Zynq-7000 hybrid embedded processor to stream 256 channels of simulated spiking and broadband neural data from a wireless receiver to downstream (Blackrock Neurotech) signal display and storage software (Heelan et al., SfN, 2017). Building on that proof-of-concept system, here we report a second-generation embedded processing system with enhancements for in-home iBCI and ongoing research. Advanced neural feature extraction and decoding approaches are being implemented on FPGA for low power computation with real-time parameter tuning. WiFi communication is now complemented by BLE 5 HID for direct neural control of Bluetooth-enabled mobile touch devices (e.g., iPad), dual USB 2.0 (for local data storage), dual Gb Ethernet, and audio and sync-pulse recording. The redesigned power system extended battery runtime while reducing heat, enabling fanless operation within safe touch temperature limits per standard IEC 60601-1 for medical devices. The new internal battery pack uses medical-grade 18650 batteries with built-in cell protection, battery charge monitoring, and hardware protection against over current, over voltage, short circuit, and over temperature conditions. A new external battery pack for extended run time uses IEC 62132-2 certified batteries for safety. An LCD screen displays system status to caregivers. Specification and design documents were maintained under Engineering Change Control processes. Ongoing research is investigating FPGA instantiation of RNN decoding, gesture decoding, state-of-the-art dimensionality reduction for longitudinal decoding stability, and evaluation in the BrainGate clinical trial. Together, these advances move closer to turnkey availability of iBCI assistive capabilities in a powerful mobile device for use at home and beyond.

Disclosures: J.D. Simeral: None. **T. Hosman:** None. **A.V. Nurmikko:** None. **D.M. Rosler:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input;, MGH is a subcontractor on an NIH SBIR with Paradromics. These entities did not support this work.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.12/JJ23

Topic: E.05. Brain-Machine Interface

Support: A. P. Giannini Foundation Postdoctoral Fellowship to N. S. Card
Pilot Award from the Simons Collaboration for the Global Brain to S. D. Stavisky
Career Award at the Scientific Interface from the Burroughs Wellcome Fund to S. D. Stavisky
Stanford Wu Tsai Neurosciences Institute to J. M. Henderson
HHMI to J. M. Henderson
Simons Foundation to J. M. Henderson
NIH-NIDCD (1U01DC019430) to J. M. Henderson

Title: Condition-invariant, rotatory, and low tangling neural ensemble dynamics in ventral motor cortex during attempted speech

Authors: *N. S. CARD[1], N. HAHN[2], F. KAMDAR[2], M. WAIRAGKAR[1], E. KUNZ[3,4], F. R. WILLETT[2,3,5], L. R. HOCHBERG[6,7,8], J. M. HENDERSON[2,4], D. BRANDMAN[1], S. D. STAVISKY[1];
[1]Neurolog. Surgery, Univ. of California, Davis, Davis, CA; [2]Neurosurg., [3]Electrical Engin., [4]Wu Tsai Neurosciences Inst., [5]Howard Hughes Med. Inst., Stanford Univ., Stanford, CA; [6]Sch. of Engin. and Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [7]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Providence VA Med. Ctr., Providence, RI; [8]Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Understanding how the brain encodes and generates coordinated movements is central to restoring motor function to individuals with paralysis. Mounting evidence suggests that populations of neurons in dorsal precentral gyrus (dPCG, motor cortex) cooperate as a dynamical system that generates precise patterns of activity that enable movements of the arm and hand. However, comparatively little is known about whether similar computational motifs extend to the neural control of speech. The neural basis of speaking is notoriously difficult to study because complex speech is a uniquely human capability, and there is a scarcity of human intracortical measurements. Here, we examine neural ensemble dynamics during attempted speaking in ventral precentral gyrus (vPCG, speech motor cortex), which is a key node in the speech network.Neuronal spiking data were recorded from BrainGate2 clinical trial participant 'T12', a 67-year-old, left-handed woman with ALS who has severe dysarthria, using 2 chronic Utah microelectrode arrays (64 electrodes each) implanted in left vPCG. Neural activity was recorded while T12 attempted to speak prompted phonemes or words in an instructed-delay task. In each experimental session, 8-50 prompts were repeated 18-72 times each. Firing rates were trial-averaged, smoothed, and aligned to either the go cue or the onset of attempted speech. We then applied dimensionality reduction techniques that were specifically designed to reveal the presence (or absence) of specific population dynamic motifs to neural data from vPCG. The motifs that we looked for included a condition-invariant signal (CIS) at movement initiation (Kaufman et al. 2016), rotatory dynamics at movement onset (Churchland et al. 2012), and minimal tangling of neural trajectories during movement execution (i.e., similar activity patterns

lead to similar future patterns; Russo et al. 2018).We identified all three computational motifs in vPCG. First, a large CIS occurred at the onset of attempted speech, regardless of the word being spoken (first CI dimension captured 29.3% of neural variance). Second, rotatory population dynamics that were significantly stronger than expected by chance were present surrounding the onset of audible speech (top jPCA plane captured 60% of neural variance, R2_skew=0.83, p=0.002). Third, neural trajectories during speech avoided tangling. These results indicate that dynamics previously observed during arm motor control in dPCG are also present during speaking in vPCG, suggesting that these computational principles may be ubiquitous throughout motor control brain areas and applicable to both arm and speech articulator control.

Disclosures: **N.S. Card:** None. **N. Hahn:** None. **F. Kamdar:** None. **M. Wairagkar:** None. **E. Kunz:** None. **F.R. Willett:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); Neuralink, Synchron, Axoft, Precision Neuro, Reach Neuro. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuralink, Enspire DBS, Maplight Therapeutics. F. Consulting Fees (e.g., advisory boards); Neuralink, Enspire DBS. **D. Brandman:** None. **S.D. Stavisky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); licensed Stanford IP related to intracortical BCIs.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.13/JJ24

Topic: E.05. Brain-Machine Interface

Support: Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R)
NIH NIDCD (U01DC017844)
NIH NINDS (U01NS123101)
AHA (19CSLOI34780000)
National Science Foundation EFRI Award (no. 1830896)
Cullen Education and Research Fund (CERF) Medical Engineering Prize for ALS
Harvard School of Engineering and Applied Sciences
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government.
CAUTION: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Designing a system for intracortical BCI control of a soft robotic arm for people with tetraplegia

Authors: *K. RAO[1], S. ALLCROFT[2,4], B. SCHORNSTEIN[2], J. GUSMAN[2,3,4], C. NICOLAS[5], L. GEREZ[6], J. ARNOLD[6], U. CIVICI[6], T. PROIETTI[6], D. PONT-ESTEBAN[6], H. YOUNG[6], T. COLE[6], E. SUITOR[6], A. KIMBERLY[6], C. LEHMACHER[6], F. KAVASSALIS[6], C. J. WALSH[6], C. E. VARGAS-IRWIN[1,3,4], L. R. HOCHBERG[4,2,5,7,3];
[1]Dept. of Neurosci., [2]Sch. of Engin., [3]Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [4]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Dept. of VA Med. Ctr., Providence, RI; [5]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [6]John A. Paulson Sch. of Engin. and Applied Sci., Harvard Univ., Cambridge, MA; [7]Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: The BrainGate team and others have previously shown that people with long-standing tetraplegia can use a neural interface to control an anthropomorphic robotic arm for 3D reach and grasp movements. However, individuals with tetraplegia consistently rank restoring movement to their own limb as a priority. Combining intracortical BCI (iBCI) technology with functional electrical stimulation (FES) has been demonstrated for reanimating paralyzed limbs, but while proof of concept demonstrations have been successful, FES can require multiple surgeries and is not suitable for certain patient populations, such as those with amyotrophic lateral sclerosis. The recent emergence of wearable soft robotics, coupled with iBCI technology, offers a promising alternative solution that addresses such challenges. Our goal is to design a wearable, textile-based, inflatable soft robotic arm (SRA) that directly restores mobility to the paralyzed arm, with the aim to provide a greater sense of embodiment. We have previously developed a soft robotic glove that enables hand extension, power grip, and precision grip by supplying compressed air to two air-tight bladders for each finger. While we have successfully demonstrated neural control of this glove for grasping movements, it remains unclear whether a neurally controlled soft robotic system can directly restore mobility to the upper arm for 3D reach and grasp movements. Here we present the development of a custom virtual environment to simulate control of an anthropomorphic limb. Using the virtual environment, we characterized information in cortical signals under open loop conditions, where the arm moves towards targets in a 3D workspace and the participant is asked to attempt the observed movements. This virtual research platform enables analysis of neural activity patterns, evaluation of linear and non-linear decoders, and customizable kinematics mimicking constraints of the soft robotic system and residual movements. In parallel, we have also developed a wearable soft exosuit to support shoulder elevation, including its necessary electromechanical control box. Compared to the previous control box for the soft robotic glove, the new prototype has been modified to support simultaneous inflation of multiple actuators in anticipation of controlling the glove and shoulder. It also includes the integration of proportional valves for shoulder movement to ensure that the degree of inflation is reflective of the decoded neural signal. The work presented here demonstrates progress towards an iBCI-SRA system that can restore intuitive, flexible, functionally useful upper extremity movement for people with tetraplegia.

Disclosures: K. Rao: None. **S. Allcroft:** None. **B. Schornstein:** None. **J. Gusman:** None. **C. Nicolas:** None. **L. Gerez:** None. **J. Arnold:** None. **U. Civici:** None. **T. Proietti:** None. **D. Pont-Esteban:** None. **H. Young:** None. **T. Cole:** None. **E. Suitor:** None. **A. Kimberly:** None. **C. Lehmacher:** None. **F. Kavassalis:** None. **C.J. Walsh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.W. is an inventor on patent and patent applications filed by Harvard University that describes the inflatable soft robotic components in this research.. **C.E. Vargas-Irwin:** None.

L.R. Hochberg: F. Consulting Fees (e.g., advisory boards); The MGH Translational Research Center has clinical research support agreements with Neuralink, Synchron, Reach Neuro, Axoft, and Precision Neuro, for which LRH provides consultative input, MGH is a subcontractor on an NIH SBIR with Paradromics.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.14/JJ25

Topic: E.05. Brain-Machine Interface

Support: Seed Grant from the ALS Association
Pilot Award from the Simons Collaboration for the Global Brain
Career Award at the Scientific Interface from the Burroughs Wellcome Fund to S D Stavisky
Stanford Wu Tsai Neurosciences Institute
HHMI
NIH-NIDCD (1U01DC019430)

Title: Speech synthesis by decoding intracortical neural activity of a person with anarthria due to ALS

Authors: *M. WAIRAGKAR[1], F. WILLETT[2], E. KUNZ[3], C. FAN[5], F. KAMDAR[6], L. R. HOCHBERG[7], J. M. HENDERSON[4], D. M. BRANDMAN[1], S. D. STAVISKY[1];
[1]Dept. of Neurolog. Surgery, Univ. of California, Davis, Davis, CA; [2]Stanford Univ. / HHMI, Stanford, CA; [3]Dept. of Electrical Engin., [4]Dept. of Neurosurg., Stanford Univ. / Wu Tsai Neurosciences Inst., Stanford, CA; [5]Dept. of Computer Sci., [6]Dept. of Neurosurg., Stanford Univ., Stanford, CA; [7]Neurology/Engineering, Massachusetts Gen. Hospital/ Brown University/ Harvard Med. School/ Providence VAMC, Boston, MA

Abstract: Neurological injury or neurodegenerative diseases such as ALS can lead to severe motor impairments including loss of speech, dramatically reducing quality of life and independence. Brain-computer interfaces (BCIs) could allow people with severe speech and motor impairment to communicate by bypassing damaged parts of the nervous system and directly decoding brain signals. Intracortical BCIs for communication have shown substantial progress in interpreting neural activity during attempted point-and-click typing and handwriting. However, these interfaces are slower than natural speech (150 words/minute). Recent BCIs have decoded speech neural correlates into words, but this approach does not capture the full expressive range of speaking. Instantaneously synthesising voice directly from neural activity would provide natural, expressive, and high-speed communication. Here, we present a speech BCI decoder to continuously synthesize speech in a person with anarthria due to ALS. Intracortical signals were recorded from BrainGate2 clinical trial participant ‘T12’, a 67-year-old woman with ALS, using 2 Utah microelectrode arrays (128 total electrodes) in ventral precentral

gyrus. Neural activity was recorded while the participant attempted to speak cued sentences. Although the participant could vocalize, her speech was unintelligible and ground truth speech was not available to train the neural decoder. To overcome this challenge, we generated target speech from the known cues using text-to-speech algorithms and time-aligned this artificial voice with the neural activity using a time warping algorithm. We trained a multi-layer transformer-based model to decode spike band power into low-dimensional spectral and pitch features of the target speech. Then, we used a vocoder to translate predicted speech features into synthesized speech waveform. Our system synthesized speech continuously from causal 200 ms sliding windows of neural activity, requiring ~7 ms of compute time per 10 ms frame. Offline speech synthesis from speech motor cortex activity yielded correlations of $r = 0.92 \pm 0.05$ between the target speech and synthesized speech across 40 Mel frequencies. Trained with 45 minutes of data, we obtained nearly intelligible speech synthesis performance across multiple intelligibility measures such as correlation coefficient, STOI (0.66±0.07) and phoneme error rate measured with ASR (0.57±0.2). Our offline results demonstrate that the major challenge of synthesizing speech from the neural activity in a person with anarthria (and thus no ground truth training data) can be overcome. We will apply this system for closed-loop BCI speech synthesis.

Disclosures: **M. Wairagkar:** None. **F. Willett:** None. **E. Kunz:** None. **C. Fan:** None. **F. Kamdar:** None. **L.R. Hochberg:** Other; Research agreements: Neuralink, Synchron, Axoft, Precision Neuro, Reach Neuro. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Advisor for Neuralink and Enspire DBS; Maplight Therapeutics. **D.M. Brandman:** None. **S.D. Stavisky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); licensed Stanford IP related to intracortical BCIs.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.15/KK1

Topic: E.05. Brain-Machine Interface

Support: NIH-NIDCD R01-DC014034
NIH-NIDCD U01-DC017844
NIH-NINDS UH2-NS095548
NIH-NINDS UO1-NS098968
Wu Tsai Neurosciences Institute at Stanford
Larry and Pamela Garlick
ALS Association Milton Safenowitz Postdoctoral Fellowship
Howard Hughes Medical Institute
Office of Research and Development, Rehabilitation R&D Service, Department of Veterans Affairs (N2864C, A2295R)
The content is solely the responsibility of the authors and does not

necessarily represent the official views of the National Institutes of Health, or the Department of Veterans Affairs or the United States Government. CAUTION: Investigational Device

Title: A brain-computer typing interface using finger movements

Authors: *N. SHAH[1], M. WILLSEY[2], N. HAHN[3], D. AVANSINO[4], F. KAMDAR[5], L. R. HOCHBERG[7], K. V. SHENOY[8], J. M. HENDERSON[6];
[2]Stanford Univ., [1]Stanford Univ., Palo Alto, CA; [3]Dept. of Neurosurg., Stanford Univ., Stanford, CA; [4]Stanford Univ., Stanford Univ., San Francisco, CA; [5]Stanford Univ., Moutnain View, CA; [6]Stanford University, Wu Tsai Neurosci. Institute; Stanford Bio-X., Stanford Univ., Stanford, CA; [7]Brown Univ., Brown Univ., Providence, RI; [8]HHMI & Stanford Univ., Stanford Univ. & HHMI, Stanford, CA

Abstract: In the era of widespread computer use, communication via text is nearly ubiquitously done via typing on a keyboard. While intracortical brain-computer interfaces (iBCIs) have shown promise for providing point-and-click typing, typing with multiple fingers simultaneously has not yet been demonstrated. We designed a 3D virtual keyboard to allow text entry via multiple finger movements, and demonstrated closed loop typing in a person with paralysis.Two 96-channel silicon microelectrode arrays were placed in the hand knob area of a clinical trial participant with C4 AIS-C spinal cord injury (T5). T5 was asked to attempt movement of the thumb, index-middle finger together, or ring-little finger together (3 individual finger groups). Decoded finger movements were visualized in 3-D using Unity. Thirty-one keys were arranged along the flexion-extension axis of each finger group, and letters/symbols with higher frequency in the English language were assigned closer to the neutral ('rest') position to reduce the average distance traveled. A temporal convolutional network decoded threshold crossings and spike band power into finger velocities, and the decoder was learned by pre-training on data from previous sessions followed by fine-tuning using calibration data collected on the day of the session. Instead of the neural network, a simpler, continuously updating linear decoder was used to generate closed-loop training and calibration data, reducing the effect of differences in the neural activity during open-loop and closed-loop finger movements. A click signal was separately decoded from the neural recordings with a logistic regression classifier.Closed-loop typing was demonstrated using a bimanual keyboard with keys distributed across both hands, each with three finger groups. T5 selected one character at a time by attempting to move the corresponding finger over the target key and then clicking with a brief attempted movement of the right leg. Using this interface,T5 could copy sentences with a speed of 30 correct characters per minute (ccpm) and accuracy of 90%, approaching the state of the art for point-and-click typing interfaces. Similar performance was demonstrated in a variant of this keyboard, where the keys were laid out for the right hand only and the clicks corresponded to left hand movement. If we allowed selection of two letters per trial using two distinct fingers, the character selection accuracy was maintained and speed increased to 40 ccpm.Overall, we present a class of simplified iBCI keyboards for typing multiple finger movements.

Disclosures: N. Shah: None. **M. Willsey:** None. **N. Hahn:** None. **D. Avansino:** None. **F. Kamdar:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); Neuralink, Synchron, Reach Neuro, Axoft, Precision Neuro. **K.V. Shenoy:** None. **J.M. Henderson:** 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.; R01DC014034, Wu Tsai Neurosciences Institute at Stanford, Larry and Pamela Garlick. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MapLight Therapeutics. F. Consulting Fees (e.g., advisory boards); Neuralink, Enspire DBS.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.16/KK2

Topic: E.05. Brain-Machine Interface

Support: Research Fellowship from the UC Davis Graduate Group in Computer Science to Xianda Hou
Career Award at the Scientific Interface from the Burroughs Wellcome Fund to S. D. Stavisky
Stanford Wu Tsai Neurosciences Institute, HHMI, Simons Foundation and NIH-NIDCD (1U01DC019430) to Jaimie Henderson

Title: Evidence of task outcome neural error signals during use of a speech brain-computer interface

Authors: *X. HOU[1,2], F. WILLETT[3], E. KUNZ[4,5], C. FAN[6], F. KAMDAR[7], L. R. HOCHBERG[8,9,10], J. M. HENDERSON[4,7], D. M. BRANDMAN[2], S. D. STAVISKY[2]; [1]Dept. of Computer Sci., [2]Neurolog. Surgery, Univ. of California Davis, Davis, CA; [3]Howard Hughes Med. Inst., [4]Wu Tsai Neurosciences Inst., [5]Dept. of Electrical Engin., [6]Dept. of Computer Sci., [7]Dept. of Neurosurg., Stanford Univ., Stanford, CA; [8]VA RR&D Ctr. for Neurorestoration and Neurotechnology, Rehabil. R&D Service, Providence VA Med. Ctr., Providence, RI; [9]Sch. of Engin. and Carney Inst. for Brain Sci., Brown Univ., Providence, RI; [10]Ctr. for Neurotechnology and Neurorecovery, Dept. of Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Self-monitoring is an important executive function that allows us to evaluate our performance while executing specific actions. In the context of brain-computer interfaces (BCIs) for restoring communication, users can evaluate the difference between their attempted action and what the BCI does. Previous research identified task outcome error-related changes in firing rates and field potentials of the dorsal precentral gyrus during BCI control of a computer cursor (Even-Chen 2017, 2018). However, it remains unknown whether similar signals exist in the human ventral precentral gyrus (vPCG) or inferior frontal gyrus (IFG). Detecting these intrinsic neural error signals could enable BCI systems to automatically make corrections (e.g., deleting an incorrect word) or update decoder weights without requiring explicit corrective user input. To test for task outcome error signals in the human cortex, we analyzed data from BrainGate2 clinical trial participant T12, a woman with ALS who has 2 chronic 64-microelectrode Utah arrays implanted in vPCG and 2 arrays in IFG. Neural activity was recorded while T12 used a

brain-to-text BCI where she tried to articulate prompted sentence and saw decoded words appear on-screen shortly thereafter (Willett*, Kunz*, Fan* 2023). We analyzed a time epoch from 500 ms before to 1000 ms after the decoded sentence was finalized on-screen. Each trial was labeled as correct or incorrect depending on whether the output matched the prompted sentence. We first used targeted dimensionality reduction on the neural data (PCA on the trial-averaged difference between correct and incorrect trials), after which single trial data were then projected into this neural subspace. Finally, a linear discriminant analysis classifier was trained to predict if a trial was incorrect using a 100 ms sliding window of spike counts and spike band power.
Offline analysis demonstrated above-chance accuracy in predicting trial outcomes starting ~300 ms after visual feedback of the decoded sentence, with a peak 88.9% accuracy at ~850 ms after feedback ($p<0.01$ shuffle test versus 63.1% chance accuracy, which reflects uneven numbers of correct and incorrect trials). While both cortical areas supported above-chance decoding, IFG activity contained much more outcome information. A limitation of the current study, which needs to be controlled for in additional experiments, is potential behavioral confounds such as if T12 e.g. shook her head after incorrect sentences. With this caveat, these findings provide preliminary evidence for the presence of error signals in IFG and vPCG and serve as a foundation for leveraging these signals to improve speech BCI performance.

Disclosures: **X. Hou:** None. **F. Willett:** None. **E. Kunz:** None. **C. Fan:** None. **F. Kamdar:** None. **L.R. Hochberg:** F. Consulting Fees (e.g., advisory boards); Neuralink, Synchron, Axoft, Precision Neuro, Reach Neuro. **J.M. Henderson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuralink, Enspire DBS, Maplight Therapeutics. **D.M. Brandman:** None. **S.D. Stavisky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); licensed Stanford IP related to intracortical BCIs.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.17/KK3

Topic: E.05. Brain-Machine Interface

Support: R01NS109257

Title: Comparison of Four Neural Decoders Across 48 Human Subjects Using a Real-Time Invasive BCI Model

Authors: *P. I. ALCOLEA[1], Z. C. DANZIGER[2];
[2]Biomed. Engin., [1]Florida Intl. Univ., Miami, FL

Abstract: Invasive brain-computer interfaces (iBCIs) convert intracortically recorded neural firing rates into direct control of assistive devices for paralyzed users; but their invasiveness severely limits how rigorously we can test iBCI design choices, such as the decoder that maps

neurons to device commands. In this work we used the joint angle BCI (jaBCI, Awasthi et al. 2022), a recently developed and validated human-in-the-loop, real-time iBCI model, to perform a rigorous comparison of the three most common iBCI decoders and a novel decoder in a cursor control task in 48 naïve human subjects over 4 days. We evaluated 1) the classic velocity Kalman filter (vKF, Wu et al. 2002) that determines velocity through a linear weighting of prior cursor kinematics and current neural firing. 2) The ReFIT decoder (Gilja et al. 2012) that adds to the vKF model implicit information about online subject intention during calibration. 3) A population vector decoder with assisted calibration (PV-A, Inoue et al. 2018) that is an affine map between neuron firing rates to cursor velocity and that, during calibration, slowly transitions between pre-determined cursor trajectories to full subject control. 4) A new direction selection decoder (DS) where neural activity maps into a selection from a small discrete menu of possible velocities. In a 2D, 8-target, center out cursor task we found that DS, PV-A, ReFIT, and vKF groups had means of 93%, 56%, 39%, and 26% targets hit, respectively. These differences were all significantly different from one another (2-way ANOVA, $p < 0.001$). Our results support prior findings in monkey that suggest ReFIT outperforms vKF in cursor control. PV-A outperforming both Kalman filter decoders indicates that it may solve the cursor stopping problem more effectively, which was the goal behind its original design. Perhaps surprisingly, we found that DS outperformed all decoders by a wide margin, suggesting discrete control is viable, or preferable, to continuous control even for continuous tasks. In addition, subjects' simulated neural activity during decoder calibration was separable by target location. However, despite all subjects driving the identical set of simulated neurons, to observe the separability across subjects required the rotation of each subject's neural latent space prior to averaging. This suggests that subject control strategy plays a large role in determining the "neural manifold," and may be important for decoder design. At a higher level, this study highlights the potential benefits of the jaBCI model to test closed-loop human iBCI use of decoders at scale and to rapidly prototype new potential decoder designs.

Disclosures: P.I. Alcolea: None. **Z.C. Danziger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US 011625099B2.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.18/KK4

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS062092

Title: Semantic decoding from natural speech production using intracranial EEG recordings

Authors: *C. PESCATORE[1], J. CAI[2], A. C. PAULK[1], A. E. HADJINICOLAOU[1], X. TIAN[1], N. KNOX[1], Z. M. WILLIAMS[2], S. S. CASH[1];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., Massachusetts Gen. Hosp., Boston, MA

Abstract: Remarkable advancements in systems neuroscience, machine learning, and neuro-engineering have yielded the scientific and technologic tools necessary to extract and decode neural signals directly from cortex. The most advanced applications of these new approaches have been in decoding motor information for restoring limb movements. Restoring fluent communication for patients with anarthria or severe dysarthria from neurologic injury or disease through speech decoding is rapidly improving as well. However, less attention has been devoted to directly decoding the semantic meaning of the words and sentences produced by patients. This approach might provide an even more natural and rapid method for enabling communication. In this study, we leverage stereo-EEG recordings of six participants during unrestricted, natural conversation to test the hypothesis that the lexical semantics of spoken words can be accurately decoded from neural recordings using machine-learning based algorithms. By using a pre-trained word2vec embedding as a representation of lexical semantics and aligning neural signals to the onset of each word, we demonstrate that the semantic meanings can be remarkably predicted at a cosine distance of an average of 0.45 lower than what is expected by chance using a fully connected neural network. These results are robust and consistent across all individuals, with a reproducible standard deviation among individuals of 0.16. However, prediction accuracy on specific words varies between individuals. We further discuss this variance of accuracies by considering the difference in electrode placement and provide possible directions to optimize the decoding performance tailored to personal specificity. Together, these findings suggest the possibility of single-word, real-time compatible semantic decoding in speech-related BCIs, providing insights on improving communication for individuals with communication disorders.

Disclosures: C. Pescatore: None. **J. Cai:** None. **A.C. Paulk:** None. **A.E. Hadjinicolaou:** None. **X. Tian:** None. **N. Knox:** None. **Z.M. Williams:** None. **S.S. Cash:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.19/KK5

Topic: E.05. Brain-Machine Interface

Support: Weill Institute for Neuroscience

Title: Leveraging beta frequency for continuous BCI robot control

Authors: *Y. GRAHAM[1,2], N. NATRAJ[1,2], S. SEKO[1,2], H. YAN[1,2], E. CHANG[1], K. GANGULY[1,2];
[1]UCSF, San Francisco, CA; [2]San Francisco Veterans Affairs Med. Ctr., San Francisco, CA

Abstract: Neurological disorders affect millions of people. They impair speech and motor functions, and greatly decrease quality of life. Brain-computer interfaces (BCIs) have the potential to improve function by translating movement-related neural signals into control signals for external assistive devices. Control of these devices can enable users to regain upper limb functionality, develop some autonomy and improve their quality of life. While there has been remarkable progress in BCI control, control of physical robotic assistive devices can still be variable, especially in real-world situations. The central hypothesis of this study is that tracking a user's internal state of active engagement with the BCI, using neurophysiological signals, can improve reliability of BCI control of a complex dexterous robot. We have found rhythmic fluctuations in the envelope of beta (13-30 Hz) power during continuous robotic control. While changes in beta are typically associated with task cues and movement onset in able bodied subjects, we found that spontaneous fluctuations in beta are also present during BCI task control; importantly, such spontaneous events appear to occur independent of any overt cues in our task and, instead, appear to represent neurophysiological correlates of fluctuating internal states over long timescales. Our study participants are a male (39 years old) and a female (48 years old), both suffered from a brainstem stroke and are currently tetraplegic. Both are implanted with a chronic ECoG array, 128 channels and 256 channels respectively, over sensory and motor cortices. Neural activity was recorded as the participants performed various robot tasks. We conducted spatial-temporal analysis of beta across the ECoG array as well as phase analysis to related beta to the kinematics of the robot. Specifically, we found localizations of beta on the array as well as infraslow (<0.3 Hz) changes in the envelope of beta power during "active" continuous online BCI control. We focused on understanding the fluctuations in beta power during continuous control in order to leverage such fluctuations to improve robustness of robotic control by gating the velocity of the robot based on the internal states.

Disclosures: Y. Graham: None. **N. Natraj:** None. **S. Seko:** None. **H. Yan:** None. **E. Chang:** None. **K. Ganguly:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.20/KK6

Topic: E.05. Brain-Machine Interface

Title: Development of a brain-machine interface for decoding of self-paced movement intention from EEG signal

Authors: *M. CERADINI[1], S. TORTORA[2,3], S. MICERA[1,4], L. TONIN[2,3];
[1]Biorobotics Inst., Sch. of Advanced Studies Sant'Anna, Pisa, Italy; [2]Dept. of Information Engin., [3]Padua Neurosci. Ctr., Univ. of Padova, Padova, Italy; [4]Bertarelli Fndn. Chair in Translational Neuroengineering, EPFL, Geneve, Switzerland

Abstract: Brain-machine interface (BMI) offer a promising and intuitive solution for individuals with motor impairments to control prosthetic or rehabilitation devices [Zhuang M, 2020]. A crucial aspect of this technology is the possibility to accurately decoding the anticipation of user's movement intentions. To address this challenge, we conducted a study involving 7 healthy subjects who performed a reach and grasp protocol while their EEG signals were recorded. In our study, we employed Power Spectral Densities (PSD), Shannon Entropy, and a hybrid combination of these two, as features to identify self-paced movement intentions. Preliminary offline analysis using machine learning (i.e., Linear Discriminant Analysis, Quadratic Discriminant Analysis, and Support Vector Machines) demonstrated classification with accuracies significantly above chance level. Building upon these findings, we have devised an enhanced online closed-loop protocol for testing our models. In this protocol, participants were seated in front of a screen with different objects to grasp within their field of view. The subjects are then instructed to focus their attention on a screen that displays instructions regarding the specific type of grasp movement they should perform, or alternatively, the instruction to rest. Following the instructional phase, a classification period ensues, during which the subject could start the movement (or do nothing in case of no-movement) while in the meantime the model decodes the subject's intention of movement, or no-intention. If the model accurately identifies the intended movement, the subject is granted permission to proceed with the corresponding action. Conversely, the trial is classified as a failure. All subjects achieved performance above chance level, demonstrating the possibility to train the subject in such a self-paced motor task and to achieve high accuracy and a low false-positive rate in the decoding of movement intention. Compare to existing studies our results are promising because we can decode the movement intention up to 1 s before the actual movement, compared to the standard of 100/200 ms [Ibáñez, 2014]. The achievement of high accuracy in decoding movement intentions holds significant implications for the translational applications of BMI [Pereira, 2021]. By accurately decoding movement intentions, it becomes possible to anticipate the intended movements and apply asynchronous classifiers specific to different types of grasps, for instance. This breakthrough has the potential to revolutionize various rehabilitation scenarios, including the control of external robotic and assistive devices, as well as neuroprosthesis.

Disclosures: **M. Ceradini:** None. **S. Tortora:** None. **S. Micera:** None. **L. Tonin:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.21/KK7

Topic: E.05. Brain-Machine Interface

Support: Dutch Science Foundation SGW-406-18-GO-086
Dutch Technology Foundation UGT7685
ERC Advanced Grant 320708

Title: Speech perception generates activity over human sensorimotor cortex which can lead to False Positive activation of a speech BCI trained on overt, whispered, and mimed speech

Authors: *A. SCHIPPERS, Z. V. FREUDENBURG, M. VANSTEENSEL, N. F. RAMSEY; UMC Utrecht Brain Ctr., Utrecht, Netherlands

Abstract: The sensorimotor cortex (SMC) has shown to be a useful neural signal source for Brain-Computer Interfaces (BCI) based on speech production. Increases in the high-frequency band (HFB) power can be used to decode overtly or covertly spoken words from the users' brain activity. However, increases in HFB power can also be generated in the SMC during speech perception, thereby posing a potential source of false positive (FP) BCI activation. Here we investigate if and how decoders used to control a speech BCI may be sensitive to FP activation during speech perception.

Three epilepsy patients were subdurally implanted with high density electrocorticography (ECoG) grids over the left SMC. Participants completed an audiovisual speech perception task and a speech production task, in which they were asked to overtly produce, whisper, and mime the same 7 sounds. Speech trials were alternated with rest. After preprocessing and the extraction of the HFB power (65 - 95 Hz) from the ECoG data, task response was determined by comparing HFB power during speech trials to those during rest using R^2 analysis. All subjects showed increased HFB power in the SMC during the speech perception task and all three conditions of the production task. To test decodability of the produced speech signals in all three conditions, spatial match-filter (SMF) and support vector machine (SVM) classifiers were trained for each of the production conditions separately. The classifiers were tested on data from the same condition (using a leave one out approach). Produced syllables in all three conditions were decoded with high accuracy (above 67%) for all subjects and both classifiers. Classifiers trained on mimed data had lowest accuracy. To test whether the classifiers would generate FPs during speech perception, each classifier (trained on speech production data) was tested on the perception data. Both classifiers generated FPs (i.e., rest being classified as speech production) in all three conditions. Interestingly, most false positives were generated using decoders based on the mimed data. However, classification performance (when excluding rest as a possible class) during perception was around chance level (14%) in all conditions, indicating that the content of the perceived sounds could not be reliably decoded.

The current study demonstrates that decoders trained on speech production can lead to false positive activation of a BCI during speech perception. Decoders trained on mimed speech are least accurate in decoding the produced sounds and most susceptible for false positive activations. This topic deserves further investigation before speech BCIs can be used reliably in naturalistic situations.

Disclosures: A. Schippers: None. **Z.V. Freudenburg:** None. **M. Vansteensel:** None. **N.F. Ramsey:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.22/KK8

Topic: E.05. Brain-Machine Interface

Title: Decoding Reach-to-Grasp Movements: A Comparative Study of TCRE and EEG for High-Performance BCI Systems

Authors: *A. RABIEE[1], S. GHAFOORI[2], A. CETERA[3], W. G. BESIO[5], R. ABIRI[4];
[1]Dept. of Electrical, Computer, and Biomed. Engin., [3]Anna Cetera, [4]Univ. of Rhode Island, [2]Univ. of Rhode Island, Kingston, RI; [5]Univ. of Rhode Island, Univ. of Rhode Island Interdisciplinary Neurosci. Program, East Greenwich, RI

Abstract: Spinal cord injuries often result in devastating hand dexterity and grasp disabilities, severely impacting affected individuals' quality of life. With the restoration of hand dexterity being a top priority in this patient population, Brain-Computer Interface (BCI) systems serve as promising solutions. However, current invasive brain-machine interfaces customarily used in BCI systems present significant drawbacks, including a lack of dexterity and generalizability, high costs, and accessibility issues. This study aims to investigate the potential of the Tripolar Concentric Ring Electrode (TCRE), a non-invasive alternative, in decoding human reach-to-grasp movements and enhancing BCI system effectiveness. We compared the performance of TCRE with conventional EEG systems using advanced signal processing methods and state-of-the-art deep learning models. Our experimental protocol involved collecting EEG and tEEG data simultaneously from specific channel locations, including F5, C3, C4, and P3 (corresponding to the Anterior Intraparietal area), as participants performed various reach-to-grasp tasks. The processed data was analyzed using Event-Related Potential (ERP) techniques, an approach known for its efficiency in detecting neural responses. Our approach goes beyond traditional analyses, employing LSTM, CNN, and RCNN deep learning models to perform binary and multiclass classification tasks. These classification tasks aim to understand the detailed neural correlates of different types of hand movements, which could pave the way for improved BCI system designs.The results revealed a clear superiority of TCRE over regular EEG. In the multiclass classification task, TCRE achieved a notable accuracy of 88.78%, compared to 83.70% with the EEG. Binary classification tasks also favored TCRE, with the highest accuracy of 88.38% and 87.46% for TCRE and EEG, respectively. These results highlight the substantial promise of TCRE as a potent, precise, and non-invasive tool for improving BCI systems' performance. Our study establishes an advanced analytical methodology for examining EEG and tEEG data, creating a new direction for further exploration in non-invasive brain-machine interface development. By demonstrating the potential of TCRE in accurately decoding reach-to-grasp movements, we open a pathway for developing more affordable, generalizable, and user-friendly BCI systems, offering a promising approach for restoring hand dexterity in patients with spinal cord injuries. This could significantly enhance their ability to perform daily activities, improve their independence, and ultimately, uplift their quality of life.

Disclosures: **A. Rabiee:** None. **S. Ghafoori:** None. **A. Cetera:** None. **W.G. Besio:** None. **R. Abiri:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.23/KK9

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS121079

Title: Pupil diameter correlates with neural population activity and performance during human intracortical brain-computer interface use

Authors: *N. G. KUNIGK[1,3,4], W. HOCKEIMER[2,3], C. GONTIER[3], B. M. DEKLEVA[2,4,3], S. M. CHASE[5,6,4], M. L. BONINGER[2,3,1], J. L. COLLINGER[2,3,1,4,5];
[1]Bioengineering, [2]Physical Med. and Rehabil., Univ. of Pittsburgh, Pittsburgh, PA; [3]Rehab Neural Engin. Labs, Univ. of Pittsburgh, Pittsburgh, PA; [4]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA; [5]Biomed. Engin., [6]Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Intracortical brain-computer interfaces (iBCIs) can improve motor function and independence after paralysis by transforming movement intent signals from the motor cortex into commands for a variety of effectors. However, recording instabilities and lack of robustness are major impediments to the widespread clinical acceptance of iBCIs. Particularly, it has been shown that the activity of the motor cortex is highly variable, context-dependent, and modulated by the mental state of the subject. Changes in mental state, such as fatigue, pain, or stress, can have a broad impact on cortical modulations and must be accounted for to maintain consistent iBCI performance. We expect that changes in subject state will impact the participant's ability to engage with the iBCI task and therefore used pupillometry to measure changes in participant arousal or effort during iBCI control. Pupil diameter has previously been shown to correlate with neural measures of engagement in non-human primates performing a BCI task in a way that was predictive of performance and learning. Here, we recorded pupillometry and neural activity from two human participants (P2 and P3) with tetraplegia who have intracortical Utah arrays implanted in primary motor cortex. Multi-unit activity from the Utah arrays as well as pupil diameter data were collected during a 2D center-out-and-back task. Neural data was collected at 50 Hz, and pupillometry data was collected at 100 Hz and downsampled. All data was z-scored based on trial averages. We found that pupil diameter showed consistent patterns of fluctuation following trial onset, generally decreasing across the course of each trial for both participants. Furthermore, the change in pupil diameter from baseline over the course of the reach phase in each trial was found to be correlated ($\rho = 0.448$ for P2 and $\rho = 0.465$ for P3) with the magnitude of a measure of "neural push" - a component identified via factor analysis of the neural data over the course of entire sets of trials - which peaks at the beginning of the reach phase of each trial and may represent a "go" command in the motor cortex. A larger range in pupil diameter over the entire trial also correlated ($\rho = 0.622$ for P2 and $\rho = 0.562$ for P3) with longer trial completion times, indicating worse performance. These results suggest a relationship between a primary component of population-level neural activity used in the decoding algorithm and the externally measured metric of pupil diameter. Incorporating quantitative measures of users' internal states may provide performance benefits both within and across sessions by enabling compensation for common internal contextual factors that may hinder performance.

Disclosures: **N.G. Kunigk:** None. **W. Hockeimer:** None. **C. Gontier:** None. **B.M. Dekleva:** F. Consulting Fees (e.g., advisory boards); Blackrock Neurotech. **S.M. Chase:** None. **M.L. Boninger:** None. **J.L. Collinger:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.24/KK10

Topic: E.05. Brain-Machine Interface

Support: National Institutes of Health (grant NINDS 5U01DC018671)
Joan and Sandy Weill Foundation
Susan and Bill Oberndorf
Ron Conway
Graham and Christina Spencer
William K. Bowes, Jr. Foundation
Rose Hills and Noyce Foundations
National Institute of General Medical Sciences (NIGMS) Medical Scientist Training Program, Grant #T32GM007618
National Science Foundation GRFP

Title: A multimodal neuroprosthesis for intelligible speech synthesis and avatar control

Authors: *K. LITTLEJOHN[1], S. METZGER[3], A. SILVA[3], D. A. MOSES[3], M. P. SEATON[3], R. WANG[3], M. E. DOUGHERTY[3], J. R. LIU[3], P. WU[2], M. BERGER[4], I. ZHURAVLEVA[2], A. TU-CHAN[3], K. GANGULY[3], G. K. ANUMANCHIPALLI[2], E. F. CHANG[3];
[1]Electrical Engin. and Computer Sci., [2]Univ. of California, Berkeley, Berkeley, CA; [3]Univ. of California San Francisco, San Francisco, CA; [4]Speech Graphics, Edinburgh, United Kingdom

Abstract: Speech neuroprostheses have thepotential to restore communication to people living with paralysis, but naturalism andexpressivity are elusive. Here, we use electrocorticography (ECoG) recordings from thespeech-motor cortex in a clinical-trial participant with severe limb and vocal paralysis to achieveintelligible, real-time speech-synthesis and facial-avatar animation. We trained andevaluated deep-learning models using neural activity collected as the participant attempted tosilently speak sentences. Learning statistical mappings between the ECoG features and the sequencesof speech-sound features in the sentences was challenged by the absence of clear timinginformation in the attempted speech. To overcome the inability to definitively know when the speech-soundfeatures began and ended, we used a conconnectionist temporal classification lossfunction during training of our neural decoders, which is commonly used in automaticspeech recognition. A Tacotron-2 and WaveGlow inspired vocoder was used to synthesizethe speech. We demonstrate intelligible and rapid speech synthesis of high-utilityphrases, in the participant's own voice, with human listeners achieving a median perceptual word errorrate of 29%. In addition to synthesizing speech, we sought to animate the

face of a virtualavatar by directly decoding articulatory gestures and animating them. For facial avatar, wedemonstrate that the speech-motor cortex can be used to control virtual orofacial movements forspeech and non-speech communicative expressions. For non-speech communicativeexpressions, the participant was trained to execute specific orofacial movements andemotional expressions, with neural data corresponding to these actions recorded. An 87.8%classification accuracy was achieved for six distinct articulatory movements and up to 96.9% forstrong-intensity emotional expressions, showing potential for restoring the ability toexpress meaningful orofacial gestures. The decoders reached high performance with fewer than twoweeks of training. Our findings introduce a new proof-of-principlespeech-neuroprosthetic approach that has significant promise to restore full, embodied communication topeople living with severe paralysis, and enhance our understanding of speech andorofacial movement encoding in the speech-motor cortex.

Disclosures: K. Littlejohn: None. **S. Metzger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a pending provisional UCSF patent application that is relevant to the neural-decoding approaches used in this work. **A. Silva:** None. **D.A. Moses:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a pending provisional UCSF patent application that is relevant to the neural-decoding approaches used in this work. **M.P. Seaton:** None. **R. wang:** None. **M.E. Dougherty:** None. **J.R. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a pending provisional UCSF patent application that is relevant to the neural-decoding approaches used in this work. **P. Wu:** None. **M. Berger:** Other; Chief technical officer at Speech Graphics Ltd in Edinburgh, Scotland. **I. Zhuravleva:** None. **A. Tu-Chan:** None. **K. Ganguly:** None. **G.K. Anumanchipalli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a pending provisional UCSF patent application that is relevant to the neural-decoding approaches used in this work. **E.F. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on a pending provisional UCSF patent application that is relevant to the neural-decoding approaches used in this work.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.25/KK12

Topic: E.05. Brain-Machine Interface

Support: NINDS 5U01DC018671
Grant #T32GM007618

Title: Persistent somatotopy drives high-performance text decoding from cortical signals in a patient with paralysis

Authors: *A. SILVA[1], S. METZGER[2], K. LITTLEJOHN[2], D. A. MOSES[2], M. SEATON[2], R. WANG[2], M. DOUGHERTY[2], J. R. LIU[2], P. WU[3], M. BERGER[4], I. ZHURAVLEVA[3], A. TU-CHAN[2], K. GANGULY[2], G. ANUMANCHIPALLI[3], E. F. CHANG[2];
[1]Neurolog. surgery, Univ. of California at San Francisco, San Francisco, CA; [2]Univ. of California San Francisco, San Francisco, CA; [3]Univ. of California, Berkeley, Berkeley, CA; [4]Speech Graphics Ltd, Edinburgh, Scotland, UK, Edinburgh, United Kingdom

Abstract: Loss of speech due to neurological injury is devastating and can severely degrade communication and quality of life. Speech neuroprostheses have the potential to naturally restore communication by decoding cortical activity into intended speech, bypassing diseased motor pathways. While prior studies have shown feasibility of this approach, results are limited by highly restrictive vocabulary sizes or low decoding rates. Further, it is unclear what speech information persists in cortical activity of patients with paralysis and would allow for further speech decoding advancement. To address these challenges, we implanted a 253-channel, high-density electrocorticography (ECoG) array in a person with severe vocal and limb paralysis, centered over the central sulcus, as part of a clinical trial. As the participant silently attempted to speak, we directly decoded cortical activity into sentences using deep-learning models and statistical natural-language models. We achieved a median word error rate of 25% at a decoding rate of 78 words per minute with a vocabulary of over 1000 words, which can cover 85% of conversational English. All results were demonstrated in real-time on sentences unseen during model training. We also trained models to classify the 26 NATO code-words; models performed at 96.8% accuracy for longer than 2 months without any retraining, demonstrating signal stability. Thorough analysis of electrodes contributing to decoding revealed cortical somatotopy of the speech articulatory muscles that persisted over 18 years after paralysis. Electrodes that contributed most to decoding performance encoded attempted movements of the speech articulators (tongue, lips, jaw, and larynx) and were largely centered on the central sulcus and anterior precentral gyrus. These results establish the feasibility of a stable, high-performance speech neuroprosthesis capable of restoring rapid communication. Performance is not driven by higher-order speech features like semantics; rather, a detailed articulatory representation persists on the sensorimotor cortex years after paralysis.

Disclosures: A. Silva: None. **S. Metzger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has a pending patent. **K. Littlejohn:** None. **D.A. Moses:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has a pending patent. **M. Seaton:** None. **R. Wang:** None. **M. Dougherty:** None. **J.R. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has a pending patent. **P. Wu:** None. **M. Berger:** A. Employment/Salary (full or part-time):; Speech graphics. **I. Zhuravleva:** None. **A. Tu-chan:** None. **K. Ganguly:** None. **G. Anumanchipalli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has a pending patent. **E.F. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); has a pending patent.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.26/KK13

Topic: E.05. Brain-Machine Interface

Support: UH3NS114439 (NINDS)
U01DC016686 (NIDCD)

Title: Decoding from a speech BCI enables control for an individual with ALS without recalibration

Authors: *S. LUO[1], M. ANGRICK[1], C. COOGAN[1], D. N. CANDREA[1], K. WYSE-SOOKOO[1], S. SHAH[1], Q. RABBANI[1], G. W. MILSAP[2], A. R. WEISS[1], W. S. ANDERSON[1], N. J. MARAGAKIS[1], L. L. CLAWSON[1], M. J. VANSTEENSEL[3], A. UCHIL[1], B. A. WESTER[2], F. V. TENORE[2], H. HERMANSKY[1], M. S. FIFER[2], N. F. RAMSEY[3], N. E. CRONE[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [3]UMC Utrecht Brain Ctr., Utrecht, Netherlands

Abstract: Brain-computer interfaces (BCIs) can be used to control assistive devices by patients with neurological disorders like amyotrophic lateral sclerosis (ALS) that limit speech and movement. For assistive control, it is desirable for BCI systems to be accurate and reliable, preferably with minimal setup time. In this study, a participant with severe dysarthria due to ALS operated computer applications with intuitive speech commands via a chronic electrocorticographic (ECoG) implant over ventral sensorimotor cortex. Speech commands were accurately detected and decoded throughout the study period without model retraining or recalibration. Use of the BCI did not require exogenous timing cues, enabling the participant to issue self-paced commands at will. These results demonstrate that a chronically implanted ECoG-based speech BCI can reliably control assistive devices over long time periods with only initial model training and calibration, supporting the feasibility of unassisted home use.

Disclosures: S. Luo: None. **M. Angrick:** None. **C. Coogan:** None. **D.N. Candrea:** None. **K. Wyse-Sookoo:** None. **S. Shah:** None. **Q. Rabbani:** None. **G.W. Milsap:** None. **A.R. Weiss:** None. **W.S. Anderson:** None. **N.J. Maragakis:** None. **L.L. Clawson:** None. **M.J. Vansteensel:** None. **A. Uchil:** None. **B.A. Wester:** None. **F.V. Tenore:** None. **H. Hermansky:** None. **M.S. Fifer:** None. **N.F. Ramsey:** None. **N.E. Crone:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.27/KK14

Topic: E.05. Brain-Machine Interface

Support: UH3NS114439 (NINDS)

Title: A quickly trainable electrocorticographic-based brain-click detector is used for high performance long-term switch-scan spelling

Authors: *D. CANDREA[1], S. SHAH[2], S. LUO[1], M. ANGRICK[2], Q. RABBANI[1], C. COOGAN[2], G. MILSAP[4], B. A. WESTER[4], W. ANDERSON[3], F. TENORE[4], N. F. RAMSEY[5], M. S. FIFER[4], N. E. CRONE[2];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Neurol., [3]Johns Hopkins Hosp., Baltimore, MD; [4]Johns Hopkins Applied Physics Lab., Laurel, MD; [5]Brain Ctr. Rudolf Magnus, Univ. of Utrecht, Utrecht, Netherlands

Abstract: Brain-computer interfaces (BCIs) can restore communication to individuals who suffer severe movement or speech impairments by interfacing neural activity to typing applications. Upper limb "brain-click" decoders provide a basic yet highly functional capability. We sought to test the limits of how long a brain-click detector could maintain performance based on limited training data, from a chronically implanted high-density electrocorticographic (ECoG) array in a human participant with ALS. Using a switch-scanning spelling application, our participant used the BCI to select appropriate letters and words to spell sentences. Our model detected nearly all attempted clicks with a low detection latency while maintaining a low false positive rate. We demonstrate that an ECoG-based brain-click detector can be quickly trained and deployed and can retain robust performance for long periods of BCI use, enabling functional text-based communication to individuals with communication impairments.

Disclosures: D. Candrea: None. **S. Shah:** None. **S. Luo:** None. **M. Angrick:** None. **Q. Rabbani:** None. **C. Coogan:** None. **G. Milsap:** None. **B.A. Wester:** None. **W. Anderson:** None. **F. Tenore:** None. **N.F. Ramsey:** None. **M.S. Fifer:** None. **N.E. Crone:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.28/KK15

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01DC019498
DoD Grant W81XWH-21-0538

Title: Transfer learning on cross-patient micro-ECoG speech data

Authors: *Z. SPALDING[1], S. DURAIVEL[1], S. RAHIMPOUR[3,7], C.-H. CHIANG[1], M. TRUMPIS[1], C. WANG[1], K. BARTH[1], S. P. LAD[3], A. H. FRIEDMAN[3], D. G.

SOUTHWELL[3,4,5], S. SINHA[8], J. VIVENTI[1,3,4,5], G. B. COGAN[1,2,3,5,6];
[1]Biomed. Engin., [2]Ctr. for Cognitive Neurosci., Duke Univ., Durham, NC; [3]Neurosurg., [4]Neurobio., [5]Duke Comprehensive Epilepsy Ctr., [6]Neurol., Duke Sch. of Med., Durham, NC; [7]Neurosurg., Univ. of Utah, Salt Lake City, UT; [8]Penn Epilepsy Ctr., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Prior work has shown that the high spatial resolution of micro-electrocorticographic (µECoG) arrays improves speech decoding from cortical sensorimotor signals. However, µECoG recording is currently limited to the intraoperative setting, making it difficult to acquire the amount of data necessary to train machine learning models that enable accurate speech decoding. We hypothesized that cross-patient transfer learning could be used to expand the amount of µECoG data available to such models. Transfer learning was performed via two methods: (1) pre-training selective layers of deep recurrent neural networks (RNNs) and (2) alignment of low-dimensional patient-specific feature manifolds to a common state-space (SS) prior to decoding. We used high-density µECoG arrays placed over the sensorimotor cortex to record cortical signals intraoperatively from four subjects during a phoneme repetition task. The high-gamma power (HG: 70-150 Hz) of recorded neural signals was used to decode spoken phonemes. Sequence-to-sequence RNNs were (1) trained on patient-specific data and (2) pre-trained across multiple patients and fine-tuned on a single patient's data. Our preliminary results using RNNs show that it is possible to distribute model training across multiple patients. Phoneme decoding accuracies were comparable between models trained with patient-specific (34.0 ± 1.0%, chance: 12.4%) and cross-patient (32.3 ± 0.9%, chance: 12.4%) data. Latent feature spaces for each patient were constructed using principal component analysis (PCA) and subsequently aligned to a common SS. Models using this SS approach showed comparable phoneme decoding accuracies when trained on patient-specific data (47.2 ± 2.1%, chance: 11.1%) and cross-patient data aligned to a common space (44.4 ± 2.9%, chance: 11.1%). Further, this alignment technique allowed models to predict phonemes with accuracies well above chance on data from patients they had not been trained on (39.1 ± 3.9%, chance: 11.1%). These results suggest that there is a shared neural representation of speech production between patients which could be used to increase the amount of training data available for machine learning models driving neural speech prosthetic systems.

Disclosures: Z. Spalding: None. **S. Duraivel:** None. **S. Rahimpour:** None. **C. Chiang:** None. **M. Trumpis:** None. **C. Wang:** None. **K. Barth:** None. **S.P. Lad:** None. **A.H. Friedman:** None. **D.G. Southwell:** None. **S. Sinha:** None. **J. Viventi:** None. **G.B. Cogan:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.29/KK16

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 2T32EB003383
UH3NS114439 (NINDS)
U01DC016686 (NIDCD)

Title: Long-term stability of ECoG recordings during speech tasks

Authors: *K. R. WYSE-SOOKOO[1], S. LUO[1], N. E. CRONE[2];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Neurol., The Johns Hopkins Hosp., Baltimore, MD

Abstract: Brain-computer interfaces (BCIs) can give individuals with severe paralysis a way to communicate beyond their physical capabilities by using their neural signals to control assistive electronics. In order to achieve long-term, reliable BCI use, stable recording of neural signals is essential. Electrocorticography (ECoG) with electrode strips has previously been able to achieve high signal stability over long periods of time, enough for 36 months of motor BCI home use in an individual with severe paralysis. This study aims to investigate, for the first time, the long-term stability of ECoG signals recorded from a chronic ECoG implant during a speech task. Signals were recorded by two 64-electrode grids placed on the ventral sensorimotor cortex of a clinical trial participant. High gamma and high frequency noise band power were extracted from ECoG recordings of syllable repetition tasks at baseline and while speaking and tracked over time across grids to give insight into signal stability. The overall rates of change as found by linear regression for each frequency band were low, and gamma band power during speaking remained well above the power of high frequency noise for all but one day. Initial individual channel analysis found that the majority of the electrodes showed no significant changes in signal to noise ratio or high gamma activation relative to baseline throughout the study. While work is being done to investigate individual channels, these results show that ECoG is a promising option for long-term, stable recording in speech BCI systems for those living with severe paralysis.

Disclosures: K.R. Wyse-Sookoo: None. **S. Luo:** None. **N.E. Crone:** None.

Poster

PSTR488. Brain-Computer/Machine Interfaces

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR488.30/KK17

Topic: E.05. Brain-Machine Interface

Support: UH3NS114439 (NINDS)

Title: A closed-loop brain-computer interface that synthesizes intelligible words for a clinical trial participant with amyotrophic lateral sclerosis

Authors: *M. ANGRICK[1], S. LUO[1], Q. RABBANI[1], D. N. CANDREA[1], S. SHAH[1], G. W. MILSAP[2], W. S. ANDERSON[1], C. R. GORDON[1], K. R. ROSENBLATT[1], L. L. CLAWSON[1],

A. UCHIL[1], N. J. MARAGAKIS[1], F. V. TENORE[2], M. S. FIFER[2], H. HERMANSKY[1], N. F. RAMSEY[3], N. E. CRONE[1];
[1]The Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Applied Physics Lab., Laurel, MD; [3]UMC Utrecht Brain Ctr., Utrecht, Netherlands

Abstract: Neurological disorders can severely impact speech communication function, rendering affected individuals completely unable to speak. Previous research has shown that brain-computer interfaces (BCIs) can be used to reconstruct spoken speech purely from invasively acquired neural activity. However, existing models in epilepsy patients have been used primarily in offline analyses that retrospectively recover acoustic speech signals while bypassing the computational and experimental challenges of real-time implementation in patients with speech impairments. In this study, we have been working with a patient diagnosed with amyotrophic lateral sclerosis (ALS) whose speaking capabilities are gradually deteriorating to build a closed-loop electrocorticographic BCI that is capable of synthesizing individual words from a fixed-size vocabulary. The study participant enrolled in an ongoing clinical trial (ClinicalTrials.gov, NCT03567213) approved by the Johns Hopkins Institutional Review Board (IRB) and the FDA under an investigational device exemption (IDE). We utilized recent advances in deep learning and speech synthesis techniques to generate high-quality acoustic speech signals that are able to preserve the participant's voice characteristics. This BCI translates speech-related cortical activity during overt speech into acoustic speech waveforms that are played back as auditory feedback. Evaluation of the intelligibility of the synthesized speech indicates that many words can be correctly recognized by human listeners. Our results provide further evidence that previous speech BCI demonstrations with epilepsy patients may be generalizable to speech-impaired individuals, particularly those with ALS.

Disclosures: **M. Angrick:** None. **S. Luo:** None. **Q. Rabbani:** None. **D.N. Candrea:** None. **S. Shah:** None. **G.W. Milsap:** None. **W.S. Anderson:** None. **C.R. Gordon:** None. **K.R. Rosenblatt:** None. **L.L. Clawson:** None. **A. Uchil:** None. **N.J. Maragakis:** None. **F.V. Tenore:** None. **M.S. Fifer:** None. **H. Hermansky:** None. **N.F. Ramsey:** None. **N.E. Crone:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.01/KK18

Topic: E.09. Motor Neurons and Muscle

Title: Rapid, high-efficiency differentiation of motor neurons from human pluripotent stem cells

Authors: J. WANG[1], J. CHAN[1], A. C. EAVES[1,2], S. A. LOUIS[1], ***E. KNOCK**[1,3];
[1]STEMCELL Technologies Inc., Vancouver, BC, Canada; [2]BC Cancer, Terry Fox Lab., Vancouver, BC, Canada; [3]Biol., Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Human motor neuron (MN) diseases include devastating disorders such as amyotrophic lateral sclerosis and spinal muscular atrophy. A reliable human MN model is critical to uncover disease mechanisms, as rodent models do not recapitulate all disease phenotypes. The aim of this study was to generate human pluripotent stem cell (PSC)-derived motor neurons and determine their suitability for disease modeling. To achieve this, human PSCs were aggregated to form embryoid bodies (EBs) with STEMdiff™ Motor Neuron Differentiation Kit in an ultra-low attachment or AggreWell™400 plate. On day 9, EBs were dissociated into single cells and replated for adherent culture. On day 14, the cells were either matured using the STEMdiff™ Motor Neuron Maturation Kit or assessed by immunocytochemistry and qPCR for motor neuron markers BIIITUB, ISL1, and HB9. The MNs cultured in maturation medium were either plated onto multielectrode array plates or co-cultured myotubes generated using MyoCult™ Differentiation Kit (Human) . A highly pure population of MNs was observed on day 14 (BIIITUB: 92.6 ± 3.7%; ISL1: 56.0 ± 13.9%; HB9: 65.2 ± 13.2%; mean ± SD; n = 6) with cervical identity by the expression of HOXA5 through qPCR. After two weeks in maturation medium, the MNs showed high expression of mature markers, including CHAT, MAP2, and SYP. Preliminary multi-electrode array recordings showed that the weighted mean firing rate (Hz) of the MNs increased from 0 Hz (day 0 in maturation medium) to 2.7 Hz (day 10 in maturation medium, mean, n = 2). Finally, MNs were successfully co-cultured with hPSC-derived myotubes for one week. Taken together, STEMdiff™ Motor Neuron Culture System provides a powerful tool to generate hPSC-derived MNs and co-culture systems for in vitro studies of human MN diseases.

Disclosures: **J. Wang:** A. Employment/Salary (full or part-time):; Stemcell Technologies Inc. **J. Chan:** A. Employment/Salary (full or part-time):; Stemcell Technologies Inc. **A.C. Eaves:** A. Employment/Salary (full or part-time):; Stemcell Technologies inc. **S.A. Louis:** A. Employment/Salary (full or part-time):; stemcell technologies Inc. **E. Knock:** A. Employment/Salary (full or part-time):; STEMCELL Technologies Inc.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.02/KK19

Topic: E.09. Motor Neurons and Muscle

Title: Rapid and consistent generation of functional motor neurons from reprogrammed human iPSCs using opti-ox technology

Authors: *M. HERRERA VAQUERO, G. MASTROGIOVANNI, P. BALFOUR, S. MILDE, L. FOULSER, S. SUR, K. LAI, M. DERMIT, M. DERMIT, V. YIANNI, J. CONDE-VANCELLS, K. FIRTH, T. OOSTERVEEN, W. BERNARD, M. METZAKOPIAN, M. KOTTER;
bit.bio, Cambridge, United Kingdom

Abstract: Motor neurons consist of distinct neuronal subtypes that control the activity of muscles and glands in direct or indirect manners. Motor neurons form a large neuronal network that receives inputs from interneurons, sensory neurons, or other motor neurons to control complex behaviours such as locomotion. Pathological perturbation of these motor circuits can lead to the development of motor neuron diseases (MNDs) such as spinal muscular atrophy and amyotrophic lateral sclerosis.
Development of therapies to treat MNDs is hampered by the limited translatability of existing preclinical animal models as well as the lack of reliable and consistent sources of in vitro models. Human induced pluripotent stem cells (hiPSCs) can be used to generate motor neurons for in vitro applications, however, current differentiation protocols are often lengthy, inconsistent, and difficult to scale. Our proprietary opti-ox™ (optimised inducible overexpression) technology enables highly controlled expression of transcription factors, which can rapidly reprogram hiPSCs into specific cell types of interest, to provide a robust, consistent, and reliable cell source for *in vitro* applications.
We have used opti-ox technology to rapidly reprogram hiPSCs into motor neurons, termed ioMotor Neurons, which are a homogenous population of cells with classical neuronal morphology and neurite outgrowth. As early as 4 days in culture, cells express the pan-neuronal markers MAP2 and TUBB3, the cholinergic markers ChAT and VAChT, and the motor neuron-specific markers MNX1 and ISL1/2, as assessed by both ICC and RT-qPCR. Bulk RNA sequencing of ioMotor Neurons demonstrates a rapid acquisition of a motor neuron phenotype. ioMotor Neurons show spontaneous neuronal activity with increasing firing rate over 40 days in culture, as shown by multielectrode array activity (MEA). Finally, next generation sequencing methods have shown consistency between three different batches produced through opti-ox mediated reprogramming.
opti-ox technology can be utilised for the scalable and consistent production of hiPSC-derived motor neurons. ioMotor Neurons have the potential to advance the development of new therapeutics for MNDs and to further our understanding of motor neuron development and maturation *in vitro*.

Disclosures: **M. Herrera Vaquero:** A. Employment/Salary (full or part-time):; bit.bio. **G. Mastrogiovanni:** A. Employment/Salary (full or part-time):; bit.bio. **P. Balfour:** A. Employment/Salary (full or part-time):; bit.bio. **S. Milde:** A. Employment/Salary (full or part-time):; bit.bio. **L. Foulser:** A. Employment/Salary (full or part-time):; bit.bio. **S. Sur:** A. Employment/Salary (full or part-time):; bit.bio. **K. Lai:** A. Employment/Salary (full or part-time):; bit.bio. **M. Dermit:** A. Employment/Salary (full or part-time):; bit.bio. **M. Dermit:** A. Employment/Salary (full or part-time):; bit.bio. **V. Yianni:** A. Employment/Salary (full or part-time):; bit.bio. **J. Conde-Vancells:** A. Employment/Salary (full or part-time):; bit.bio. **K. Firth:** A. Employment/Salary (full or part-time):; bit.bio. **T. Oosterveen:** A. Employment/Salary (full or part-time):; bit.bio. **W. Bernard:** A. Employment/Salary (full or part-time):; bit.bio. **M. Metzakopian:** A. Employment/Salary (full or part-time):; bit.bio. **M. Kotter:** A. Employment/Salary (full or part-time):; bit.bio.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.03/KK20

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant NS112910
DoD Grant W81XWH2010186
LSU Health Shreveport Center for Brain Health (CBH) Grant in Aid

Title: Modeling movement disorders using patient-derived motor neurons

Authors: *B. DING;
Biochem. and Mol. Biol., LSU Hlth. Sci. Ctr. at Shreveport, Shreveport, LA

Abstract: The limited access to patient brain tissues greatly impedes progress in neurological disease research. Although animal models offer insights into disease mechanisms, significant species-dependent differences exist, and they only reflect limited aspects of the pathophysiology of human diseases. It is believed that these species-dependent differences contribute to the high failure rate in clinical trials that have been based on successful results in animal models. Excitingly, the generation of patient-specific neurons overcomes this limitation and provides an unprecedented approach in deciphering the molecular pathogenesis underlying neurological diseases. Recently, we reported two techniques for generating patient-specific motor neurons (MNs): direct conversion of patient fibroblast cells using lentiviral delivery of transcription factors, and induction and differentiation of patient induced pluripotent stem cells (iPSCs). By using patient-specific neurons, we modeled the movement disorder DYT1 dystonia and successfully replicated disease-dependent cellular deficits, including deformed nuclei, impaired neurodevelopment, disrupted nucleocytoplasmic transport, and the unexpected finding of nuclear Lamin B1 mislocalization. TEM and immunogold labeling indicated that the dysregulated nuclear Lamin B1 led to the thickening of the nuclear lamina and deformation of the nucleus in DYT1 neurons. Through proteomic studies, we further identified that mislocalized nuclear Lamin B1 extensively disrupted critical factors and signaling pathways involved in various biological processes and neuronal functions. Our study demonstrates the high value of patient-derived neurons in modeling neurological diseases and provides novel insights into the pathogenesis of dystonia.

Disclosures: B. Ding: None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.04/Web Only

Topic: E.09. Motor Neurons and Muscle

Title: Mapping the dendrite topography of facial motor neurons in larval zebrafish

Authors: *K. L. MCARTHUR;
Southwestern Univ., Georgetown, TX

Abstract: During early development, the cell bodies of hindbrain neurons exhibit spatial topography that predicts their functional properties. For example, in larval zebrafish (Danio rerio), dorsal facial motor neurons generate bursts of activity that coincide with attempted whole-body movements, while ventral facial motor neurons generate rhythmic bursts that drive rhythmic ventilatory behaviors of the jaw and operculum (McArthur & Fetcho 2017). What is the mechanism linking cell body location to differences in patterned activity? One possibility is that cell body topography correlates with dendrite topography, such that neurons with dorsal and ventral cell body locations can sample different sets of synaptic inputs in the developing hindbrain. To test this hypothesis, I explore the relationship between cell body position and dendrite position, using single-cell fluorescent labeling. Zebrafish facial motor neurons were labeled in two ways: 1) with dextran-conjugated fluorescent dye, via single-cell electroporation at 4 days post-fertilization (n=10); and 2) via sparse transgenic expression of fluorescent protein (n=10). Labeled neurons were imaged as z-stacks at 5 days post-fertilization. Each neuron's dendritic arbor was traced using ImageJ. While there is significant overlap of dendritic arbors overall, the dendrites extending from the dorsal-most and ventral-most cell bodies occupy distinct dorsal and ventral regions of the caudal hindbrain neuropil, respectively. These results are consistent with the hypothesis that neurons with different cell body positions can sample different synaptic inputs, leading to topographic differences in patterned activity. Future studies will use retrograde labeling to determine if distinct neuronal populations project to dorsal and ventral regions of the facial motor neuron dendritic zone. These studies will further our understanding of the strategies used by developing circuits in the hindbrain, to recruit distinct motor neuron populations to specific functional roles.

Disclosures: K.L. McArthur: None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.05/LL1

Topic: E.09. Motor Neurons and Muscle

Support: BR4910/2–2 (SPP1935)

Title: Hnrnpr regulates translation and localization of mrnp particles in axons of motoneurons

Authors: *A. ZARE[1], S. SALEHI[2], J. BADER[3], C. SCHNEIDER[4], U. FISCHER[4], M. BRIESE[2], M. SENDTNER[2];
[1]Inst. of Clin. Neurobio., Univ. Hosp. of Würzburg, würzburg, Germany; [2]Clin. Neurobio.,

Julius-Maximilians Univ. of Würzburg, Würzburg, Germany; [3]Dept. of Proteomics and Signal Transduction, Max Planck Inst. of Biochem., Martinsried, Germany; [4]Dept. of Biochem., Univ. Wuerzburg, Würzburg, Germany

Abstract: Neuronal function critically relies on precise spatial and temporal control of translation. During development, control of local protein synthesis allows neurons to modulate axonal outgrowth and growth cone plasticity and to fine-tune cellular processes necessary for maintaining neuronal homeostasis. Deficiency of axonal RNA transport and concomitant local mRNA translation have been implicated in the pathogenesis of motoneuron disorders including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Heterogeneous nuclear ribonucleoprotein R (hnRNP R) is an RNA-binding protein that regulates mRNA transport, stability and translation in the cytosol. In motoneurons, hnRNP R knockdown leads to axon growth defects. To elucidate hnRNP R functions *in vivo*, we generated hnRNP R knockout mice (C57BL/6) by CRISPR/Cas9-mediated genome engineering. Mutant mice showed neuromuscular junction denervation leading to impaired motor behavior. Motoneurons cultured from hnRNP R knockout mice exhibit reduced axonal synthesis of cytoskeletal components and components of the translation machinery. These findings point to a function of hnRNP R in controlling the local synthesis of key factors required for establishing and maintaining neuromuscular innervations.

Disclosures: A. zare: None. **S. Salehi:** None. **J. Bader:** None. **C. Schneider:** None. **U. Fischer:** None. **M. Briese:** None. **M. Sendtner:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.06/LL2

Topic: E.09. Motor Neurons and Muscle

Support: U-RISE NIGMS# 1T34GM145529 01
NIGMS Grant T34GM145529, Orville Edward Egbert, M.D. Endowment fund

Title: The impact of mutated HSP27 in motor neuropathy

Authors: *G. MARTINEZ[1], R. BERNAL[2], P. SABANDAL[4], K.-A. HAN[3];
[1]Biol. Sci., The Univ. of Texas at El Paso, El Paso, TX; [2]Chem., [3]Biol. Sci., Univ. of Texas At El Paso, El Paso, TX; [4]Univ. of Texas, El Paso, El Paso, TX

Abstract: The Charcot-Marie-Tooth (CMT) disease is a spectrum of genetic disorders affecting the peripheral nerves thereby causing muscle weakness and movement difficulty There are many types of CMT and mutations in the small heat-shock protein 27 (HSP27) are linked to the type 2F. It is, however, unknown whether diverse HSP27 missense mutations cause motor neuropathy

in distinct mechanisms. To address this gap in knowledge, we generated the transgenic flies expressing five different missense mutations (R127W, S135F, R136W, T151I, and P182L) in motor neurons and characterized their phenotypes. To monitor motor neuropathy, we used a negative geotaxis assay and a newly developed mating assay. We found that the flies with HSP27 mutations exhibited no detectable changes in locomotor activity but showed dampened capacity for copulation. Remarkably, the flies with S135F, R136W or P182L mutation showed the most severe deficit in copulation. We are currently characterizing the NMJ of the Muscle of Lawrence used for copulation. The findings of this study will help advance our understanding of the pathogenesis of CMT2F and may pinpoint novel treatment targets.

Disclosures: G. Martinez: None. **R. Bernal:** None. **P. Sabandal:** None. **K. Han:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.07/LL3

Topic: E.09. Motor Neurons and Muscle

Support: NIH/HINDS Grant R01-NS098780

Title: The role of Srp54 in motor neuron development in zebrafish and implications for spinal muscular atrophy

Authors: *N. LOSIEVSKI[1], P. KAMATH[2], A. EVEREST[3], T. L. GALLAGHER[4], A. KESSLER[3], C. E. BEATTIE[3], S. L. AMACHER[5], S. J. KOLB[6];
[1]Neurol., [2]Biol. Chem. and Pharmacol., [3]Neurosci., [4]Mol. Genet., [5]Biol. Chem. and Pharmacology; Mol. Genet., [6]Biol. Chem. and Pharmacology; Neurol., The Ohio State Univ., Columbus, OH

Abstract: Spinal muscular atrophy (SMA) is a motor neuron disease typically caused by a mutation in the *survival of motor neuron 1* (*SMN1)* gene that reduces the amount of SMN protein throughout an organism. All cells require SMN to survive, but the first observed symptoms in SMA patients are dysfunction and death of motor neurons. It is unknown why motor neurons are especially sensitive to deficient functional SMN protein. SMN oligomerizes to form the SMN complex whose canonical function is small nuclear ribonucleoprotein (RNP) assembly. Additionally, the SMN complex has been implicated in other RNP assembly processes that may contribute to motor neuron selectivity in SMA. To determine what SMN associations may be crucial to motor neuron health, we used a transgenic zebrafish that expresses tagged SMN protein under a motor neuron-specific promoter. Coimmunoprecipitation of tagged SMN showed that signal recognition particle 54 (SRP54) protein can associate with SMN in vertebrate motor neurons, supporting a previous study that suggested SMN promotes SRP biogenesis *in vitro*. SRP54 is part of the SRP complex, an RNP responsible for processing a subset of secreted and

integral membrane proteins that contain a signal sequence. Perhaps deficient SMN impacts SRP assembly or function which disrupts the processing of motor neuron-specific proteins and results in motor neuron death. To test if loss of Srp54 affects motor neuron dysfunction and death, we assessed axon morphology in a *srp54-/-* zebrafish line at 30 hours postfertilization (hpf), a time when most motor axons have extended through the muscle. However, zebrafish embryos may contain maternally-contributed Srp54 protein at 30 hpf and it is anticipated that lingering Srp54 would maintain motor axon morphology. Preliminary motor axon length and branching measurements suggest *srp54-/-* embryos do not have significant motor axon defects, so it is predicted that there is residual maternally-derived Srp54 at this time point. Srp54 protein levels will be measured at different time points to investigate if maternal Srp54 protein is depleted later than 30 hpf. Results will reveal when Srp54 depletion occurs and the optimal time point for motor axon morphological assessment. This work will further assess whether an association between SMN and SRP is relevant to the motor neuron selectivity in SMA.

Disclosures: **N. Losievski:** None. **P. Kamath:** None. **A. Everest:** None. **T.L. Gallagher:** None. **A. Kessler:** None. **C.E. Beattie:** None. **S.L. Amacher:** None. **S.J. Kolb:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.08/LL4

Topic: E.09. Motor Neurons and Muscle

Support: CIHR Postdoctoral Fellowship

Title: Developmental mechanisms for firing hysteresis in motoneurons of the postnatal mouse

Authors: *S. A. SHARPLES, G. B. MILES;
Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Intrinsic properties of spinal motoneurons support a range of functions that enable the generation of flexible movement. Critical for all motor behaviours is the maintenance of postural tone, which emerges toward the end of the second postnatal week in mice and is supported by sustained discharge of motoneurons. Persistent inward currents (PICs) conducted by sodium and calcium channels are believed to be critical for sustained motoneuron discharge. The capacity for sustained firing can be assessed using patch clamp electrophysiology during triangular current ramps, where the influence of PICs presents as sustained hysteresis firing behaviour. Here we used whole cell patch clamp electrophysiology to study the maturation of PICs measured in voltage clamp and the associated hysteresis firing behaviours measured in current clamp from lumbar motoneurons across developmental stages in mice when hindlimb weight bearing emerges. PIC amplitude was largest in high threshold motoneurons, which also displayed prominent sustained firing hysteresis behaviours. The amplitude of PICs increased following the

emergence of hindlimb weight bearing, which was paralleled by an increase in sustained firing behaviours. Pharmacological blockade of Nav1.6 channels reduced PIC amplitude at both pre- and post-weight bearing stages whereas blocking L-type calcium channels only reduced PIC amplitude after the emergence of weight bearing. Unexpectedly, blocking the channels mediating PICs did not influence the hysteresis behaviours. Instead, we found activating persistent outward M-type potassium currents increased firing hysteresis by delaying the onset of firing, with no influence on firing offset. Further experiments using muscarine, a neuromodulator that is known to increase PIC amplitude and decrease the M-current, produced a reduction in sustained hysteretic firing behaviour. These results demonstrate that PICs and hysteresis behaviours in motoneurons do indeed increase around the emergence of hindlimb weight bearing, however our pharmacological studies point to non-PIC-dependent mechanisms for the genesis of sustained hysteresis firing behaviours.

Disclosures: S.A. Sharples: None. **G.B. Miles:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.09/Web Only

Topic: E.09. Motor Neurons and Muscle

Support: CIHR Grant PS180430
NIH Grant NS104436
WCHRI Innovation Grant
NSERC PDF-557796-2021

Title: The effect of development and biological sex on motor unit firing properties

Authors: *A. YACYSHYN, G. MOHAMMADALINEJAD, B. AFSHARIPOUR, J. DUCHCHERER, M. GORASSINI;
Univ. of Alberta, Edmonton, AB, Canada

Abstract: To date, there are no estimates of the contribution of persistent inward currents (PICs) to self-sustained firing of motoneurons (MN) in a pediatric population or whether they differ between males and females throughout development to young adulthood. Recent work has shown that despite a lack of sex-related differences in peak discharge rates in young (21-33yrs), healthy adults at 30% of their maximal voluntary contraction (MVC), females exhibited larger estimated PICs (ΔF) compared to males. Whether this is true at younger ages (<21 years) is unknown. The purpose of this study, using non-invasive high density surface EMG (HDsEMG), is to examine if MN firing properties and the contribution of PICs to self-sustained firing change throughout development, from 7-28 years of age, and whether sex-related difference exist. MU spike trains were decomposed from the tibialis anterior using HDsEMG and blind source

separation algorithms for 4 isometric triangular contractions (10s up, 10s down) to a peak of 10, 20, and 30% MVC. MU counts, start, peak, and end MU firing rates, and ΔF were assessed with unpaired samples t-tests between sexes within a developmental group. Groups were divided into young (7-17yrs) male (n=13) and female (n=7), and older (18-28yrs) male (n=5) and female (n=7). Young males and females had equivalent MU counts for all intensities (~8.3 MUs/contraction); older males had 60% more MUs ($p \leq 0.021$) than older females for all intensities (~20.1 vs ~8.1 MUs/ contraction, respectively). Start firing rates in older males were ~14% faster than older females at 10% MVC ($p=0.048$), but were equivalent for all other rate measures across all intensities. Young female peak firing rates were ~12% faster than young males at 10% MVC ($p=0.008$) but were equivalent for all other rate measures across all intensities. All rate data had an inverse relationship with age. All ΔF values were equivalent between sexes ($p>0.160$). These preliminary results indicate that MU decomposition is easier in older males compared to older females and younger children of both sexes, potentially due to a sex- and development-related differences in muscle fibre composition characteristics. Sex-related differences in firing rates at only 10% MVC indicates that differences in intrinsic motoneuron properties may be washed out by higher synaptic inputs at the stronger contraction intensities. The inverse relationship of firing rates or ΔF and age is consistent with continued developmental changes in intrinsic MN properties. Finally, the lack of sex-related differences in discharge rate hysteresis (ΔF) conflicts with previous findings, potentially due to differences in methodology.

Disclosures: A. Yacyshyn: None. **G. Mohammadalinejad:** None. **B. Afsharipour:** None. **J. Duchcherer:** None. **M. Gorassini:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.10/LL5

Topic: E.09. Motor Neurons and Muscle

Support: Natural Sciences and Engineering Research Council

Title: Does regional recruitment and differential control of motor units during postural control persist during the ageing process?

Authors: *J. W. COHEN[1], T. IVANOVA[2], J. GARLAND[2];
[1]Hlth. Sci., Univ. of Western Ontario, London, ON, Canada; [2]Western Univ., London, ON, Canada

Abstract: Ageing is associated with changes in the neuromuscular system such as degeneration of motor and sensory neurons leading to functional deteriorations. Previously, we observed that young adults recruit motor units (MU) in distinct regions of the triceps surae during a leaning task in multiple directions. Further, we observed that young adults preferentially controlled

separate motoneuron pools by modulating the firing rates, and intermittency of motoneurons in distinct subpopulations. The aim of this study was to examine if ageing of the neuromuscular system affects regional recruitment and this MU control strategy.We recruited 32 participants (18 old adults (aged 77 ± 5 years) & 14 young adults (aged 25 ± 2 years) to perform a multi-directional leaning task in standing. Participants stood on a force platform and maintained their center of pressure (CoP) in 5 different leaning directions. High-density surface electromyography recordings were decomposed into single MU action potentials. The average rectified value (ARV) of the EMG, the barycenter, the average firing rate (AFR), coefficient of variation of the Interspike interval (CoV) and firing intermittency were calculated on the MU spike trains. The barycenter, defined as the weighted mean of the maximal ARV across columns and rows, was used to evaluate the regional recruitment of single MUs. A MU tracking analysis was used to identify groups of MUs as being common (active in 2 or more leaning directions) or unique (active only in a single leaning direction). Statistical analyses were performed using multiple linear mixed model regressions.There was an effect of age on the location of active MU and their firing behaviours. The shifting in the location of the barycenter of unique units with direction was significantly less in older than young adults ($p < 0.001$). The AFRs were significantly lower across target directions in older adults ($p < 0.001$). Also, the AFR in unique units significantly modulated across directions ($p < 0.001$) in young but not older adults. The CoVs were significantly higher in the older adults compared to the young adults ($p < 0.001$). Interestingly, the degree of intermittent activity in the unique units increased across directions in the older adults and decreased in the young adults ($p < 0.001$) Finally, older adults had worse balance performance evaluated by larger CoP 95% ellipsel area, total path lengths, and velocity ($p < 0.001$). The results provide evidence that the aged neuromuscular system may partially lose the ability to preferentially recruit MUs regionally and may control distinct motoneuron pools within the ankle plantarflexors using a different motor control strategy.

Disclosures: **J.W. Cohen:** None. **T. Ivanova:** None. **J. Garland:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.11/LL6

Topic: E.09. Motor Neurons and Muscle

Support: NIH R01MH085927
R01NS109388

Title: Locomotion interneurons control cholinergic motor neurons by acting on two different acetylcholine receptors in C. elegans

Authors: *S. RIAZ, L. NIU, Z.-W. WANG;
Neurosci., Univ. of Connecticut Sch. of Med., Farmington, CT

Abstract: Each animal species often possesses multiple genes for receptor subunits of a single neurotransmitter. To comprehend the functioning of a specific neural circuit, it is essential to identify the postsynaptic receptors and demonstrate their physiological functions. In the case of *C. elegans*, a bilateral pair of locomotion interneurons known as AVA plays a central role in generating backward movements by regulating a particular type of cholinergic motor neurons called A-MNs. Previous studies have revealed that spontaneous postsynaptic currents (sPSCs) recorded from A-MNs sorely depend on acetylcholine release from AVA, and LGC-46 is a postsynaptic receptor in A-MNs. Furthermore, A-MNs express an extrasynaptic receptor consisting of five different subunits (ACR-2, ACR-3, ACR-12, UNC-38, and UNC-63). In this study, we investigated whether the subunits implicated in the putative extrasynaptic receptor might also function as postsynaptic receptors by recording sPSCs from a representative A-MN and comparing the sPSC frequency among wild-type and mutant strains of specific receptor genes. Our findings indicate that the *acr-2(n2420)* gain-of-function (*gf*) mutant exhibited a considerable increase in sPSC frequency and an almost paralytic behavior. These phenotypes of the *acr-2(gf)* could be largely alleviated by loss-of-function (*lf*) mutations of *acr-12*, *unc-38*, or *unc-63*, all of which showed reduced sPSC frequencies compared to the wild-type. Importantly, double mutants of *lgc-46(lf)* and either *acr-12(lf)*, *unc-38(lf)*, or *unc-63(lf)* completely lacked sPSCs, indicating the existence of two distinct populations of acetylcholine receptors. Consistent with their function as postsynaptic receptors, GFP-tagged receptor subunits appeared as discrete puncta in A-MNs, often localized in close proximity to a presynaptic marker expressed in AVA. Collectively, our results demonstrate that AVA controls A-MNs through two distinct populations of postsynaptic receptors, one with LGC-46 as a key component and the other with ACR-2, ACR-12, UNC-63, and UNC-38 as key components.

Disclosures: **S. Riaz:** None. **L. Niu:** None. **Z. Wang:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.12/LL7

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant NS110577-04
NIH/NIBIB/P41/EB018783
NYS SCI Research Trust Fund C32241GG
Other supports/Albany Samuel S. Stratton VA Medical Center

Title: Are muscarinic m2 acetylcholine receptors on spinal motoneurons involved in electrical sensorimotor cortex (SMC) stimulation (ECS)-induced plasticity of motor output?

Authors: *Y. WANG[1], B. HERRON[1,2], Y. CHEN[1], A. VATO[1,2], J. S. CARP[1,2], J. R. WOLPAW[1,2];
[1]Albany Stratton VA Ctr., Albany, NY; [2]State Univ. of New York, Albany, NY

Abstract: The muscarinic m2 acetylcholine receptor (m2R) modulates spinal cord nociceptive responses (Gomeza et al 1999) and neonatal rhythmic locomotion (Nascimento et al 2019), but it is not clear whether m2Rs affect locomotion in adult animals (Jordan et al 2014). Using adult rats, we have established an ECS protocol that produces a long-term soleus H-reflex (SOL HR) increase (Chen et al 2007). This ECS protocol also decreases $GABA_B$ receptor ($GABA_BR$) and G-protein-coupled inwardly rectifying potassium channel 2 (Girk2) but does not change cholinergic C-terminal innervation of SOL motoneurons (Wang et al 2012, 2020, 2021). Because m2R can directly interact with $GABA_B$Rs in regulating neuronal Girk 2 trafficking (Boyer SB 2009), we are now examining the influence of ECS on m2R and Girk2 expression and their relationships with $GABA_B$Rs on SOL motoneurons.Ten rats were implanted with SOL EMG electrodes and posterior tibial nerve stimulating cuffs in their right (R) legs and ECS electrodes over left (L) hindlimb SMC areas. ECS (25-Hz train of 0.1-ms biphasic pulses for 1 s every 10 s) were delivered 24 hr/d X 30 d, producing no visible behavioral responses. The R SOL HR was recorded throughout. After 30 days, the ECS rats and ten naïve control (NC) rats were injected with cholera toxin subunit B in SOL muscles and perfused 3 days later. L4-S1 lumbar sections were cut for immunofluorescence staining of m2R and $GABA_B$Rs and mRNA *in situ* hybridization of Girk 2, SOL motoneuron immunoreactivity (IR) and mRNA were quantified in a blinded fashion. In rat lumbar spinal cord, m2R puncta distribution is closely associated with $GABA_B$2-IR on motoneuron somatic and dendritic surfaces. On SOL motoneuron somatic membrane, m2R-IR averaged 79.3± 3.9(SE) % of NC (p<0.001 vs NC, n=121), GirK2-IR averaged 88.3±2.7% of NC (p<0.01 vs NC, n=107), and Girk2 mRNA averaged 74±4% of NC (p<0.001 vs NC, n=72). These preliminary results reveal that ECS treatment produces inhibitory effects on motoneuron surface expression of m2 receptor and Girk2 channels and suggest that ECS attenuates Girk2 mediated coordination between m2 and $GABA_B$ receptors in spinal cord motor circuitry.

Disclosures: Y. Wang: None. **B. Herron:** None. **Y. Chen:** None. **A. Vato:** None. **J.S. Carp:** None. **J.R. Wolpaw:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.13/LL8

Topic: E.09. Motor Neurons and Muscle

Support: HD36020(XYC)
NS22189(JRW)
NS061823(XYC&JRW)

NS110577 (JRW,YW,JSC,AV)
NICHD/1P41EB018783(JRW)
NYS Spinal Cord Injury Research Trust Fund C32241GG(BJH)
Stratton VA Medical Center, Albany, NY

Title: Weak electrical stimulation of rat sensorimotor cortex changes the soleus H-reflex & soleus motoneurons

Authors: *Y. CHEN[1], Y. WANG[2], B. HERRON[3], X. PU[4], A. VATO[6], J. S. CARP[5], J. R. WOLPAW[7];
[1]Albany Stratton VA Med. Ctr., Albany, NY; [2]Albany Stratton VA Med. Ctr., Albany Stratton VA Ctr., Albany, NY; [3]Univ. At Albany, Sch. of Publ. Hlth., State Univ. of New York, Univ. of Albany, Albany, NY; [5]Stratton VA Med. Ctr., [4]Stratton VA Med. Ctr. / NCAN, Albany, NY; [6]Albany VA Med. Ctr., Albany VA Med. Ctr., Albany, NY; [7]Jonathan r Wolpaw, Natl. Ctr. for Adaptive Neurotechnologies, Delmar, NY

Abstract: Operant conditioning of the soleus (SOL) H-reflex (HR) induces spinal motoneuron (MN) and interneuron plasticity and improves treadmill locomotion in rats with incomplete spinal cord injury (J Neurosci 26:12537-12543, 2006). The corticospinal tract and sensorimotor cortex (SMC) are essential for HR conditioning (J Neurophysiol 78: 1730-1734, 1997; 87:645-652, 2002; 96: 119-127, 2006). Weak electrical stimulation (ECS) of rat SMC increases the HR. We are now exploring the time course of this HR change and its concurrent effects on neurotrophic factor expression in SOL MNs.
Under anesthesia, Sprague-Dawley rats are implanted with EMG electrodes in the right SOL muscle, a nerve stimulating cuff on the right posterior tibial nerve, and epidural stimulating electrodes over left SMC. After control HR data is collected, each rat receives weak SMC ECS (25-Hz, 40-ms bipolar pulses for 1s every 10s) for 30 d. ECS amplitude (<30 μA) is continually adjusted to maintain a small stable SOL motor evoked potential. HR data are collected 24/7 throughout. When ECS ends, both SOL are retrogradely labeled with cholera toxin subunit B and the rat is perfused 4 days later. SOL MNs are identified in L4-5 spinal segments. ECS effects on SOL MN tropomyosin receptor kinase B and C (TrkB & TrkC) proteins and their mRNAs are assessed by single molecule in situ hybridization; their values are expressed as % of average values for Naive Control (NC) rats.
Data from 24 rats to date confirmed that weak ECS for 30 days increases SOL HRs: final HR for days 28-30 averaged 189.1(±16.2SE)% of pre-ECS baseline ($p<0.001$); background EMG level and M-wave size did not change. The 30-day course of H-reflex increase was similar to that seen with operant up-conditioning of the SOL HR.
In ECS rats, TrkB and TrkC immunofluorescent reactivity (IR) increased in SOL MNs bilaterally ($p<0.02$). TrkB/TrkC mRNAs increased in right SOL MNs of ECS rats; somatic mRNA increased to 113(±5SE)% and 117(±6SE)% of NC values for TrkB and TrkC, respectively ($p=0.04$). ECS-induced HR increase in right SOL MNs was positively correlated with the increases in TrkC-IR ($R=+0.71$, $p=0.03$) and its mRNA ($R=+0.81$, $p=0.02$).
These results indicate that the ECS protocol up-regulates neurotrophic factor receptors TrkB and TrkC and their gene expression. Additional chronic data collection and analyses should provide further insight into the long-term spinal effects of ECS protocols. Current studies focus on the possibility that ECS might constitute a novel therapeutic approach to improving locomotor function impaired by spinal cord injury or disease.

Disclosures: **Y. Chen:** None. **Y. Wang:** None. **B. Herron:** None. **X. Pu:** None. **A. Vato:** None. **J.S. Carp:** None. **J.R. Wolpaw:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.14

Topic: E.09. Motor Neurons and Muscle

Support: NIH1R01 NS110577
NIH/NIBIB/P41/EB018783
NYS SCI Research Trust Fund NS22189
NYS SCI Research Trust Fund C32241GG

Title: Electrocortical stimulation induces diverse transcriptional effects in the spinal cord.

Authors: *B. HERRON[1,2], Y. WANG[2], Y. CHEN[2], J. CARP[2], A. VATO[2], J. WOLPAW[1,2];
[1]State Univ. of New York, Univ. of Albany, Albany, NY; [2]Stratton VA Med. Ctr., Albany, NY

Abstract: Electrocortical stimulation (ECS) has local effects, and it also has remote effects on regions to which the stimulated region connects. We are studying ECS short-term and long-term effects on gene expression in the spinal cord. Our experimental paradigm delivers weak ECS to the rat sensorimotor cortex and monitors the H-reflex of soleus motoneurons as a biomarker of remote effects. We have now performed global gene expression studies on 18 lumbar spinal cord samples from 9 ECS-treated rats and comparable samples from 6 untreated control rats using the TempOSeq platform. Differential gene expression analysis revealed more than 700 genes that were differentially expressed between ECS rats and Control rats ($P<0.05$ after correction for multiple tests). These included 331 genes that were up-regulated in ECS rats and 379 that were down-regulated. Pathway analysis of differentially expressed genes revealed several pathways that appear to be affected by ECS; including pathways that are known to contribute to the regulation of neurotransmitter transport and/or release. Some affected pathways are involved in synaptic function (e.g., syt1 and Slc10a4); others are related to signal transduction (e.g., MAPK pathway, PI3/AKT, and G protein signaling). Furthermore, in five additional rats perfused several months after ECS ended (ECS long-term rats), expression of 144 genes differed from Control rats ($p<0.05$). Such long-term changes in gene expression may underlie the remarkable long-term persistence of the H-reflex increase produced by ECS. These results indicate that weak ECS of rat SMC for 30 days has broad genomic effects on lumbar spinal cord. These genomic changes may directly or indirectly induce plasticity in spinal cord motor circuitry that modifies motor function. Ongoing work seeks to verify these initial results and to identify genomic changes that underlie significant functional effects.

Disclosures: **B. Herron:** None. **Y. Wang:** None. **Y. Chen:** None. **J. Carp:** None. **A. Vato:** None. **J. Wolpaw:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.15/LL9

Topic: E.09. Motor Neurons and Muscle

Support: NINDS R01 NS104436
Advance Clinical Translational Network AWD12047
URI College of Pharmacy Seed Grant

Title: Primary Afferent Depolarization as a driver of spasticity in cerebral palsy

Authors: *E. MENA-AVILA[1], E. REEDICH[1], L. GENRY[1], D. J. BENNETT[2], M. A. GORASSINI[3], K. QUINLAN[1];
[1]George and Anne Ryan Inst. for Neurosci., Univ. of Rhode Island, Kingston, RI; [2]Univ. Of Alberta, [3]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Cerebral palsy (CP) arises from a variety of perinatal insults and results in permanent, lifelong motor deficits, most often spasticity. Spastic CP is thought to arise from damage to the cortex and corticospinal tracts (CSTs) which results in downstream disinhibition of spinal motoneurons and hyperreflexia, although the specific mechanism has never been directly tested. Rabbits subjected to hypoxia-ischemia (HI) in late gestation show hypertonia and hyperreflexia, hallmarks of spastic CP. One way CSTs modulate reflex circuits is through primary afferent depolarization (PAD). Nociceptors and CST projections converge onto spinal GAD2+ GABAergic interneurons that mediate PAD. Whether perinatal insults that weaken the CST alter PAD has never been investigated. We hypothesize that CST impairment allows expansion of nociceptive innervation of GAD2+ neurons and promotes irradiation of reflexes to more distant spinal segments. Pregnant New Zealand White rabbits undergo HI procedures. Under anesthesia a Fogarty balloon catheter is inflated to occlude uterine arteries for 40 minutes at 70-80% gestation, causing HI in developing fetuses. In sham animals anesthesia is delivered at the same developmental time point without HI, and naïve controls have no interventions performed. Kits are born naturally at term. Isolated midsagittal hemisected spinal cords of neonatal rabbits (ages postnatal day 1-5) from either sex were used. To evoke monosynaptic reflexes (MSR) and dorsal root potentials (DRPs) dorsal roots are stimulated. To explored reflex irradiation both dorsally and ventrally, dorsal or ventral roots in several adjacent segments are recorded simultaneously. Consistent with observations in CP patients, reflex amplitude and irradiation is larger in HI rabbits. To measured reflex irradiation, peak amplitude of MSRs and area under the curve (AUC) of DRPs are compared as percentage of the response evoked in the in the 1st segment (seg). Low-threshold DRP irradiation is larger in HI rabbits (2nd seg: 55 ± 49, n = 3, 3rd seg 36 ± 24, n

= 3, all % 1st seg AUC) compared to sham rabbits (2nd seg: 15 ± 4, n = 4, 3rd seg 13 ± 6, n = 4, all % 1st seg AUC). MSRs evoked by 2.5 x threshold show a larger spread irradiation in HI rabbits (2nd seg: 54 ± 39, n = 2, 3rd seg 17 ± 10, n = 2, and 4th seg 12, n = 1, all % 1st seg peak amplitude) compared to control rabbits (2nd seg: 40 ± 27, n = 2, 3rd seg 13 ± 6, n = 2, and 4th seg 7 ± 3, n = 2, all % 1st seg peak amplitude). With this data we can conclude that DRP and MSR spread is augmented in HI rabbits. Furthermore, spinal circuits and sensory afferents are dysfunctional (not just the brain!). Further examination of pathophysiological changes in spinal circuits in CP is needed.

Disclosures: **E. Mena-Avila:** None. **E. Reedich:** None. **L. Genry:** None. **D.J. Bennett:** None. **M.A. Gorassini:** None. **K. Quinlan:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.16/LL10

Topic: E.09. Motor Neurons and Muscle

Support: NINDS R01 NS104436
Advance Clinical Translational Network AWD12047
URI College of Pharmacy Seed Grant
Startup funds from the College of Pharmacy, the Provost Office and The George and Anne Ryan Institute for Neuroscience
RI-INBRE Summer Undergraduate Research Fellowship Program - Rhode Island Institutional Development Award (IDeA) Network of Biomedical Research Excellence from NIH NIGMS P20GM103430

Title: Motor unit pathophysiology in a rabbit model of cerebral palsy

Authors: *E. REEDICH[1,2], D. SANDERS[1,2,3], E. MENA AVILA[1,2], A. MADDEN[1,2], C. HAMILTON[1,2,3], L. T. GENRY[1,2,4], L. A. DOWALIBY[1,2], K. QUINLAN[1,2], M. MANUEL[1,2]; [2]George and Anne Ryan Inst. for Neurosci., [3]INBRE SURF Program, [4]Interdisciplinary Neurosci. Program, [1]Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral palsy (CP) is the most common motor disability in children, with a prevalence of 1:500 live births. Hallmarks of spastic CP are hyperreflexia and hypertonia, which are typically accompanied by muscle weakness and fatigue. It is unclear how CP-causative injuries like hypoxia-ischemia (HI) lead to motor deficits. Motor unit (MU) development occurs in the perinatal period when CP-causative injuries occur, and depends on spinal motoneuron (MN) activity, which is enhanced in the HI rabbit model of spastic CP. Perinatal MN hyperexcitability likely affects the development of MU physiological types (S, slow; FR, fast fatigue-resistant; and FF, fast fatigable), but the effect of prenatal HI injury on MU development

is unexplored. We hypothesize that increased MN drive after prenatal HI injury causes MUs to develop atypically, establishing a neuromuscular system amenable to spasticity, weakness, and fatigue. To test our hypothesis, we are recording single MUs *in vivo* in anesthetized control and HI rabbits using the split ventral root method, and characterizing MU contractile properties throughout the early postnatal period. We expect that HI MUs will generate smaller forces and exhibit greater fatigability than control MUs. Measuring muscle fiber type composition provides further insight into muscle force-generating capacity and fatigability. We are using immunostaining to label type I, IIa, IIx, and IIb myofibers and are evaluating differences in fiber type distributions of control and HI rabbit muscles. Our preliminary analysis indicates that the lateral gastrocnemius muscle contains a greater proportion of type IIb myofibers in HI rabbits compared to control. Lastly, we are assessing neuromuscular maturity in control versus HI rabbits by tracking the emergence of mono-innervation at the neuromuscular junction (NMJ), an anatomical hallmark of maturity. Our preliminary data suggests that NMJs undergo delayed maturation in HI rabbit skeletal muscle compared to control. Overall, this project will determine whether aberrant MU development and electrophysiology after prenatal HI injury contribute to motor dysfunction in the rabbit model of spastic CP.

Disclosures: **E. Reedich:** None. **D. Sanders:** None. **E. Mena Avila:** None. **A. Madden:** None. **C. Hamilton:** None. **L.T. Genry:** None. **L.A. Dowaliby:** None. **K. Quinlan:** None. **M. Manuel:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.17/LL11

Topic: E.09. Motor Neurons and Muscle

Support: CONACYT 808827 to ZFL
Conacyt CF1311312 DLCQ

Title: Polypyrrole implant on ventral root avulsion: effect on the electrophysiological characteristics of peripheral nerves in the rabbit

Authors: Z. FLORES-LOZADA[1], A. FLORES-HERNANDEZ[1], J. MORALES CORONA[3], I. JIMENEZ-ESTRADA[4], R. ZEMPOALTECA[2], ***D. CORONA QUINTANILLA**[2];
[1]Posgrado en Ciencias Biológicas, [2]Univ. Autónoma de Tlaxcala, Tlaxcala, Mexico; [3]Física, Univ. Autónoma Metropolitana, Iztapalapa, Mexico; [4]IPN Ctr. Invst & Adv Studies, Mexico City, Mexico

Abstract: Spinal cord injuries are mainly caused by road accidents. The damage to the spinal cord can be its compression, stretching or break of the spinal cord roots. The rupture or avulsion of the spinal roots causes sensory and motor dysfunctions. When this injury occurs in the anterior

root it is denominated ventral root avulsion (VRA). The VRA occurs mainly at the lumbosacral level (L6-S2) and in these spinal segments the locomotion and posture functions are regulated. To date, there is no approved or completely successful treatment that helps to restore motor functions lost after VRA, so this research proposes the use of a biomaterial as a repair strategy. Polypyrrole (PPy) is a biomaterial that has shown a regenerative potential for various disorders of the central nervous system, including VRA injury. The aim of this study was to determine whether PPy implantation restores the characteristics of compound action potential (CAPs) of the femoral and peroneal nerve in rabbits after VRA. The virgin female rabbits divided into 3 groups: 1) Sham (n=4), 2) VRA (n=4) and 3) VRA+PPy (n=4) were used. The animals underwent right unilateral avulsion surgery of the 6 lumbar. After of 30 days, the CAPs were recorded in vitro. In the recordings it is observed that VRA causes a modification in the CAPs. The VRA decreases the amplitude and duration of CAPs, possibly due to the damage to the myelin and axons. But, in the VRA+PPY group, the CAPs seem to maintain their electrophysiological properties, akin to the CAPs of the Sham group. The results of the recordings suggests that the L6 VRA affects the excitability of the fibers and the PPy implant seems to be protecting the functional characteristics of the nerves, with these first observations we can infer that the PPy implant strategy immediately after VRA promotes the functional recovery of the denervated nerves.

Disclosures: **Z. Flores-Lozada:** None. **A. Flores-Hernandez:** None. **J. Morales Corona:** None. **I. Jimenez-Estrada:** None. **R. Zempoalteca:** None. **D. Corona Quintanilla:** None.

Poster

PSTR489. Motor Neurons: Development, Identification, Intrinsic Properties, and Modulation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR489.18/LL12

Topic: E.09. Motor Neurons and Muscle

Title: Differential Effects of Caffeine on the Excitability of Human Spinal Motoneurons

Authors: C. TAYLOR[1], F. NEGRO[2], ***C. K. THOMPSON**[1];
[1]Temple Univ., Philadelphia, PA; [2]Univ. degli Studi di Brescia, Univ. of Brescia, Brescia, Italy

Abstract: Human spinal motoneurons are partially governed by the activation of persistent inward currents that contribute to changes in excitability and elicit prolonged output of motoneurons. Persistent inward currents are regulated by monoamines, such as serotonin. Caffeine, a widely consumed performance-enhancing supplement, has been shown to stimulate the release of monoamines. However, little is known on how caffeine may directly affect motoneuron excitability and discharge characteristics. We utilized a double-blind, inactive placebo-controlled, crossover study design (clinical trial: NCT04891393) to examine the effects of caffeine (3 mg/kg) on motoneuron excitability and discharge characteristics. Utilizing high-density electromyography from the right tibialis anterior (TA) and soleus (SOL) muscles of 20

neurologically intact adults, we decomposed and tracked populations of concurrently active motor units during sub-maximal (20%) isometric contractions of the ankle before and at 30 min intervals post ingestion of either caffeinated or decaffeinated coffee. In the caffeine group, we observed significant changes in mean arterial pressure and heart rate post ingestion. Likewise, linear mixed effects models demonstrate, significant interactions, within the caffeine group, over time, within the TA motor pool for changes in estimated motoneuron excitability, discharge rate, and the variability of inter-spike intervals. No changes are observed within the SOL or following ingestion decaffeinated coffee. These findings suggest that caffeine differentially affects the excitability of human lower extremity motor pools.

Disclosures: C. Taylor: None. **F. Negro:** None. **C.K. Thompson:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.01/LL13

Topic: F.01. Neuroethology

Support: Howard Hughes Medical Institute

Title: Defensive decision making in Drosophila melanogaster is shaped by shifting brain states

Authors: *S. ASINOF[1], G. CARD[2];
[1]Janelia Res. Campus, Herndon, VA; [2]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: *Drosophila melanogaster* respond to threats by rapidly selecting from a suite of defensive behaviors such as running away, freezing in place, or taking flight. Our previous work characterized feedforward neuronal pathways that detect approaching predators and determined how some features of a threat, such as size and rate of angular expansion, interact within the fly's innate neural architecture to influence the fly's choice. However, it remains unclear whether the behavioral choices resulting from this fixed neural circuitry are therefore predictable. We approached this question by analyzing our collection of high-speed video recordings of the responses of tens of thousands of flies to looming stimuli. We then developed a novel computational method to automatically annotate their movements. Surprisingly, we were unable to predict the flies' choices with high accuracy using information about the threatening stimulus or the flies' initial behavior prior to the stimulus, suggesting that other factors contribute to their decision. We modified our assay to observe the responses of individual flies to repeated looming stimuli and discovered that there was more variability in defensive choices between individuals than among trials for a single individual. In fact, we found that individual *Drosophila* chose the same behavior repeatedly on a time scale of minutes to hours, but the consistency did not last across days. By driving activity in a population of modulatory neurons we were able to institute persistent changes in defensive action selection. Taken together, these findings suggest that

defensive decision-making in *Drosophila melanogaster* is not a rote, reflexive computation but is instead shaped substantially by slow fluctuations in brain state.

Disclosures: S. Asinof: None. **G. Card:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.02/LL14

Topic: F.01. Neuroethology

Support: Howard Hughes Medical Institute

Title: An action selection role for giant interneurons during predation

Authors: *C. M. CHAI[1,2], C. M. MORROW[2], D. D. PARIKH[2], C. R. VON REYN[2,3], A. LEONARDO[2], G. M. CARD[1,2];
[1]Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY; [2]HHMI Janelia Res. Campus, Ashburn, VA; [3]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Philadelphia, PA

Abstract: Large axon-diameter descending interneurons are metabolically costly but transmit information rapidly from sensory neurons in the brain to motor neurons in the nerve cord. Though often considered isolated command neurons triggering fast-reaction-time, all-or-none escape responses, giant interneurons are just one of multiple parallel pathways enabling selection between behavioral alternatives. However, such degeneracy among escape circuits makes it unclear if and how giant neurons benefit prey fitness under naturalistic conditions. The fruit fly *Drosophila melanogaster* selects between executing a faster short mode escape or a slower but more stable escape takeoff sequence when confronted with an oncoming visual threat, and this decision depends on the relative spike timing of the Giant Fiber (GF) interneurons. Here, we competed flies with genetically-silenced GFs against intact flies in a behavioral arena with wild-caught damselfly predators and find that GF silencing decreases fly survival. High-speed videography of predation events demonstrates that this decrease is a direct consequence of GF-silenced flies failing to escape when challenged with damselfly attack speeds and approach distances that would normally elicit successful escapes. Our findings further indicate that GFs promote fly survival by influencing action selection, rather than reducing reaction time, as a means to enhance escape performance during realistically complex predation scenarios. We conclude that despite the presence of parallel descending escape pathways, GF-mediated fast escapes are still strongly accountable for successful predator evasion and prey survival under ecologically relevant conditions.

Disclosures: C.M. Chai: None. **C.M. Morrow:** None. **D.D. Parikh:** None. **C.R. von Reyn:** None. **A. Leonardo:** None. **G.M. Card:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.03/LL15

Topic: F.01. Neuroethology

Support: Wellcome Trust collaborative award 220343/Z/20/Z
Wellcome Trust collaborative award 221300/Z/20/Z
Medical Research Council MC-U105188491
Walter Benjamin DFG fellowship (to TS)
Howard Hughes Medical Institute

Title: Transforming descending input into behavior: The organization of premotor circuits in the Drosophila Male Adult Nerve Cord connectome

Authors: *H. S. J. CHEONG[1,2], K. EICHLER[3], T. STÜRNER[4,3], S. K. ASINOF[2], A. S. CHAMPION[3,4], E. C. MARIN[3], T. B. ORAM[2], M. SUMATHIPALA[2], L. VENKATASUBRAMANIAN[3,4], S. NAMIKI[2,5], I. SIWANOWICZ[2], M. COSTA[3], S. BERG[2], J. JANELIA FLYEM PROJECT TEAM[2], G. S. X. E. JEFFERIS[4,3], G. M. CARD[2,1];
[1]Columbia Univ., New York, NY; [2]Janelia Res. Campus, Ashburn, VA; [3]Univ. of Cambridge, Cambridge, United Kingdom; [4]MRC Lab. of Mol. Biol., Cambridge, United Kingdom; [5]Univ. of Tokyo, Tokyo, Japan

Abstract: In both vertebrates and *Drosophila*, motor signals are transmitted by descending neurons (DNs) from the brain to the nerve cord, where they synapse onto motor neurons (MNs) and premotor circuits to carry out muscle control and generate behavior. Dissecting the organization of DNs onto premotor pathways and MNs is thus crucial to understand how animals control and coordinate their motor output. The recent release of the densely-reconstructed *Drosophila* Male Adult Nerve Cord connectome (MANC, Takemura et al., 2023), accompanied with detailed neuron annotation (Marin et al., 2023), now allows us to broadly examine DN-to-MN pathways across the *Drosophila* nerve cord and describe their organization at the synaptic level. Here, we proofread and curated all DNs and MNs in MANC, and identified 29% of DNs and 55% of MNs by light level or EM matching. Then, we carried out large-scale analyses examining DN-to-MN information flow, premotor network structure and network recurrence across the VNC. Lastly, we examined with finer detail circuits downstream of key DNs of interest that may play roles in motor control in flight, walking and escape behaviors. Overall, DN-to-MN connectivity commonly occurs at relatively short path lengths across motor systems, although direct connectivity is uncommon. VNC networks are organized into communities that are specialized in controlling individual motor systems such as those of the legs, wings, halteres and abdomen, as well as communities that likely exert second-order motor control through other communities. Our work here thus lays the foundation for researchers to conduct further investigation into VNC circuits of interest.

Disclosures: **H.S.J. Cheong:** None. **K. Eichler:** None. **T. Stürner:** None. **S.K. Asinof:** None. **A.S. Champion:** None. **E.C. Marin:** None. **T.B. Oram:** None. **M. Sumathipala:**

None. **L. Venkatasubramanian:** None. **S. Namiki:** None. **I. Siwanowicz:** None. **M. Costa:** None. **S. Berg:** None. **J. Janelia FlyEM Project Team:** None. **G.S.X.E. Jefferis:** None. **G.M. Card:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.04/LL16

Topic: F.01. Neuroethology

Support: DFG-grant Bu857/15-1
DFG-grant IT/9-1

Title: Drosophila curve walking: the breaking of symmetry in motor control

Authors: *E. GOROSTIZA, R. CUSTÓDIO, T. BOCKEMÜHL, K. ITO, A. BUSCHGES; Univ. of Cologne, Cologne, Germany

Abstract: Locomotion is a fundamental feature for countless animal species. Terrestrial animals, such as arthropods, execute coordinated movements of their limbs to change their locations. Kinematic parameters and movement sequences of the limbs and joints must be modified to fulfill actual behavioral demands. Insects, for example, adjust the step cycle period and stance duration of their legs in order to control speed during straight walking, and their leg coordination patterns change gradually with speed (e.g. Wosnitz et al. 2013).
However, only limited insight exists for the behavioral goals that require more complex alterations and interleg coordination, e.g., turning during locomotion (e.g. Dürr, 2005). To address this question, we use the fruit fly because of its highly versatile behavioral performance and the availability of neurogenetic toolbox for analyzing the underlying neural mechanisms. Curve walking presents an interesting readout of the motor system in insects. During straight walking, contralateral pairs of legs in each body segment produce similar but antiphasic steps. For curve walking, on the contrary, the motor system must break contralateral symmetry and generate different modifications in stepping kinematics between left and right legs. To address these questions, we analyzed 416 videos of free-walking flies with nonlinear trajectories, and found three major strategies to produce curve walking; adjustments, arcs, and turns. Adjustments and arcs can be performed with higher forward speeds than turns, and their pivot point is far from the body, while for turns the pivot point is very close to the animal. Kinematic parameters from arcs resemble those from straight walking, while turns present distinctive differences, e.g., in stance durations. We also found differences in the temporal phase relationship between legs on the inner and the outer side of the curves. Our analyses suggest that contralateral asymmetries required for curve walking increase when forward walking speed decreases through the different strategies. Lastly, we established a paradigm to study the role of different neuronal groups in curve walking command and modulation.
Supported by DFG-grant Bu857/15-1 and IT/9-1, NeuroNex

Disclosures: **E. Gorostiza:** None. **R. Custódio:** None. **T. Bockemühl:** None. **K. Ito:** None. **A. Buschges:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.05/LL17

Topic: F.01. Neuroethology

Support: NSF GRFP
NIH Grant R01NS116595

Title: Investigating the neural implementation of roll control in fruit flies

Authors: *B. K. LUDLOW[1], H. TEOH[1], S. C. WHITEHEAD[1], B. H. DICKERSON[2], E. EHRHARDT[3], W. KORFF[4], D. STERN[4], M. H. DICKINSON[5], I. COHEN[1];
[1]Physics, Cornell Univ., Ithaca, NY; [2]Neurosci., Princeton Univ., Princeton, NJ; [3]Inst. of Zoology, Univ. of Cologne, Cologne, Germany; [4]Janelia Res. Campus, Ashburn, VA; [5]Caltech, Pasadena, CA

Abstract: Like balancing a meter stick on the tip of your finger, flapping flight is an inherently unstable dynamical system. This instability drives the need for fast corrections to mid-flight perturbations, such as a gust of wind or approaching fly swatter. Fruit flies have one of the fastest corrective responses in the animal kingdom-beginning within five milliseconds-which, when combined with a relatively sparse flight motor system, makes them an excellent model system to study the neural basis for fast motor control. We investigated the neural mechanism for stability about the body roll axis, which is the fly's most unstable degree of freedom. Previous work in tethered flight has found asymmetric activity in the first axillary steering muscles during fictitious roll maneuvers. Using precisely targeted split-Gal4 lines, we can optogenetically activate and silence individual steering muscle motor neurons bilaterally within freely-flying flies. I have found that activation of either the first axillary steering muscle motor neurons produces a large decrease in the wing stroke amplitude. If activated unilaterally, this would produce a differential between left and right stroke amplitude previously shown to be present during roll maneuvers in free flight. These results have been further validated by quasi-steady aerodynamic simulations developed within our lab. However, when these same motor neurons are silenced, flies maintain stable flight and are even able to perform robust corrective responses to external roll perturbations; this is different from previous results for pitch control, which show that the loss of certain steering muscles can cause major deficits in pitch stability. We believe this indicates redundancy within the roll controller system, perhaps as an adaptation in case of muscle fatigue or injury. Our results further the understanding of motor control and how it can be maintained even in the event loss of function within a sparse motor system.

Disclosures: **B.K. Ludlow:** None. **H. Teoh:** None. **S.C. Whitehead:** None. **B.H. Dickerson:** None. **E. Ehrhardt:** None. **W. Korff:** None. **D. Stern:** None. **M.H. Dickinson:** None. **I. Cohen:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.06/LL18

Topic: F.01. Neuroethology

Support: NIH Grant 1R01NS116595-01

Title: Uncovering tergopleural muscles' role in fruit fly's reflex response toward pitch instability.

Authors: *H. TEOH[1], S. C. WHITEHEAD[2], K. LUDLOW[1], E. EHRHARDT[3], G. M. CARD[4], W. KORFF[5], D. STERN[5], M. H. DICKINSON[2], I. COHEN[1];
[1]Cornell Univ., Ithaca, NY; [2]Caltech, Pasadena, CA; [3]Inst. of Zoology, Univ. of Cologne, Cologne, Germany; [4]Zuckerman Inst., Columbia Univ., New York, NY; [5]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: An extreme form of locomotion, such as the flapping flight, requires a flying insect to continuously adjust its wing motion via a dozen pairs of steering muscles to maintain its aerial stability. Previous studies have shown that, in fruit flies, the reflexes used to counteract the pitch instabilities are fast, robust, and well-described by a proportional-integral (PI) controller model that predicts the modulation of wing stroke amplitude based on the body pitch (I term) and body angular velocity (P term), with each term modulated by the basalar b1 and b2 motor units. Are there other steering muscles that are involved in a pitch correction maneuver? We discovered the fruit fly's tergopleural (tp) muscles, often associated with courtship, also play a role in the pitch correction maneuver. In our behavioral assay, targetting the tp motor neuron with split-GAL4 driver lines, a freely flying fruit fly's tp muscle was first optogenetically suppressed/activated before a mechanical perturbation about its pitch axis with varying strength was applied. We found that silencing the tp muscle led to an under-corrected maneuver for big perturbations; inversely, activating tp causes an over-correction for small perturbations. However, wing stroke angle response to pitch perturbation remains intact compared to the control group fruit flies. This observation hints at the necessity of going beyond a simple PI model to understand the correction dynamics fully. A more thorough analysis of control fruit flies' data revealed that a fruit fly utilizes an additional degree of freedom - wing rotation angle- when the perturbation strength is above a certain threshold, implying an additional correction mode. Via quasi-steady aerodynamic calculations, we showed that the wing rotation generates additional drag-based corrective torque to assist a significantly perturbed fruit fly in regaining its original stable orientation. These new insights allowed us to find a relationship between tp muscle activity and wing rotation angle, consistent with the fly's correction performance. Our ongoing endeavor involves extending the current control theory framework to better model fruit flies' reflex response toward pitch

instability. Our work provides additional insight into the neuromuscular underpinning of flight control.

Disclosures: H. Teoh: None. **S.C. Whitehead:** None. **K. Ludlow:** None. **E. Ehrhardt:** None. **G.M. Card:** None. **W. Korff:** None. **D. Stern:** None. **M.H. Dickinson:** None. **I. Cohen:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.07/LL19

Topic: F.01. Neuroethology

Support: NIH Grant UF1NS115817

Title: Circuitry and molecular composition of the octopus axial nerve cord

Authors: *C. S. OLSON[1], C. W. RAGSDALE[2];
[1]Univ. of Chicago Committee On Computat. Neurosci. ., Chicago, IL; [2]Univ. of Chicago Committee On Neurobio., Chicago, IL

Abstract: Octopuses have the remarkable ability to move their arms with near infinite degrees of freedom. Mediating movement is a massive axial nerve cord (ANC), equivalent to a spinal cord, running down the center of all eight arms. The ANC can be subdivided into territories. Aborally, that is, more distant from the sucker, lies the cerebrobrachial tract (CBT), which connects the arms and the brain. Oral to the CBT, there is a U-shaped cell body layer (CBL) surrounding neuropil (NP). Beyond this subdivision, little is known about the composition of ANC. We investigated the molecular identity of cells in the ANC of Octopus bimaculoides with in situ hybridization for neuronal subtype markers. We found positive expression in the CBL for cholinergic (*VACHT, CHAT, SLC5A7*), GABAergic (*GAD, VIAAT*), motor (*NKX6, LHX3, MNX*), and sensory (*PIEZO, DRGX*) neuron markers. Some of these markers (*VACHT, CHAT, SLC5A7, PIEZO*) were broadly expressed, whereas others showed a more restricted districting (*NKX6, MNX, DRGX, LHX3, GAD, VIAAT*). Those demonstrating restricted labeling either formed clusters (*LHX3, MNX*) or were limited to distinct CBL territories (*NKX6, DRGX, GAD, VIAAT*). To uncover circuit connections, we developed a slice culture method for neuronal tracing with fluorescently labeled dextrans. Amputated arms from five animals were cut into 0.5-1cm slices, and the ANC of each explant (n=36) was injected with dextrans. Injections into central NP provided strong labeling of the cell bodies directly lateral to the injection site, with limited transport orally or aborally. Deposits on one side of the NP demonstrated strong ipsilateral labeling in the CBL, with limited contralateral connections. Combined with gene expression experiments, these results establish that there are multiple subdivisions within the ANC, and provide a circuit framework for understanding how arm sensorimotor information is processed and integrated.

Disclosures: **C.S. Olson:** None. **C.W. Ragsdale:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.08/LL20

Topic: F.01. Neuroethology

Support: ONR MURI Grant N00014-19-1-2373

Title: Modeling Octopus Arm Coordination with Recurrent Inhibition and Serial CPG Circuitry

Authors: *E. GRIBKOVA[1,2,3], R. GILLETTE[1,2,4];
[2]Neurosci. Program, [3]Coordinated Sci. Lab., [4]Dept. of Mol. and Integrative Physiol., [1]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: We have developed a simple interactive arm model with a lateral inhibitory network for sucker circuitry and a serially CPG-based network for inter-segmental connections. Octopus' arms show autonomous sensorimotor processing like the vertebrate spinal cord; however, the underlying circuitry is less well-known. Notably, isolated arms show much original behavior, like precise orienting of suckers to chemotactile stimuli, grasping and recruitment of neighboring suckers, bending of the arm, and still more complex movements [1].
Precise sucker orienting to stimuli suggests that neuronal circuitry uses lateral inhibition, crucial in most sensory systems for contrast enhancement, edge detection, and sensory discrimination. Further, coordinated movements in isolated arms suggest that motor programs are intrinsic to arm circuitry itself with descending modulation from CNS, much like vertebrate spinal cord [2]. Our model estimates averaged source direction location of single and multiple chemical stimuli and performs coordinated arm movements.
The sensory network model for the sucker is inspired by putative lateral inhibition for chemotactile processing in the sea slug *Pleurobranchaea*'s oral veil [3]. The sucker network has three circular layers of leaky integrate-and-fire (LIF) neurons in a winner-take-all network, promoting stimulus discrimination and contrast enhancement [4-5]. The population activity of the innermost motorneuron layer is used to estimate the most salient stimulus source location and to guide sucker movement along a chemical gradient. Notably, the network is configurable as non-spiking for similar processing.
The arm's inter-segmental network is modeled on the lamprey spinal cord [6], with simple segmental CPGs. Inter-segmental connections provide local ipsilateral excitation and more global contralateral inhibition across segments. In each CPG, a modified version of extended generalized LIF (E-GLIF) neurons produce burst dynamics with spike frequency adaptation and post-inhibitory rebound [7]. This architecture simulates undulatory motor behavior, as in lamprey, and more complex movements of octopus' arm.

References:

[1] Fouke KE, Rhodes HJ. (2020). *Biol Bull. 238*(1), 1-11.

[2] Grillner S, El Manira A. (2019). *Physiol Rev*.
[3] Yafremava LS, Gillette R. (2011). *J Neurophysiol 105*(6), 2885-2890.
[4] Gardner EP, Martin JH. (2000). *Prin Neural Sci*, *4*, 411-429.
[5] Glackin C. et al. (2011, July). In *The 2011 Internat Joint Conf Neural Networks* (pp. 1003-1009). IEEE.
[6] Grillner S. et al. (1995). *Trends Neurosci*, *18*(6), 270-279.
[7] Geminiani A et al. (2018). *Front Neuroinformatics*, *12*, 88.

Disclosures: E. Gribkova: None. **R. Gillette:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.09/LL21

Topic: F.01. Neuroethology

Support: NSF DBI2015317
NSF Grant No. DGE174501

Title: Neuromechanical model of feeding in Aplysia californica

Authors: *M. J. BENNINGTON[1], A. S. LIAO[2], R. SUKHNANDAN[2], B. KUNDU[6], S. M. ROGERS[6], J. P. GILL[7], G. P. SUTTON[6], H. J. CHIEL[7,8,9], V. A. WEBSTER-WOOD[3,4,5];
[2]Dept. of Mechanical Engin., [3]Mechanical Engin., [4]Dept. of Biomed. Engin., [5]McGowan Inst. for Regenerative Med., [1]Carnegie Mellon Univ., Pittsburgh, PA; [6]Sch. of Life Sci., Univ. of Lincoln, Lincoln, United Kingdom; [7]Dept. of Biol., [8]Dept. of Neurosciences, [9]Dept. of Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Understanding how multifunctional behaviors arise in animal systems requires knowledge of both neural circuits and how they interact with the animal's musculostructural periphery. However, these circuits are often studied in isolation, removing crucial proprioceptive feedback pathways. Elucidating the interactions between the nervous system and the periphery requires constructing biomechanical models of the animal’s body with sufficient detail to capture the behaviorally relevant degrees of freedom and dynamics. However, increases in biological accuracy need to be balanced with the increased computational resources required. One model system for investigating multifunctionality and required model complexity is the feeding apparatus of the marine mollusk *Aplysia californica*. Previous works have modeled this system at the component [2], sub-system [3,4], and system level [5,6,7]. While these models can provide neuromechanical insights and qualitatively match animal data, sub-system models cannot test hypotheses about full-system behaviors. Furthermore, current system-level models often lack behaviorally relevant degrees of freedom and have not reported quantitative comparisons with animal data.
This work introduces a new, system-level neuromechanical model of the feeding apparatus.

Anatomical elements abstracted in previous models [5-7] were replaced with known anatomical features, and additional behaviorally-relevant degrees of freedom were introduced. Neuron elements and phenomenological connections were added to a previously developed Boolean neural model [5,7] to control the biomechanical model. The parameters of this new system model were hand-tuned first to qualitatively reproduce the three key behaviors of biting, swallowing, and rejection and then to match behavioral data. Quantitative analysis showed that the model could reproduce many key behavioral kinematic and dynamic parameters satisfactorily. A timing analysis revealed that despite the increased biomechanical complexity, the model could still run multiple times faster than real-time, on par with previous models [5]. The model showed significant disagreement with animal data in the magnitude of grasper translation, revealing where additional complexity is required in the model. The results from this model will be used to inform the creation of future robotic and computational models of the feeding apparatus that can then be used to generate and test novel neuromechanical hypotheses.
References: [1] Chiel and Beer, 1997 [2] Yu et al., 1999 [3] Sutton et al., 2004 [4] Novakovic et al., 2006 [5] Webster-Wood et al., 2020 [6] Liao et al., 2021 [7] Dai et al., 2022

Disclosures: M.J. Bennington: None. **A.S. Liao:** None. **R. Sukhnandan:** None. **B. Kundu:** None. **S.M. Rogers:** None. **J.P. Gill:** None. **G.P. Sutton:** None. **H.J. Chiel:** None. **V.A. Webster-Wood:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.10/LL22

Topic: F.01. Neuroethology

Support: MRC(UK) Grant Number (MR/T046619/1), part of the NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program
NSF DBI 2015317 as part of the NSF/CIHR/DFG/FRQ/UKRI-MRC Next Generation Networks for Neuroscience Program.
Royal Society, UK UF130507

Title: The scaling of muscle activation time constants is the primary determinant of the duration of movements in a quasi-static system: scaling of feeding behavior cycles in the marine mollusc Aplysia californica

Authors: *S. M. ROGERS[1], J. P. GILL[2], A. SKALSKI DE CAMPOS[2], K. WANG[2], I. KAZA[2], V. FAN[2], H. J. CHIEL[2], G. P. SUTTON[1];
[1]Univ. of Lincoln, Lincoln, United Kingdom; [2]Case Western Res. Univ., University Heights, OH

Abstract: The time it takes to perform a behavior is governed by the time constants of muscle activation and the mechanical forces experienced during movements. Slow movements by small

animals are dominated by elastic forces and are thus quasi-static (i.e., always near mechanical equilibrium). Muscular forces and elastic forces resisting movement both scale identically, proportional to mass$^{2/3}$, and therefore the duration of behavior should depend entirely on the time constant of muscle activation. We measured the duration of feeding behaviors in the marine mollusc *Aplysia californica* across changes in body size spanning three orders of magnitude to test this hypothesis. The time taken for muscles to produce maximum force as animals attempted to feed on tethered inedible seaweed was used as an *in vivo* approximation of the muscle force exerted during isometric contraction, which is necessary to measure the dynamics of muscle activation. The timing of muscle activation scaled with mass$^{0.3}$. The total duration of biting behaviors, incorporating both retraction and retraction, showed an identical scaling with mass$^{0.30}$, indicating a lack of additional mechanical effects. The duration of swallowing behavior, in which the animals worked to ingest a seaweed strip, however, exhibited a shallower scaling of mass$^{0.17}$. The shallower scaling is possibly because the anterior retractor muscle undergoes allometric growth during development, which we measured using micro computed tomography scans (micro-CT) of buccal masses of different sizes. We suggest that the muscles of larger animals did not need to activate as fully as those in smaller animals to produce equivalent forces during swallowing. These results indicate that the time constants of muscle activation may be the most important determinant of the scaling of behavioral durations in quasi-static systems.

Disclosures: **S.M. Rogers:** None. **J.P. Gill:** None. **A. Skalski De Campos:** None. **K. Wang:** None. **I. Kaza:** None. **V. Fan:** None. **H.J. Chiel:** None. **G.P. Sutton:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.11/LL23

Topic: F.01. Neuroethology

Support: NIH grant U01MH114812
NIH grant U19MH114830
NIH grant P51OD010425
NIH grant OD010938-40
NIH grant D024628-02
NIH grant NS092988
NIH grant HG011641
NSF grant EF-2021785
Dutch Research Council (NWO) Gravitation Program BRAINSCAPES 024.004.012
Dutch Research Council (NWO) Applied and Engineering Sciences (AES) grant 3DOMICS 17126
UK Dementia Research Institute

Title: Transcriptomic and epigenomic data for 26 species provide insight into the evolution of mammalian motor cortex

Authors: *M. WIRTHLIN[1], N. JOHANSEN[1], N. JORSTAD[2], A. YANNY[1], A. A. DE SOUSA[1], D. BERTAGNOLLI[1], A. B. CHAKKA[1], R. CHAKRABARTY[1], S.-L. DING[1], R. FERRER[1], J. GOLDY[1], N. GUILFORD[1], J. GUZMAN[1], V. OMSTEAD[1], T. PHAM[1], C. RIMORIN[1], S. C. SEEMAN[1], N. TASKIN[1], M. TIEU[1], A. TORKELSON[1], N. J. WEED[1], K. UNGERLIDEN[3], G. BALMUS[3], T. BARRETT[4], D. C. BOLSER[5], K. L. DREW[6], D. FITZPATRICK[7], S. M. FREEMAN[8], A. C. HALLEY[9], G. D. HORWITZ[10];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Genentech, Inc., South San Francisco, CA; [3]UK Dementia Res. Inst. at Univ. of Cambridge, Dept. of Clin. Neurosciences, Univ. of Cambridge, Cambridge, United Kingdom; [4]Lovelace Biomed., Albuquerque, NM; [5]Physiological Sci., Univ. of Florida, Gainesville, FL; [6]Inst. Arctic Biol, Univ. Alaska, Fairbanks, AK; [7]Max Planck Florida Inst., Jupiter, FL; [8]Biol., Utah State Univ., Logan, UT; [9]Ctr. for Neurosci., Univ. of California Davis, Davis, CA; [10]Physiol. and Biophysics, Univ. of Washington, Seattle, WA

Abstract: Understanding how mammalian brains have evolved to execute diverse motor behaviors is a fundamental question in neuroscience. The answer to this question depends on identifying the genetic and molecular processes that have guided the evolution of the complex neural architecture underlying these behaviors. However, exploring this problem is challenging due to the limited availability of transcriptomic and epigenomic resources beyond traditional model species.

To address this gap, we conducted 10X single nucleus RNA-seq of mammalian motor cortex (M1) across 26 species, encompassing 13 primates and 4 rodents, as well as diverse taxa including scandentians (northern treeshrew), lagomorphs (European rabbit), chiropterans (Egyptian fruit bat), carnivorans (cat, coyote, and ferret), artiodactyls (pig), cingulates (nine-banded armadillo), and marsupials (Virginia opossum). Additionally, we performed single nucleus ATAC-seq for 18 of these species and utilized PacBio IsoSeq to enhance transcriptomic annotations for six targeted species with new reference genomes. Analyzing over 1.5 million sequenced cells, we generated a consensus M1 cell type taxonomy, providing a foundation for investigating the genomic evolution of mammalian brains.

Our analyses revealed lineage-specific trends in cellular composition and cytoarchitectonics of M1 across species. Notably, we discovered that while a 2:1 excitatory-to-inhibitory neuron ratio for M1 was previously considered to be a primate-specific trait, this ratio can be found among other mammals as well, while the 5:1 ratio found in murine rodents is particularly high. We also identified conserved and divergent patterns of gene expression, including primate lineage- and human-specific features. This included subclass-level markers across species, as well as transcription factors driving changes in gene regulatory networks within M1 cell types. In addition, we developed and tested a viral tool highly specific for layer 5 extra-telencephalic projection neurons, demonstrating conserved activity across mammals. Finally, we investigated the convergent evolution of cell type-specific molecular features related to trait differences, focusing on long-range projection neuron types in M1 layer 5, such as Betz cells. We anticipate that this multi-species, multi-omic dataset will provide a valuable resource for further investigations into the molecular basis of trait evolution and are eager to collaborate with a wide range of researchers from genomics, cell biology, neuroscientific, and ethological perspectives who are interested in expanding the diversity of species included in single cell genomics.

Disclosures: **M. Wirthlin:** None. **N. Johansen:** None. **N. Jorstad:** A. Employment/Salary (full or part-time):; Genentech, Inc., South San Francisco, CA, USA. **A. Yanny:** None. **A.A. de Sousa:** None. **D. Bertagnolli:** None. **A.B. Chakka:** None. **R. Chakrabarty:** None. **S. Ding:** None. **R. Ferrer:** None. **J. Goldy:** None. **N. Guilford:** None. **J. Guzman:** None. **V. Omstead:** None. **T. Pham:** None. **C. Rimorin:** None. **S.C. Seeman:** None. **N. Taskin:** None. **M. Tieu:** None. **A. Torkelson:** None. **N.J. Weed:** None. **K. Ungerliden:** None. **G. Balmus:** None. **T. Barrett:** A. Employment/Salary (full or part-time):; Lovelace Biomedical; Albuquerque, NM. **D.C. Bolser:** None. **K.L. Drew:** None. **D. Fitzpatrick:** None. **S.M. Freeman:** None. **A.C. Halley:** None. **G.D. Horwitz:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.12/LL24

Topic: F.01. Neuroethology

Title: Stereotyped spontaneous eyeblink timing patterns during sports driving are not limited to elite drivers

Authors: *R. NISHIZONO, N. SAIJO, M. KASHINO;
NTT Communication Sci. Labs., Atsugi, Japan

Abstract: A previous study (Nishizono et al., 2023) revealed that elite racecar drivers blink at similar positions on the race circuit during sport driving a real Formula car. The clear pattern was associated with car acceleration and presumably corresponding to the task demand. Hoppe et al. 2018 found that humans learn to blink in response to environmental demands during the visual detection task. This raises the question on whether the eyeblink patterns during sports driving by elite drivers are formed through learning and a sign of expertise and the pattern may result from proficiency through long-term training of the sport or detailed memory of the courses. If the contribution of expertise on eyeblink timing modulation is large, non-expert drivers would not exhibit any clear eyeblink patterns, or even if it exists, the patterns would not be reasonable regarding the task performance. To examine the effect of expertise on eyeblink timing, we observed three male non-expert drivers' (age range: 38-47 years) blinking and driving behavior using a race-driving simulator in this study. They held valid driving licenses but were not accustomed to sports driving both in real and virtual environments. The simulator simulated real-world physics, giving visual, sound, and force feedback from the steering wheel but not haptic or motion feedback from the seat. We tasked them with free practice sessions similar to the previous study, repeating a few laps with a few minute breaks between targeting shorter lap completion times as long as they did not crash. We simultaneously recorded eye videos and car control behavior, and eyeblink timings were determined through image processing. We examined whether they exhibited eyeblink signatures analogous to sports driving by expert drivers, including reproducibility of eyeblink locations within the driver and course combinations among multiple laps and modulation of eyeblink generation probability based on car

acceleration. The results revealed that they blinked at significantly similar positions for all drivers and course combinations. Furthermore, the relationship between eyeblink timings and vehicle acceleration adhered to the expected pattern regarding the effect of suppressing or facilitating eyeblink generation. These findings, which align with previous research conducted in real-world driving scenarios by elite drivers, revealed that expertise is not necessary for eyeblink pattern formation in a functional way in sports driving.

Disclosures: R. Nishizono: None. **N. Saijo:** None. **M. Kashino:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.13/LL25

Topic: F.01. Neuroethology

Support: ERC Synergy Grant 'BrainPlay - the self-teaching brain'
Humboldt-Universität zu Berlin

Title: Showering, hose tool use and hose clamping in Asian elephants

Authors: *L. URBAN[1,2], L. V. KAUFMANN[3,5], R. BECKER[6], A. OCHS[6], M. BRECHT[3,4]; [1]Bernstein Ctr. For Computat. Neuroscinece Berlin, Berlin, Germany; [3]Bernstein Ctr. for Computat. Neurosci., [4]NeuroCure Cluster of Excellence, [2]Humboldt-Universität zu Berlin, Berlin, Germany; [5]Sch. of Mind and Brain, Berlin, Germany; [6]Zoologischer Garten Berlin, Berlin, Germany

Abstract: Animal tool use is widespread, but many animal tools are rather simple. Here, we investigate the use of a water hose as a tool by an Asian elephant at the Berlin Zoo. Hoses are relatively complex tools, because of their length, their flexibility and the water flow through the hose. Elephants love water, their trunk can spray water like a hose and they rely on water not just for drinking but also for body care. At the Berlin Zoo one elephant named Mary showers herself with a water hose. This behavior is not seen in the other four elephants at the zoo (Cows: Pang Pha, Anchali, Carla, Bull: Victor). We found that Mary grasps the water hose differently depending on which part of her body she is showering. The hose grasping behavior is lateralized and on average she grasps about 15 cm further back from the tip when she showers her right side (54 cm) compared to showering her left side (40 cm). Most interestingly, she grasps the hose even further back (62 cm) and uses the water hose as an extension, when showering her back. Mary stops showering when we turn off the water flow. In ongoing experiments, we offer her different kinds of water hoses that differ in diameter and stiffness. By coincidence we found that another elephant, the youngest one, Anchali, sometimes clamps the hose when Mary showers. Anchali shows a complex kink and clamp behavior, which can disrupt water flow. Specifically, she lifts the hose, kinks it, changes her grip on the kinked part and squeezes the kink. We have also seen Anchali perform a ‘trunkstand’ on the water hose and this can also lead to a disruption

of water flow. We are trying to find out whether these behaviors are performed with the purpose of stopping the water flow through the hose.

Disclosures: L. Urban: None. **L.V. Kaufmann:** None. **R. Becker:** None. **A. Ochs:** None. **M. Brecht:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.14/LL26

Topic: F.01. Neuroethology

Support: NIH P01HD064653

Title: Experience with tool use modulates visuomotor neuronal response to tool action observation in monkey primary and premotor cortex

Authors: G. COUDE[1], S. KIRCHHERR[1], *P. FERRARI[1], M. BIMBI[1], J. BALDI[2], G. CAPPELLARO[1], J. BONAIUTO[1], **P. F. FERRARI**[1];
[1]CNRS, Bron, France; [2]Inst. of Cognitive Sci. Marc Jeannerod, Lyon, France

Abstract: Previous studies have shown that neurons in ventral premotor cortex display plastic changes in their firing as a consequence of visual experience. In particular, neurophysiological experiments on tool use in macaque monkeys have shown that visual exposure to tools and sensorimotor training aimed at using tools are linked with changes in the visual responses of neurons in premotor and parietal cortical regions. However, it is not clear to what extent these changes are a consequence of visual exposure or motor learning. In this study, we assessed the changes in the visual discharge of primary and premotor neurons following visual exposure to actions made with a tool, and following a specific sensorimotor training where the monkey gradually learned how to use the same tool. To achieve this aim, free floating microelectrode arrays were chronically implanted under the dura in different frontal brain regions of two macaque monkeys. Three arrays of a total of 96 electrodes were implanted in the ventral premotor and primary cortices. The neural activity was recorded daily for several weeks during a task where the monkey had to observe an experimenter using a rake to retrieve an object. In the first four weeks, the monkeys had to observe the experimenter using the rake but never experienced using it themselves. Later, in separate sessions, the monkeys were trained to use the rake. Here we compared the neural activity recorded in 10 sessions before and after training (i.e. when the monkeys themselves could use the rake successfully 75% of the trials). The results show systematic changes in neuronal visual activity after having learned to use the rake. Interestingly, in both monkeys, the activity in at least 80% of the channels in the ventral premotor cortex became more tuned to the observation of tool actions. These preliminary findings suggest that motor experience, as opposed to mere visual familiarization, plays an

important role in scaffolding the neuronal changes necessary to form new motor representations that can be activated during action observation.

Disclosures: G. Coude: None. **S. Kirchherr:** None. **P. Ferrari:** None. **M. Bimbi:** None. **J. Baldi:** None. **G. Cappellaro:** None. **J. Bonaiuto:** None. **P.F. Ferrari:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.15/LL27

Topic: F.01. Neuroethology

Support: NIH R01NS121220

Title: A cryptic extension of the Tritonia escape swim motor program rhythm is maintained for several minutes in newly identified cerebral ganglion neurons.

Authors: *E. HILL, J. WANG, W. N. FROST;
Ctr. for Brain Function and Repair, Rosalind Franklin Univ., North Chicago, IL

Abstract: The neural network that produces the stimulus-elicited escape swim of the marine mollusk *Tritonia diomedea* consists of a well-described set of neurons that includes afferent, trigger- and gating-type command, central pattern generator, and efferent flexion neurons. As such it represents one of the better-understood behavioral networks in neuroethology. The swim motor program, consisting of alternating dorsal and ventral phases, is typically 4 - 7 cycles in duration. Recently, optical recording with a VSD revealed that many pedal ganglion neurons participate in this motor program in a variable manner - they join the rhythm late, leave it early, or fire on or skip internal cycles only. Furthermore, many neurons change their level of participation from trial-to-trial, revealing the presence of a surprisingly dynamic process controlling who's in and out of the active network. The CPG interneurons all make monosynaptic connections onto the pedal flexion neurons. However, since the CPG neurons fire reliably on all swim cycles, the mechanisms governing the varying allocation of downstream neurons remains an open question. Here we used sharp electrodes to extend our knowledge of the cerebropleural ganglion neurons that may play a role in the above dynamic allocation of neurons to the swim motor program. First, we encountered several new dynamically-participating neurons and documented their positions with dye-injections and/or photographs of electrode positions, facilitating their re-identification in subsequent preparations. Next we found that many of these new neurons receive input from the swim CPG neurons (C2, DSI and VSI), with some making functional connections back to the CPG and also onto the flexion neurons in the pedal ganglion. Some connections were multicomponent, with a fast depolarization followed by slow inhibition, or vice versa, which seem well suited to explain the firing profiles of some classes of dynamically participating neurons (e.g. join late or leave early). Observed experience-dependent plasticity at some of these synapses seems appropriate to explain some of the changes in

neuronal allocation observed in our prior studies of learning. Finally, one or more neurons in the dorsal cerebral ganglion had an unexpected property: in many instances they continued to display rhythmic membrane potential oscillations with the period of the motor program that typically continued for several minutes after the motor program had ceased. One intriguing possibility is that such neurons may maintain a running template of the motor program disconnected from the behavior, keeping the animal in a swim-readiness state in case of further attack.

Disclosures: **E. Hill:** None. **J. Wang:** None. **W.N. Frost:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.16/LL28

Topic: F.01. Neuroethology

Support: MEXT KAKENHI Grant Number 21K06259
MEXT KAKENHI Grant Number 21H05295
JST SPRING Grant Number JPMJSP2119

Title: Identification of brain neurons correlated with wind-elicited escape movements in crickets

Authors: *H. CHIDA[1], H. OGAWA[2];
[1]Biosystem Sci, Grad Sch. Life Sci, Hokkaido Univ., Sapporo, Japan; [2]Dept. of Biol. Sciences, Fac. of Sci., Hokkaido Univ., Sapporo, Japan

Abstract: Animals compute the location of a stimulus source from sensory inputs and exhibit a goal-oriented behavior based on that spatial information. However, the neural circuits linking the sensory and motor pathways to control the oriented movements remain unclear. To explore the whole picture of sensory-motor association in the goal-oriented behavior, we used the wind-elicited escape behavior of crickets, in which crickets move precisely in the opposite direction to the airflow stimulus. Crickets detect airflow in their surroundings with abdominal mechanosensory organs called cerci. Sensory information, such as direction and speed of airflow, is processed by a local circuit in the terminal abdominal ganglion and conveyed to the brain by ascending projection neurons. Previous studies revealed that descending signals from the brain to the thoracic ganglia are crucial for the directional control in the escape behavior, suggesting that the neural circuits to process the airflow information and to direct the escape movements are located within the brain. To investigate the neural circuit underlying this oriented escape behavior, we simultaneously recorded the activity of brain neurons and the escape movements in the head-fixed cricket using a spherical treadmill system. A glass-microelectrode was inserted into the lateral accessory lobe (LAL), which is considered an insect brain region involved in sensory integration and the motor control of the locomotion. We have identified three brain neurons, including two local interneurons and one descending interneurons, that were

correlated with the escape behavior. For one of the local interneurons, its firing activity was greatly suppressed 66 ms before the escape behavior was initiated in response to airflow. In contrast, when the cricket did not escape, the inhibitory responses of this neuron was weaker, and its latency was longer. The other local interneuron showed transient excitatory responses only when crickets did not escape. These results suggest that these local interneurons are involved in decision of whether the crickets escape or not. In addition, we found the descending neuron that extended its axon to thoracic ganglia and exhibited either increase or decrease of firing activity depending on the turning direction in the escape movements. It is likely that this neuron provides the descending commands for the escape direction to the thoracic ganglia. The neural circuits consisting of local interneurons would determine the content of the escape behavior, including whether to respond or not, and based on that determination the descending signals would be generated in LAL to control the oriented movement.

Disclosures: H. Chida: None. **H. Ogawa:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.17/MM1

Topic: F.01. Neuroethology

Support: NIH Grant U19NS104653
NIH Grant 1R01NS124017
NSF Grant IIS-1912293
Simons Foundation SCGB 542943SPI

Title: Neural mechanisms of behavioral variability and strategy selection in larval zebrafish

Authors: *V. WANG[1], A. CHEN[2], M. DUQUE RAMIREZ[3], K. HERRERA[4], F. ENGERT[5]; [1]Harvard, Cambridge, MA; [2]Janelia Res. Campus, Ashburn, VA; [3]Mol. Cell Biol., [4]Harvard Univ., Cambridge, MA; [5]Harvard Univ., Cambridge.

Abstract: Optimal motor strategies depend on context and require exploration of an organism's motor repertoire. Using larval zebrafish, we will identify the neural mechanisms of behavioral variability and strategy selection. Head-fixed, tail-free larval zebrafish exhibit a robust optomotor response to drifting gratings. In closed loop experiments, where swim bouts result in the expected visual flow, fish generate stereotyped, 1 Hz bouts of forward swimming. In open loop experiments, where tail flicks do not affect the movement of the grating, fish exhibit increased variability in their swim bouts. We will use functional whole-brain imaging during this behavioral assay to characterize the circuits responsible for exploration of motor strategies and to identify brain-wide dynamics that encode this exploration state. We will perturb elements of this circuit to manipulate the level of behavioral variability. This model will allow us to understand how the brain injects stochasticity into an innate sensorimotor transformation.

Disclosures: **V. Wang:** None. **A. Chen:** None. **M. Duque Ramirez:** None. **K. Herrera:** None. **F. Engert:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.18/MM2

Topic: F.01. Neuroethology

Support: NIH Grant R35GM124735
NIH T32 grant for UCSF
Financial support by family

Title: Dynamics of the escape response in C. elegans revealed by calcium imaging through two-photon Bessel illumination

Authors: *M. PATEL;
previously, UCSF, San Francisco, CA

Abstract: Escaping imminent life-threatening danger is innate to all organisms. To build towards obtaining a comprehensive understanding of how the brain quickly transforms inputs to outputs, we performed high-speed calcium imaging of majority of *C. elegans* neurons paired with optogenetic stimuli via *psra-6*::ReaChR that mimic the escape response in paralyzed preparation. With the advent of two-photon Bessel illumination that has imaging speeds of 60-100 Hz for 80-100 neurons per plane, we observed sequential pulse responses in motor neurons and interneurons that significantly increased probability of neural correlates of movement states by which the nematode can steer away from danger.

Disclosures: **M. Patel:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.19/MM3

Topic: F.01. Neuroethology

Support: MSIT Grant 2019R1A2C100255514
KIOM Grant KSN1812181

MAFRA Grant 322096-051SB010
MSIT Grant K2022K2A9A2A0601867911

Title: Activation of a hypothalamus-habenula circuit by mechanical stimulation inhibits cocaine addiction-like behaviors

Authors: *H. KIM[1], H. JANG[2], D. AHN[2], H. KIM[3], B. LEE[4];
[1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Yonsei university, seoul, Korea, Republic of; [3]Physiol., Kyungpook Natl. Univ., Seoul, Korea, Republic of; [4]Dept of Physiol., Dept of Physiol., Seoul, Korea, Republic of

Abstract: Mechanoreceptor activation modulates GABA neuron firing and dopamine (DA) release in the mesolimbic DA system, an area implicated in reward and substance abuse. The lateral habenula (LHb), the lateral hypothalamus (LH), and the mesolimbic DA system are not only reciprocally connected, but also involved in drug reward. We explored the effects of mechanical stimulation (MS) on cocaine addiction-like behaviors and the role of the LH-LHb circuit in the MS effects. MS was performed over ulnar nerve and the effects were evaluated by using drug seeking behaviors, optogenetics, chemogenetics, electrophysiology and immunohistochemistry. Mechanical stimulation attenuated locomotor activity in a nerve-dependent manner and 50-kHz ultrasonic vocalizations (USVs) and DA release in nucleus accumbens (NAc) following cocaine injection. The MS effects were ablated by electrolytic lesion or optogenetic inhibition of LHb. Optogenetic activation of LHb suppressed cocaine-enhanced 50 kHz USVs and locomotion. MS reversed cocaine suppression of neuronal activity of LHb. MS also inhibited cocaine-primed reinstatement of drug-seeking behavior, which was blocked by chemogenetic inhibition of an LH-LHb circuit. These findings suggest that peripheral mechanical stimulation activates LH-LHb pathways to attenuate cocaine-induced psychomotor responses and seeking behaviors.

Disclosures: **H. Kim:** None. **H. Jang:** None. **D. Ahn:** None. **H. Kim:** None. **B. Lee:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.20/MM4

Topic: F.01. Neuroethology

Support: NIH grant 1R15NS125565-01
State of New Jersey Commission on Spinal Cord Research grant CSCR14ERG002

Title: Descending input determines the direction of motor output propagation in a distributed-pacemaker model of the C. elegans locomotion circuit

Authors: H. ANWAR[1], S. SAGHAFI[1], L. DENG[1], J. DENHAM[2], N. COHEN[3], C. O. DIEKMAN[1], ***G. HASPEL**[1];
[1]New Jersey Inst. of Technol., Newark, NJ; [2]Univ. of Leeds, Leeds, United Kingdom; [3]Univ. Leeds, Univ. Leeds, Leeds, United Kingdom

Abstract: A neuronal network might generate a repertoire of motor patterns that are determined by neuromodulation and descending input. Most motor circuits include neural oscillators, yet oscillating activity needs to be shaped into the different coordinated motor outputs. The locomotion circuit of *Caenorhabditis elegans* generates distinct forward and backward motor patterns that correlate to specific input from five pairs of premotor interneurons. It is a useful model to study the selection and generation of motor patterns because undulatory locomotion is compactly described as a propagating alternation of muscular activity, the circuit is relatively small, and synaptic connectivity data is available.
We developed an ordinary differential equation model of the locomotion circuit encompassing all 7 motoneuron classes with neuroanatomically-grounded synaptic connections. We populated the network with two kinds of nodes: passive (non-oscillatory motoneurons and muscle cells) and endogenously oscillating motoneurons. We systematically screened all 128 configurations of oscillator and passive motoneuron classes. For each configuration, we varied synaptic parameters and tested whether the network produces propagating dorsoventral alternation of muscular activity in forward or backward directions when relevant descending input was applied.
73 configurations with 1-7 oscillating motoneuron classes generated patterns that propagated either forward or backward patterns, but only 31 configurations with 3-6 oscillating classes generated both (with the same parameter values). After parameter optimization, we used a neuromechanical model to verify coordinated outputs, and synaptic ablations to explore successful solutions. We found which pacemaker-distribution configurations generate bidirectional locomotion motor patterns along a wider range of synaptic parameters. For those configurations, we discovered how direction is determined by descending input that either shifts the duty cycle or changes the amplitude of specific motoneuron classes.
Our results demonstrate the conditions in which a distributed-pacemaker network architecture could underlie the generation of multiple motor outputs and how descending input can select among them.

Disclosures: H. Anwar: None. **S. Saghafi:** None. **L. Deng:** None. **J. Denham:** None. **N. Cohen:** None. **C.O. Diekman:** None. **G. Haspel:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.21/MM5

Topic: E.09. Motor Neurons and Muscle

Support: Grant PROSNI-UdeG 2023 To GCH

Title: Behavioral impact of β-caryophyllene on GABAA-dependent locomotion in Caenorhabditis elegans

Authors: *G. CAMARGO HERNÁNDEZ[1], S. SANCHEZ ENRIQUEZ[2], D. W. AGUILAR OCAMPO[5], A. S. BARBA PERALTA[3], M. MACIAS CARBALLO[2], A. PEREZ LARIOS[4], A. CASTILLO ROMERO[6], J. C. ROLON DIAZ[2], E. A. RIVERA LEON[1], L. HERNANDEZ[7]; [1]Ciencias de la Salud, [2]Dept. de Clínicas, [3]Licenciatura en Cirujano Dentista, [4]Dept. de Ingenierías, CUAltos UNIVERSIDAD DE GUADALAJARA, Guadalajara, Mexico; [5]Maestría en Microbiología Médica, [6]Dept. de Microbiología y Patología, [7]CUCS-Universidad de Guadalajara, Guadalajara, Mexico

Abstract: The occurrence of reactive oxygen species (ROS) is a normal feature of aerobic life. However, environmental contaminants, such as sunlight (i.e., UV exposure), smoke, ozone, herbicides, etc., are additional sources of ROS as well. Although these compounds have an important role in several biological systems, their excessive production could result in oxidative stress (OS) which is an imbalance between pro-oxidant and antioxidant compounds, always in favor of the former. OS is associated with a vast series of pathologies which have a subtle beginning, become chronic, and end by being incapacitating and extremely costly, such as premature aging, atherosclerosis, chronic degenerative diseases (Parkinson's and Alzheimer's disease, amyotrophic lateral sclerosis, diabetes), and cancer. The ability of the organism to cope with oxidative stress depends on a wide range of sophisticated stress response mechanisms through which cells and tissues adapt to, repair, and eliminate injured molecules. The *C. elegans* strain used in this study included the following: Bristol N2 (wild type), which were provided by the Caenorhabditis Genetics Center (Minneapolis, MN, USA). The aim of this study was: To evaluate the effect of β-caryophyllene, against oxidative damage in the whole animal and in the GABAergic nervous system in model organism *Caenorhabditis elegans.* For this purpose, we tested the effect of exposure to β-caryophyllene, and HS on the occurrence of a shrinking response (SR) after nose touch stimulus in N2 (WT) worms. Results: As expected, the CTL group responded normally to nose touch stimulus in 92.9± 2.1% of trials, a similar result to those formerly reported (Hart et al. 1999; Kaplan and Horvitz 1993). However, among CTL animals with a defective response, we could not observe shrinking after stimulus. Therefore, in CTL group likewise in β-caryophyllene group worms, SR was not considered to be present. Conclusions: Heat stress induces inactivation of GABAA receptor function in the model organism *C. elegans.* The slowing down of the sigmoid movement of the group exposed to *β-caryophyllene* is related to an Analgesia effect in the Model Organism *C. elegans.*

Disclosures: G. Camargo Hernández: None. **S. Sanchez enriquez:** None. **D.W. Aguilar ocampo:** None. **A.S. Barba peralta:** None. **M. Macias carballo:** None. **A. Perez larios:** None. **A. Castillo romero:** None. **J.C. Rolon diaz:** None. **E.A. Rivera leon:** None. **L. Hernandez:** None.

Poster

PSTR490. Neuroethology: Control of Motor Circuit

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR490.22/MM6

Topic: F.01. Neuroethology

Support: Canadian Institutes of Health Research (CIHR)
Natural Sciences and Engineering Research Council of Canada (NSERC)
to DM: fellowship by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – project number 442662585

Title: Assessing sensory processing alterations in the Cntnap2 knockout rat using startle and sound scaling of the acoustic startle response.

Authors: *A. EL-CHEIKH MOHAMAD, D. MÖHRLE, F. HADDAD, A. ROSE, B. L. ALLMAN, S. SCHMID;
Anat. & Cell Biology, Schulich Sch. of Med. & Dent., Univ. of Western Ontario, London, ON, Canada

Abstract: The acoustic startle response and prepulse inhibition of startle (PPI) are paradigms classically used in the assessment of sensory filtering and sensorimotor gating mechanisms. These highly translational paradigms are of particular interest to clinical and preclinical research as individuals with neurodevelopmental disorders often have alterations in sensory processing. However, classical PPI testing and data analysis methods are insufficient in fully evaluating changes to startle by a prepulse. Modifications to classical protocol have been suggested that allow for fitting of a startle response curve to a sigmoidal regression function; parameters extracted from this function can be used to examine prepulse-induced scaling of the baseline startle curve, as well as pathology related differences in startle. In the current study, adult male and female *Cntnap2* wildtype (WT; M = 15, F = 13) and knockout (KO; M = 14, F = 12) Sprague-Dawley rats were used to validate modifications to the testing paradigm and evaluate the scaling components involved in the increased startle and altered PPI of this model. Data was quantified in both the classic percent (%) PPI and advanced curve fitting methods. Consistent with previous studies, using classical analysis methods *Cntnap2* KOs were found to have increased baseline startle responses and disrupted PPI in comparison to *Cntanp2* WTs. Furthermore, for both genotypes, %PPI was not found to be dependent on baseline startle at stimulus intensities typically employed in PPI testing. Advanced analyses using the modified protocol indicate that startle scaling (a decrease in startle reactivity) and sound scaling (an attenuation of sound processing) are two independent processes, with both components present in *Cntanp2* WTs. In contrast, *Cntanp2* KOs appeared to have intact startle scaling and altered sound scaling. As startle scaling is likely related to motor output and sound scaling to sound processing, this modified approach may provide further information about possible causes for the disrupted sensorimotor gating of this model.

Disclosures: A. El-Cheikh Mohamad: None. **D. Möhrle:** None. **F. Haddad:** None. **A. Rose:** None. **B.L. Allman:** None. **S. Schmid:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.01/MM7

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIDA R01DA020140
Caroline Frederick Holdship Trust, PNC Charitable Foundation
NIH 1P50HD105328

Title: Genetics & the social brain: Requirement of transcription factor Foxp2 for neuronal circuit function and medial amygdala driven innate social behavior

Authors: *N. PRAKASH[1], A. SCHREIBER[1], L. WANG[2], S. SEBAOUI[1], M. J. HERRERO[3], S. FELSEN[1], N. CAMPBELL[1], L. ZWEIFEL[4], J. CORBIN[1];
[1]Children's Natl. Med. Ctr., Washington, DC; [2]Children's Natl. Hosp., Children's Natl. Hosp., Washington, DC; [3]Univ. San Sebastian, Santiago, Chile; [4]Univ. of Washington, Seattle, WA

Abstract: Innate social behaviors are crucial to survival and reproductive success. In rodents, pheromonal cues drive social behaviors via the accessory olfactory system, which includes the medial amygdala (MeA). Previously, we discovered that a major subpopulation, of MeA output neurons, is marked by *Foxp2*, a transcription factor that is expressed from E11.5 through adulthood. In other brain regions Foxp2 plays a critical role in circuit formation and circuit plasticity. To study the function *Foxp2* in MeA-driven behaviors and brain function, we examined the phenotype of whole-body and MeA-specific *Foxp2* loss of function in adults. *Foxp2*$^{+/-}$ whole-body mutant mice display sex-specific behavioral deficits including decreased male territorial aggression, increased maternal aggression, reduced maternal care and a female-specific social interaction deficit. Unaltered number and distribution of Foxp2+ neurons in the adult MeA in these mutants suggests that the behavioral changes likely result from disruptions in neuronal and/or circuit activity, as demonstrated by altered calcium activity in MeA slices. Together, our results to date reveal a critical role for *Foxp2* in social behaviors and MeA circuit function. Using CRISPR mutagenesis and conditional knockout approaches, we are currently exploring the consequences of MeA-specific loss of function on the formation and maintenance of MeA neuronal function, connectivity and role in innate social behaviors.

Disclosures: N. Prakash: None. **A. Schreiber:** None. **L. Wang:** None. **S. Sebaoui:** None. **M.J. Herrero:** None. **S. Felsen:** None. **N. Campbell:** None. **L. Zweifel:** None. **J. Corbin:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.02/MM8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NRF-2021M3E5D2A01023887

Title: Rats show differential defensive responses toward a headed than a headless artificial predatory agent

Authors: *J. JEONG, K. JO, J.-S. CHOI;
Korea Univ., Seoul, Korea, Republic of

Abstract: In nature, rats display innate defensive behaviors in response to predatory threats. To survive, they must quickly recognize a predator's attack and respond with optimal defensive strategies, all while continuously monitoring the predator's state. While it's well-established that the presence of a relatively larger terrestrial animal, such as a cat or its odor, effectively triggers defensive behavior in rats, the influence of a predator's shape on modulating these defensive responses is not well characterized. In our current study, we designed a 4-wheeled, omnidirectional robot predator equipped with an inflatable head, mimicking a predatory entity. Rats were exposed to both a "headed" and "headless" version of this robot in a large open field (2053mm x 1405mm), and their defensive and evasive behaviors were monitored. All rats underwent a training session comprising three phases: headless1, headed, and headless2. Throughout the session, rats were exposed to different versions of a moving robot, depending on the phase. Following a three-week period, the rats were divided into two groups (headed vs. headless) and exposed to either the headed or headless version of the robot. We evaluated fear and anxiety levels based on their velocity (reflecting freezing behavior) and the time spent in the center area of the field. During the training session, rats demonstrated a significant decrease in movement velocity during the headed phase, along with a significant reduction in time spent in the center area, indicating increased anxiety. In line with the training session outcomes, further tests showed that the headed group maintained greater distance from the robot compared to the headless group. Interestingly, we observed a novel defensive behavior we termed "run and look back (RLB)", which is characterized by a momentary pause during flight to monitor the robot's movements. We noted that the headless group exhibited more RLBs than the headed group. This result suggest that a particular physical component of the predator might be utilized by the rats for determining the optimal escape strategy and defensive behaviors.

Disclosures: J. Jeong: None. **K. Jo:** None. **J. Choi:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.03/MM9

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Masupirdine (a pure 5-HT_6 receptor antagonist) for the treatment of agitation in patients with dementia of Alzheimer’s type: Phase-3 study design

Authors: *R. NIROGI, J. RAVULA, S. JETTA, V. GOYAL, P. JAYARAJAN, V. BENADE, A. SHINDE, R. SUBRAMANIAN, A. MOHAMMED, V. JASTI;
Suven Life Sci. Ltd., Hyderabad, India

Abstract: Masupirdine (SUVN-502) is a pure, potent and orally active serotonin-6 (5-HT_6) receptor antagonist. In animal models of aggression like resident-intruder task and dominant-submissive assay, administration of masupirdine attenuated aggressive behaviours. Treatment with masupirdine also significantly modulated the cortical levels of dopamine and norepinephrine. In addition, post hoc analyses of the Phase-2 study of masupirdine in Alzheimer's disease (AD) patients (NCT02580305) showed potential beneficial effects in reducing agitation/aggression symptoms. Contingent on the observations from the animal models and AD patients, a Phase-3, double-blind, randomized, placebo-controlled, parallel group, global study to evaluate the efficacy, safety, tolerability, and pharmacokinetics of masupirdine in patients with agitation in dementia of the Alzheimer's type (ClinicalTrials.gov Identifier: NCT05397639) has been initiated. The study will recruit participants (male or female, 50-90 years of age, both inclusive) with agitation as per International Psychogeriatric Association (IPA) provisional consensus definition of agitation in cognitive disorders and having the diagnosis of Alzheimer's dementia. The study will enrol ~375 participants from USA and Europe. Participants will be randomly assigned in a 1:1:1 ratio to receive either 50 mg or 100 mg masupirdine or placebo for 12 weeks. Cohen-Mansfield Agitation Inventory items score aligning to the IPA agitation criteria domains and the Modified Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change score as related to agitation are the primary and key secondary endpoint, respectively. Subject enrolment is expected to be completed by Q3 2024. Phase-3 study data readout is anticipated in Q1/Q2 2025.

Disclosures: **R. Nirogi:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **J. Ravula:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **S. Jetta:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Goyal:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **P. Jayarajan:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Benade:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Shinde:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **R. Subramanian:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **A. Mohammed:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd. **V. Jasti:** A. Employment/Salary (full or part-time):; Suven Life Sciences Ltd.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.04/MM10

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: The mice demonstrated adaptability and flexibility in assessing the risk of a novel predator

Authors: *T. LI[1], M. LIU[2], R. YUAN[3], C. LI[3], L. REN[3], K. YUAN[4];
[1]Biomed. Engin., Tsinghua Univ., Beijing, China; [2]Dept. of Biomed. Engin., Tsinghua Univ., Beijing City, China; [3]Tsinghua Univ., Beijing, China; [4]Tsinghua Univ., Beijing City, China

Abstract: Prey animals like mice perform risk-assessment to detect and analyze potential threats when they encounter novel objects. However, the behavioral strategies exploited by prey animals to assess the risk of a freely-moving novel predator and change of risk-assessment behavioral patterns with time remained to be characterized. To address these questions, we developed a prey (mouse)-novel predator (rat) interaction assay that only allows mouse to have full access to freely-moving rat. Initially, the mouse spent most of their time observing the rat from a distance. After a few minutes of continuous observation, the mouse chose to approach the rat and take a closer observation when the rat did not show large body movements and was not paying attention to the mouse During the approach or close observation, rat's sudden large movement or turning of the head towards the mouse would interrupt mouse's ongoing behaviors promptly and induce retreat. When the mouse gradually realized that the rat was not going to chase it, the mouse increased the frequency of approach and close observation. Meanwhile, the mouse demonstrated less avoidance, less distant risk-assessment and more grooming. Nonetheless, rat's turning of the head towards the mouse was still able to induce mouse's defensive behaviors. Thus, during novel predator-oriented risk-assessment, the mouse demonstrated remarkable adaptability and flexibility to ensure that they could gather information relevant to rats while not being preyed on. We concluded that the risk-assessment behavioral paradigm we developed could be used to interrogate the neural mechanisms underlying adaptive and flexible animal behaviors.

Disclosures: T. Li: None. **M. Liu:** None. **R. Yuan:** None. **C. Li:** None. **L. Ren:** None. **K. Yuan:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.05/MM11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS-1456471
NIH Grant 1R34NS118412-01

Title: Natural Behavioral State Confers Nociceptive Tolerance to Manduca sexta

Authors: *G. KONDAKATH, A. VELIKO-SHAPKO, I. M. MESSINGER, B. A. TRIMMER; Biol., Tufts Univ., Medford, MA

Abstract: Strong and potentially harmful stimuli are detected by a specialized sensory system termed nociception. Activation of nociception typically evokes avoidance and defensive behaviors. The tobacco hornworm (*Manduca sexta*) caterpillar, a notoriously slow crawler,

produces sudden movements in response to strong mechanical and thermal stimuli. This includes defensive “strikes” towards posterior stimuli and avoidance “withdraws” away from anterior stimuli. Both behaviors can be sensitized by strong or repetitive stimuli but there are no studies on the mechanisms that reduce nociception. Here, we report a natural behavioral state that makes *Manduca* unresponsive to external stimuli, potentially providing a model to study how nociception is modulated. When gently disturbed, *Manduca* caterpillars ‘freeze,’ and stay motionless for extended periods. In this state they adopt a characteristic posture with their head and thorax curled ventrally to resemble the Sphinx. Using fifth-instar *M. sexta* larvae, we have explored three key questions about the sphinx state: (a) how is it induced, (b) how does it affect nociception, and (c) is the brain essential for its initiation? We found that the sphinx state can be triggered by vibrating the substrate and by mild mechanical stimuli (stroking the larval body with a paintbrush) but is most effectively produced by translational (back and forth) movement of the substrate. Next, we observed that during the sphinx state, *Manduca* was significantly less responsive (both strike and withdrawal behaviors) to noxious thermal stimuli. Finally, we showed that surgically separating the cerebral ganglion and sub-esophageal ganglion (SEG) or making an incision between the SEG and prothoracic ganglion, prevents larvae from entering the sphinx state. This suggests that the brain is necessary for the larvae to enter the sphinx state and that the SEG is not sufficient to facilitate this transition. Overall, our results demonstrate that *Manduca* can downregulate its responses to noxious stimuli which makes it a tractable model system for studying the neural mechanisms of nociception modulation. Future experiments will determine the adaptive significance of this posture and examine how sensory and motor circuits are changed to ensure that behaviors remain appropriate in different contexts.

Disclosures: G. Kondakath: None. **A. Veliko-Shapko:** None. **I.M. Messinger:** None. **B.A. Trimmer:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.06/MM12

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Postpartum resource scarcity alters maternal defensive behavior in rats

Authors: *S. KU[1], M. DUPUIS[1], J. FLOWERS, III[1], C. ARDEKANI[1], D. A. BANGASSER[2]; [1]Psychology and Neurosci., Temple Univ. Grad. Neurosci. Program, Philadelphia, PA; [2]Dept. of Psychology and Neurosci., Temple Univ., Atlanta, GA

Abstract: Postpartum affective disorders (PADs), such as postpartum depression and anxiety, are debilitating diseases with limited treatments. Two risk factors for PADs are postpartum stress and low socioeconomic status (SES). We mimic these factors using a model of resource scarcity: the limited bedding and nesting manipulation (LBN). LBN dams reside on a metal grate with insufficient nesting material during the beginning of the postpartum period to restrict their access

to bedding. LBN dams have higher levels of stress hormones and we previously found they display increased pup-directed behavior (passive nursing, blanket nursing, arched back nursing, licking, and grooming of pups) and decreased self-care (self-grooming, eating, drinking, and resting outside the nest). Here we leveraged our LBN model to expand current knowledge and understand how postpartum resource scarcity affects maternal defensive behavior. On postnatal day (PND) 2, Long Evans dams (100-150 days old) were placed in either standard housing (ample bedding, 2 nestlets, and 1 enrichment, n = 10) or LBN (n = 8) housing conditions. On PND10, dams were put through a resident/intruder task to elicit aggression, where a younger male intruder rat was placed in the dams' home cage for 15 minutes. Prior research typically focuses on singular measures to score aggressive behavior, such as attack duration or type. Here, we present a comprehensive characterization of offensive and defensive fluctuations in aggression by compiling information from three categories: 1) locus of attack on the intruder (flank, abdomen, crotch, head/neck, rump, back) 2) dam positioning relative to the intruder (lateral, face-on, posterior), and 3) type of attack (bite, kick, wrestle, standing boxing, pin, shove). Though data analysis is still ongoing, preliminary findings indicate that LBN dams had a longer latency to attack ($p = 0.08$) with a trend towards shorter total attack durations ($p = 0.056$), suggesting reduced overall aggression. LBN dams show shorter offensive attack durations (bite, wrestles, pins, $p = 0.03$) and reduced pinning ($p = 0.02$), an overtly dominant form of aggression. There were no differences between control and LBN dams in defensive attack durations ($p = 0.87$). We are currently conducting whole brain cFOS analyses to determine how LBN-induced patterns of defensive behavior lead to differentially activated circuits. Together, these studies will reveal mechanisms by which resource scarcity alters maternal affective behavior and may help lead to novel targets for treating PADs, particularly symptoms regarding overprotective or anxious parenting.

Disclosures: S. Ku: None. **M. Dupuis:** None. **J. Flowers:** None. **C. Ardekani:** None. **D.A. Bangasser:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.07/MM13

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Northwestern University Office of Undergraduate Research

Title: Acute stress enhances male aggression in adult WMI males

Authors: *A. H. HARTER, C. K. KIM, A. YAMAZAKI, M. NEMESH, L. LEE, E. E. REDEI; Northwestern Univ., Evanston, IL

Abstract: Stress hyperreactive male Wistar Kyoto (WKY) More Immobile (WMI) rats display heightened aggressive behavior toward non-threatening juveniles immediately after a 30 min

restraint stress (RS). WKY Less Immobile (WLI) males with and without stress as well no stress WMIs and females of either strain do not show enhanced aggression. The current study is aimed to investigate whether stress-induced aggressive behavior is dependent on the age of the WMI male, adolescence vs. adulthood, and the age of the intruder, juvenile or same-age. WMI and WLI males remained in the home cage (controls) or were exposed to 30 minutes RS. Immediately following RS, social interaction (SI) tests were conducted on all groups. This procedure was carried out at early adolescence (30 days old), and at late adolescence (45-50 days old), with a same-age intruder. In adulthood (over 60 days of age), the tests were repeated with a same-age intruder, and finally with juveniles (25-28 days old). The four-minute SI tests were recorded and analyzed for behaviors related to anxiety (grooming), activity (rearing), social interaction (olfactory investigation), and aggression (confrontation). No strain differences were observed in either adolescents or adult males in grooming, rearing, or olfactory investigation behaviors. In contrast, adult, but not adolescent, WMIs showed increased aggressive confrontation after stress, while WLIs showed a decrease in this behavior against both same-age and juvenile intruders (same-age, strain x stress, $F[1,31]=10.42$, $p<0.01$; juvenile, strain x stress, $F[1,31]=21.53$, $p<0.01$). Testosterone levels at the end of the experiment were significantly lower in WMIs compared to WLIs regardless of stress. Plasma corticosterone levels were not significantly different between no stress WLI and WMI males and showed the same elevated levels in stressed animals of both strains. The data suggests that the increased stress reactivity of the WMI male is likely contributing to its enhanced aggression. However, pubertal changes other than testosterone levels are involved.

Disclosures: **A.H. Harter:** None. **C.K. Kim:** None. **A. Yamazaki:** None. **M. Nemesh:** None. **L. Lee:** None. **E.E. Redei:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.08/MM14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Gustavus Adolphus College funding

Title: Adolescent development of female-female interactions in a neutral arena in Syrian hamsters: A continued analysis

Authors: *K. C. DE LORME[1], **A. JOERGER**[1], M. SMERILLO[1], A. COX[1], B. M. ARSZNOV[2];
[1]Dept. of Psychological Sci., Gustavus Adolphus Col., Saint Peter, MN; [2]Psychology, Minnesota State Univ. Mankato, Mankato, MN

Abstract: Behavior from this previously presented study has been reexamined to include new behaviors and assess interrater observation. Across development, Syrian hamsters learn

appropriate displays of complex agonistic behaviors to communicate social status, ensure successful reproduction, and defend territory and resources. Specific agonistic behaviors that change during adolescence include frequency and body location of attacks, offensive posturing, defensive posturing, and flank marking. The development of agonistic behavior has been studied extensively in males using the resident-intruder paradigm; however, fewer studies have investigated how hamsters interact in a neutral territory, especially in females. Here, we investigated how female hamsters differ in their display of agonistic behavior towards each other in a neutral arena across adolescence. To do this, 24 age- and weight-matched female hamsters were divided into three age groups: prepubertal (postnatal day (P)27), adolescent (P45), and adult (P62). All pairs were placed in a neutral arena for 10 minutes that neither female had occupied prior to testing and digitally recorded for later quantification using Behavioral Observation Research Interactive Software. Behaviors quantified included: offensive and defensive postures, flank marking, vaginal marking, grooming, social contact, rearing, and front, side, and rear attacks. Prepubertal females displayed higher frequencies and longer bouts of both offensive and defensive posturing compared to adolescents and adults. Adolescent females reared more than prepubescent and adult females, and adults vaginally marked more than prepubescent females with adolescents in between. Surprisingly, there was no significant difference in flank marking between the groups. There was also no difference between groups in location of attacks or overall attacks. In sum, these data are in partial agreement with previous studies of hamster agonistic behavior development. The biggest departures are seen in scent marking and topography of attacks. It is possible that there was no difference in flank marking due to adults using vaginal marking in addition to flank marking, which was not seen in prepubescent females. Additionally, the neutral arena does not elicit attacks as readily as the resident-intruder paradigm, and thus, the typical change in body location of attacks across development was not found due to very low amounts of attacks overall. We are currently investigating whether these female hamsters have differing *c*-Fos expression in the prefrontal cortex and medial amygdala during agonistic interactions.

Disclosures: **K.C. De Lorme:** None. **A. Joerger:** None. **M. Smerillo:** None. **A. Cox:** None. **B.M. Arsznov:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.09/MM15

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSFC Grant No.32100817
China Postdoctoral Science Foundation Grant No. 2020TQ0333
China Postdoctoral Science Foundation Grant No.2020M681416
National Science and Technology Innovation 2030 Major Program Grant No.2021ZD0203200-03

Title: Esr1 dependent wiring of the male aggression circuit

Authors: *X. ZHA[1], X. LIU[2], L. WANG[3], J.-K. LIN[4], X. XU[5,2];
[1]Ctr. for Excellence in Brain Sci. and Intelligence Technol. (Institute of Neuroscience), Shanghai, China; [2]Ctr. for Excellence in Brain Sci. and Intelligence Technol. (Institute of Neuroscience), Chinese Acad. of Sci., Shanghai, China; [3]Peking Univ., Beijing, China; [4]Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Ctr. for Excellence in Brain Sci. and Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; [5]Chinese Acad. of Sci., Chinese Acad. of Sci., Shanghai, China

Abstract: Perinatal estrogen is known to shape the developing brain to establish neural pathways involved in male-specific aggressive behaviors. However, the precise neural mechanisms underlying this process remain poorly understood. In our study, we designed a genetic approach that enabled us to selectively delete estrogen receptor alpha (Esr1), the primary mediator of estrogen's effects, in specific molecularly defined hypothalamic neurons and to track the Esr1-deleted cells to investigate their role in the neural circuitry. Our findings revealed that developmental deletion of Esr1 in SF-1 lineage of hypothalamic neurons resulted in abnormal connectivity between these neurons and upstream regions known to promote aggression, such as the posterior amygdala (PA) and lateral septum (LS). Additionally, we observed that deleting Esr1 during development, but not in adulthood, led to a decrease in the firing rate of the targeted neurons in response to electrical stimulation. Moreover, these neurons lost their ability to induce aggressive behavior when activated in adult males. Notably, we discovered that perinatal estrogen treatment in female newborns masculinized the firing rate of neurons in the ventromedial hypothalamus (VMHvl) and endowed them with the capacity to induce male-like aggression when stimulated. Additionally, developmental Esr1 deletion disrupted sodium channel currents, and pharmacological blockade of sodium channels reduced aggressive behavior in adult males. These findings highlight the sex-specific wiring of the aggression circuit, influenced by perinatal estrogen and Esr1 signaling. They provide valuable insights into the significance of sex-specific neural development for understanding sex differences in neurodevelopmental processes and psychiatric disorders.

Disclosures: **X. Zha:** None. **X. Liu:** None. **L. Wang:** None. **J. Lin:** None. **X. Xu:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.10/MM16

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Whitehall grant 2019-12-43
Klingenstein Simons Fellowship in Neuroscience
(Simons Collaboration on the Global Brain #542969
McKnight Foundation

Ben Barres postdoctoral fellowship
NIH R01NS050835
PhD scholarship SFRH/BD/51715, Fundac¸ a˜ o para a Cieˆ ncia e a Tecnologia
NIH R01NS049488
NIH R01HD109519
Stanford SoM Dean's Fellowship
NARSAD Young Investigator Grant

Title: Hypothalamic neurons that mirror aggression

Authors: *T. YANG[1], D. W. BAYLESS[1], Y. WEI[1], D. LANDAYAN[1], I. MARCELO[4], Y. WANG[1], L. A. DENARDO[5], L. LUO[2], S. DRUCKMANN[3], N. M. SHAH[1];
[1]Psychiatry and Behavioral Sci., [2]Biol., [3]Neurobio., Stanford Univ., Stanford, CA;
[4]Champalimaud Fndn., Champalimaud Fndn., Lisbon, Portugal; [5]Physiol., UCLA, Los Angeles, CA

Abstract: Studies of social behaviors in mice, such as mating and aggression, have provided understanding of the neural and molecular mechanisms underlying the transformation of sensory and physiological signals into social behaviors. For example, males show territorial aggression toward other males whereas they mate with females. Previous studies have shown that progesterone receptor (PR)-expressing neurons, which co-express estrogen receptor alpha (Esr1), in the ventrolateral subregion of the ventromedial hypothalamus (VMHvl) are critical for male aggression. VMHvl^{PR} neurons are widely considered to constitute the "attack center", which has been assumed to be a final output and therefore impervious to social context. Contrary to this assumption, however, my previous study showed that VMHvl^{PR} neurons are sensitive to social context, sensed by a specific chemosensory pathway, such that even their forced activation failed to elicit an attack when the animal was in someone else's territory (Yang et al., 2017). In nature, territorial aggression is usually advertised because the winner wants to emphasize dominance not only to the loser but also to other potential competitors. Given the central role of VMHvl^{PR} neurons in aggression and their sensitivity to social context, I sought to test whether these cells were sensitive to fights between other individuals. Using fiber photometry as well as miniscope calcium imaging, I discovered that VMHvl^{PR} neurons in fact mirror aggression between other individuals (Yang et al., 2023). The same individual VMHvl^{PR} neurons were co-activated during the performance and the observation of aggressive behaviors, such as attacks and tail rattling. This mirroring property was not seen during non-aggressive behaviors, nor was it detected in an important brain region for sex recognition and aggression. I further identified that this mirroring property requires visual input rather than pheromonal cues and was observed even in socially naive mice who had never engaged in or been trained to fight. To examine the functional relevance of mirroring aggression, I used the genetically encoded TRAP2 strategy, to tag these aggression-mirroring VMHvl^{PR} neurons. Expression of DREADDs, chemogenetic actuators, in mirror-TRAPed neurons demonstrated that these cells were both necessary and sufficient for aggression. My work reveals mirror neurons in the mouse brain, in the hypothalamus which is deeply conserved in vertebrates, for an innate social behavior, and it further shows functional relevance for mirror neurons. Together, I have established a novel, genetically tractable platform to dissect how neural circuits can mirror behavior.

Disclosures: **T. Yang:** None. **D.W. Bayless:** None. **Y. Wei:** None. **D. Landayan:** None. **I. Marcelo:** None. **Y. Wang:** None. **L.A. DeNardo:** None. **L. Luo:** None. **S. Druckmann:** None. **N.M. Shah:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.11/MM17

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Knut and Alice Wallenbergs Stiftelse (2020-0054)
Vetenskapsrådet Distinguished Professor Program (2021-00671)
Wallenberg AI, Autonomous Systems and Software Program

Title: Development of intermale aggression requires contextually appropriate PMvDAT cell activity

Authors: *L. HEIKKINEN[1,2], D. MASINI[1], N. XU[3], C. BROBERGER[1];
[1]Stockholm Univ., Stockholm, Sweden; [2]Karolinska Institutet, Stockholm, Sweden; [3]KTH Royal Inst. of Technol., Stockholm, Sweden

Abstract: A majority of male rodents will vigorously attack a male conspecific introduced in its home cage, as observed in the resident-intruder (R/I) paradigm. This behaviour, once manifest, remains stable over time. However, aggression phenotype is initially undistinguishable and requires multiple exposures to novel mice to settle at a level that is then maintained through adult life. This “aggression career” suggests that learning and plasticity may be key features of innate social behaviour, yet the neural mechanisms of this phenomenon remain obscure. Mouse intermale aggression depends critically on a population of dopamine transporter -expressing neurons of the ventral premammillary nucleus of the hypothalamus (PMvDAT cells; Stagkourakis et al., 2018). The excitability of these cells differs significantly between aggressive and non-aggressive mice. Here, we asked if PMvDAT activity is necessary and/or sufficient as the animal develops a stable aggression phenotype over repeated intruder encounters.Repeated R/I tests were performed in initially naïve male DAT-Cre C57BL/6J mice while we manipulated PMvDAT neuronal activity prior to or during the first experiences of a resident mouse with an intruder. We first inhibited PMvDAT cells during three consecutive RI trials followed by three RI trials with no neuronal modulation. Separately, we stimulated PMvDAT cells while the animal was temporarily alone in its home cage across three stimulation sessions, followed by three days of RI testing with no neuronal modulation. To assess the role of PMvDAT neurons during the development of an aggressor phenotype, we expressed either halorhodopsin or inhibitory DREADDs in PMvDAT cells of adult male mice. Establishment of an aggression phenotype was prevented by PMvDAT inhibition but took place during subsequent trials with no neuromodulation. We next tested the activation of PMvDAT cells in mice with no RI experience using channelrhodopsin-2 or excitatory DREADDs Unexpectedly, repeated stimulation of

PMvDAT cells in the same environment in the absence of an intruder prevented the later development of a typical R/I aggressor phenotype. Together these findings suggest that PMvDAT activity during the peer encounter is required for development of intruder-directed aggression, and that the activation must be coupled with a specific social context. Further inspection of the observed behaviors revealed that the properties of non-aggressive social micro-behaviours predict the presence or absence of aggression, suggesting the categorical shift in expression of an aggression phenotype is accompanied by changes in the content of other social behaviours.

Disclosures: L. Heikkinen: None. **D. Masini:** None. **N. Xu:** None. **C. Broberger:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.12/MM18

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Oxytocin treatment rescues irritability-like behavior in Cc2d1a conditional knockout mice

Authors: *K.-H. CHENG;
Natl. Cheng Kung Univ. Col. of Med., Tainan, Taiwan

Abstract: Irritability, a state of excessive reactivity to negative emotional stimuli, is common in individuals with autism spectrum disorder (ASD). Although it has a significant negative impact of patients' disease severity and quality of life, the neural mechanisms underlying irritability in ASD remain largely unclear. We have previously demonstrated that male mice lacking the Coiled-coil and C2 domain containing 1A (CC2D1A) in forebrain excitatory neurons recapitulate numerous ASD-like behavioral phenotypes, including impaired social behaviors and pronounced repetitive behaviors. Here, by using the bottle-brush test (BBT) to trigger and evaluate aggressive and defensive responses, we show that *Cc2d1a* deletion increased irritability-like behavior in male but not female mice, which is correlated with reduced number of oxytocin (OXT)-expressing neurons in the paraventricular nucleus (PVN) of the hypothalamus. Intranasal OXT administration or chemogenetic activation of OXT neurons in the PVN rescues irritability-like behavior in *Cc2d1a* conditional knockout (cKO) mice. Administration of a selective melanocortin receptor 4 agonist, RO27-3225, which potentiates endogenous OXT release, also improves irritability-like behavior in *Cc2d1a* cKO mice, an effect blocked by a specific OXT receptor antagonist, L-368,899. We additionally identify a projection connecting the posterior ventral segment of the medial amygdala (MeApv) and ventromedial hypothalamus (VmH) in executing irritability-like behavior during the BBT. Chemogenetic suppression of the MeApv-VmH pathway blocks irritability-like behavior in *Cc2d1a* cKO mice. Together, these results reveal the molecular and neural circuit mechanisms for irritability in ASD and provide translatable insights into the development of OXT-based therapeutics for clinical interventions.

Disclosures: K. Cheng: None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.13/MM19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 2 U19 NS107616-06

Title: Oxytocin control of the hippocamposeptal neural circuit regulates social hierarchy and its implication for alzheimer's disease pathology

Authors: *G. ZHAO[1], C. WANG[1], K. LOUIE[2], J. LIU[3], R. W. TSIEN[3];
[1]New York Univ., New York City, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY;
[3]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY

Abstract: Oxytocin is a neuropeptide produced in the hypothalamus that plays central roles in reproduction, social behaviors, and emotion. However, its function in defined neural circuits controlling social dominance is not well understood. Perturbed oxytocin levels have been found in postmortem brain tissue of patients with Alzheimer's Disease (AD) (Mazurek, M. et al, 1987); social dysfunction is also highly prevalent in AD patients. The hippocampus is known to be one of the most vulnerable brain regions susceptible to amyloid pathology (Hampel, H. et al, 2021) and the lateral septum (LS) is the major subcortical target downstream of hippocampal inputs. Moreover, oxytocin receptors are enriched in the lateral septum (LS), which concurrently receives significant inputs from PVN oxytocin-expressing neurons (Horai, M. et al, 2020). Here, we examine the role of oxytocin in ion channels, neuronal activity and synaptic transmission in the LS and social ranking established by a tube test paradigm. We are also in the process of incorporating these data in an agent-based model, enabling large-scale simulation of changing social hierarchy dynamics to test whether hypothesized mechanisms align with empirical data. Manipulation of the oxytocin system in the LS is achieved using oxytocin receptor f/f (OXTR f/f) mice to assess the behavioral impact of brain-region specific oxytocin receptor knock down. The tube test is a novel assay that assesses social dominance and hierarchy using round-robin competition within group-housed mice (Fan, Z. et al, 2019). Injection of Cre adenovirus into the LS of OXTR f/f mice perturbs stabilized tube test social rankings, followed by re-establishment of altered social hierarchies, while similar injection in GFP controls leaves rankings unaffected. We hypothesize that oxytocin signaling in the LS is important for social recall. Since re-stabilized hierarchies are different, oxytocin signaling in the LS neurons may be critical in reliable conveyance of hippocampally encoded social salience to other brain structures that are downstream projection sites of LS. Future tests will aim to assess how this path guides behavioral outputs in wild-type and AD model mice and to determine whether oxytocin signaling is impaired within the context of amyloid or tau pathology.

Disclosures: **G. Zhao:** None. **C. Wang:** None. **K. Louie:** None. **J. Liu:** None. **R.W. Tsien:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.14/MM20

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH U19NS107616

Title: A dedicated oxytocin circuit in the hypothalamus controls social avoidance learning

Authors: *T. OSAKADA, Y. JIANG, R. YAN, D. WEI, R. TABUCHI, B. DAI, X. WANG, J. LIU, R. W. TSIEN, A. C. MAR, D. LIN;
Neurosci. Inst., NYU Langone Hlth., NY, NY

Abstract: Many animals live in complex social groups. To survive, it is essential to know who to avoid and who to interact. Although naïve mice are naturally attracted to any adult conspecifics, a single defeat experience could elicit social avoidance towards the aggressor for days. The neural mechanisms underlying the behavior switch from social approach to social avoidance remains incompletely understood. Here, we identify oxytocin neurons in the retrochiasmatic supraoptic nucleus (SOR^{OXT}) and oxytocin receptor (OXTR) expressing cells in the anterior subdivision of ventromedial hypothalamus, ventrolateral part ($aVMHvl^{OXTR}$) as a key circuit motif for defeat-induced social avoidance learning. After defeat, $aVMHvl^{OXTR}$ cells drastically increase their responses to aggressor cues. This response change is functionally important as optogenetic activation of $aVMHvl^{OXTR}$ cells elicits time-locked social avoidance towards a benign social target whereas inactivating the cells suppresses defeat-induced social avoidance. Furthermore, OXTR in the aVMHvl is itself essential for the behavior change. Knocking out OXTR in the aVMHvl or antagonizing the receptor during defeat, but not during post-defeat social interaction, impairs defeat-induced social avoidance. $aVMHvl^{OXTR}$ receives its private supply of oxytocin from SOR^{OXT} cells. SOR^{OXT} is highly activated by the noxious somatosensory inputs associated with defeat. Oxytocin released from SOR^{OXT} depolarizes $aVMHvl^{OXTR}$ cells and facilitates their synaptic potentiation, and hence, increases $aVMHvl^{OXTR}$ cell responses to aggressor cues. Ablating SOR^{OXT} cells impairs defeat-induced social avoidance learning whereas activating the cells promotes social avoidance after a subthreshold defeat experience. Altogether, our study reveals an essential role of SOR^{OXT}-$aVMHvl^{OXTR}$ circuit in defeat-induced social learning and highlights the importance of hypothalamic oxytocin system in social ranking and its plasticity.

Disclosures: **T. Osakada:** None. **Y. Jiang:** None. **R. Yan:** None. **D. Wei:** None. **R. Tabuchi:** None. **B. Dai:** None. **X. Wang:** None. **J. Liu:** None. **R.W. Tsien:** None. **A.C. Mar:** None. **D. Lin:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.15/MM21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NASEM Ford Fellowship
R15MH122946

Title: Androgen Receptor Knockdown in the Medial Amygdala Leads to Increased Condition Defeat in Syrian Hamsters

Authors: *C. WHITTEN[1], A. GILLESPIE[2], T. BRUNO[1], M. COOPER[2];
[2]Psychology, [1]Univ. of Tennessee Knoxville, Knoxville, TN

Abstract: Social stress is an aversive emotional experience that leads to individual differences in fear and anxiety. Environmental factors such as dominance status can alter the way individuals react to and cope with stressful events. We have previously found that dominant male hamsters show a longer latency to submit, a reduction in defeat-induced social avoidance, and increased androgen receptor (AR) expression in the posterior dorsal (MePD) and ventral (MePV) medial amygdala compared to subordinates. These findings suggest AR activity in the MePD and MePV may be necessary for status-dependent changes in stress-related behavior. To test this possibility, animals received stereotaxic surgery with bilateral MePD/MePV infusion of a short hairpin adeno-associated virus to knockdown AR (AR-shRNA) or a non-functional scrambled virus (SCRM). Animals were then euthanized at two, three-, or six-weeks to measure AR knockdown efficiency. Separate groups of animals received AR-shRNA or SCRM infusion prior to social defeat stress and testing for anxiety-like behavior. We found that AR-shRNA infusion leads to greater AR knockdown in MePD/MePV after two- and three-weeks compared to SCRM infusion, although AR expression increases after six weeks. Knockdown of AR in the MePD/MePV did not alter aggression, however, it did increase the condition defeat response following acute social defeat stress. This work highlights that androgen receptor expression within the MePD and MePV can promote resistance to social defeat stress and may be an important factor related to stress related psychopathologies.

Disclosures: C. Whitten: None. **A. Gillespie:** None. **T. Bruno:** None. **M. Cooper:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.16/MM22

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R15 Grant MH122946

Title: Neurons that Play Together Stay Together: Effects of Social Play Deprivation on Perineuronal Net Expression

Authors: *J. KELLY[1], C. J. WHITTEN[2], B. KILGORE[3], A. GILLESPIE[3], Y. SINGAMANENI[3], A. TEMPLE[3], M. A. COOPER[4];
[1]Univ. of Tennessee, Knoxville, Knoxville, TN; [2]Univ. of Tennessee Knoxville, Knoxville, TN; [4]Dept. of Psychology, [3]Univ. of Tennessee, Knoxville, TN

Abstract: Social play is a crucial developmental experience for species-typical neural and behavioral development. Social play deprivation during adolescence potentiates the effects of social defeat stress in adult Syrian hamsters (Mesocricetus auratus). Play deprivation decreased pruning of apical dendrites in the infralimbic (IL) and prelimbic (PL) regions of the medial prefrontal cortex. Increases in stress susceptibility may be driven by plasticity in the IL and PL and their downstream targets including the basolateral amygdala (BLA) and the CA2 region of the hippocampus. Perineuronal nets (PNNs) are extracellular structures known to preferentially surround parvalbumin (PV) neurons and stabilize synapses by gating experience-dependent neuroplasticity. We investigated how adolescent social play deprivation alters PNN expression on PV neurons, and how changes in PNN expression are associated with susceptibility to social defeat stress. We reared Syrian hamsters from postnatal day 21-42 either in peer isolation, peer isolation with 1-hour of social play daily, or group-housed with litter-mates. In adulthood we exposed subjects to acute social defeat stress and assessed their defeat-induced anxiety in conditioned defeat and social avoidance tests. We found that play-deprived hamsters showed the most defensive and submissive behavior during conditioned defeat tests, and that 1-hour of daily social play was sufficient to rescue this increase in a conditioned defeat response. Hamsters that received 1-hour of social play daily had reduced co-expression of PNN and PV in the IL and greater PNN density in the CA2 compared to other treatment groups. Play-deprived hamsters had increased expression of PNNs on PV neurons in the BLA, and PNN/PV co-expression was associated with social approach during a social avoidance test. Our findings demonstrate that social play during adolescence generates long-term changes in neural plasticity in limbic regions that modulate responses to social defeat stress in adulthood. This work delineates a critical role for social play in the development of neural ensembles regulating adult social behavior.

Disclosures: J. Kelly: None. **C.J. Whitten:** None. **B. Kilgore:** None. **A. Gillespie:** None. **Y. Singamaneni:** None. **A. Temple:** None. **M.A. Cooper:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.17/MM23

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R15 MH122946

Title: Social dominance alters perineuronal net expression in a sex dependent manner in the ventral medial prefrontal cortex and basolateral amygdala

Authors: *A. F. RADFORD, C. J. WHITTEN, M. K. HOOKER, M. A. COOPER; Psychology, Univ. of Tennessee, Knoxville, Knoxville, TN

Abstract: The emergence of dominance relationships promotes experience-dependent neural plasticity that modulates future agonistic behavior and stress vulnerability. A key neural circuit that mediates stress vulnerability and undergoes status-dependent neural plasticity includes ventral medial prefrontal cortex (vmPFC) neurons projecting to the basolateral amygdala (BLA). Perineuronal nets (PNNs) are extracellular structures that surround cell bodies and their proximal dendrites to constrain stress-related neural plasticity and support stable cellular networks. Because dominant animals show greater resistance to social stress than subordinates, we hypothesize that dominant animals will have more PNNs in the PFC, while subordinates will have more PNNs in the BLA. We used adult male and female Syrian hamsters (*Mesocricetus auratus)* to investigate whether the development of a dominance relationship alters the expression of PNNs within the BLA as well as the infralimbic (IL) and prelimbic (PL) regions of the vmPFC. We created dominance relationships by exposing pairs of hamsters to two weeks of daily aggressive interactions in a resident-intruder format. Controls were tested similarly, but animals were exposed to a partner across a clear and perforated barrier to prevent status formation. We collected brains 60 minutes after the last aggressive interaction and performed immunofluorescence staining for wisteria floribunda agglutinin to quantify PNN expression. Hamsters readily formed dominance relationships and dominant males and females attacked their opponents more quickly and at higher rates compared to subordinates. Aggression in female hamsters was dependent on their estrous cycle, such that dominant females attacked at a higher rate during diestrus. Subordinate females had significantly more PNNs in the PL than dominant females, although PNN expression did not differ in the IL or BLA. Meanwhile, dominant males had significantly more PNNs in the BLA than control males, while PNN expression did not differ in the IL or PL. Interestingly, dominant females and males showed a negative correlation between attack rate and the number of PNNs in both IL and PL. These findings suggest that more aggressive animals have reduced PNN expression within the vmPFC, which may allow for greater neural plasticity in cortical circuits regulating stress resistance. Overall, development of dominance relationships may cause neural plasticity that increases stress coping rather than blocking stress responses.

Disclosures: A.F. Radford: None. **C.J. Whitten:** None. **M.K. Hooker:** None. **M.A. Cooper:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.18/MM24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: ERC-2022-ADG Project 101097411– Territory

Title: Neural encoding of social threat behaviours in the ventromedial hypothalamus of male and female mice

Authors: *S. DEB, M. E. MASFERRER, C. T. GROSS;
Epigenetics and Neurobio. Unit, European Mol. Biol. Lab., Monterotondo, Italy

Abstract: Defensive behaviors are a critical part of the strategies animals employ to cope with threatening stimuli. In social animals like humans and mice, conspecific threats can arise between territorial males or by the advances of males towards an unreceptive female. Defensive strategies towards social threats include avoidance, escape, submissive postures, and in some cases, defensive attack. The ventrolateral division of the ventromedial hypothalamus (VMHvl) has been shown to be essential for both defensive and aggressive behaviors of mice toward social threats. Moreover, neurons in VMHvl modulate their activity during the initiation and execution of both defensive and aggressive behaviors to social threats. However, it is still not clear how these functionally modulated units interconnect within the VMHvl to select and support appropriate defensive behaviors. Here we used optrodes to carry out *in vivo* single unit electrophysiology in the VMHvl of male and female mice during exposure to a social threat. To measure the full repertoire of social avoidance behaviors we designed a novel circular arena apparatus where pairs of mice (male-male or female-male) can interact freely and exhibit robust avoidance and escape behavior. Classes of single units were identified that encoded different aspects of defensive behaviors. Importantly, our circular arena allowed us to measure defensive behaviors in non-receptive females avoiding and escaping advances from males. Finally, we will present preliminary data in which we have used optrode stimulation to identify the functional identity and connectivity of the recorded units.

Disclosures: S. Deb: None. **M.E. Masferrer:** None. **C.T. Gross:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.19/MM25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: EIPOD4
ERC-2022-ADG Project 101097411– Territory

Title: Dynamic integration of space and social status in the mammalian hypothalamus

Authors: *D. BATTIVELLI, L. BOLDRINI, M. JAISWAL, L. SPAGNOLETTI, P. PATIL, E. ENGELEN, C. T. GROSS;
EMBL, Monterotondo, Italy

Abstract: Work has shown that the ventrolateral division of the ventromedial hypothalamus (VMHvl) encodes a generalized state of social threat and is necessary and sufficient for producing both social aggression and avoidance. However, it remains unclear how the selection between aggression and avoidance occurs and how this selection might support the animal's adaptation to its environment. We have shown that neurons in VMHvl that fire in the context of social defeat are reactivated when the animal explores the location where the defeat occurred, even in absence of the aggressor. At the neural population level, following social defeat VMHvl firing becomes tuned to the context where the animal is located, discriminating between the home cage and the chamber where the aggression took place. We hypothesize that the VMH may encode a functional map of territorial space that guides context-appropriate social threat responses. To test this hypothesis, we developed an experimental apparatus that allows for the study of territorial behavior under semi-natural conditions. Pairs of male mice in the apparatus exhibited a stereotyped evolution of dominance hierarchies and the context-specific expression of social aggression and avoidance. Moreover, we found that, unlike outbred mice, laboratory inbred mice were unable to express stable territorial behaviors in the apparatus. Finally, we developed a novel dual-color fluorescence detection method to track urine marking patterns in freely interacting mice. We are using this apparatus to test several hypotheses about the neural control and encoding of territorial behaviors in mice.

Disclosures: D. Battivelli: None. **L. Boldrini:** None. **M. Jaiswal:** None. **L. Spagnoletti:** None. **P. Patil:** None. **E. Engelen:** None. **C.T. Gross:** None.

Poster

PSTR491. Defensive Behavior and Aggression

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR491.20/NN1

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: A brainstem neural circuit for instinctive assessment and escape in mice.

Authors: *I. P. AYUSO-JIMENO[1], P. RONCHI[2], F. TORRAO[1], T. SALEMI[1], C. GALLORI[1], T. WANG[1], C. T. GROSS[1];
[1]EMBL, Monterotondo, Italy; [2]Electron Microscopy Core Facility, EMBL, Heidelberg, Germany

Abstract: To survive animals have to finely regulate their threat assessment and escape behavior. In mice, the brainstem dorsal periaqueductal gray (dPAG) generates innate defensive behaviors towards a multitude of threats, including predators, prey, and aggressive conspecifics. dPAG integrates threat information coming from diverse inputs with cognitive information coming from cortical areas such as the anterior cingulate cortex (ACC). Glutamatergic layer 5

neurons in ACC project to dPAG where they inhibit the expression of defensive behavior. In vivo single unit recordings in dPAG identified two major neuron cell types that are modulated during approach to a threat and during escape from that threat, referred to as Assessment+ and Flight+ cells, respectively. However, so far it is not clear if Assessment+ and Flight+ cells correspond to neuron classes that can be distinguished by their connectivity or gene expression patterns, leaving it unclear how they may cooperate to regulate support or trigger defensive behaviors. Here, we performed in vivo single unit calcium miniscope imaging in Vglut2+ and Vgat+ neurons in dPAG to determine 1) how Assessment+ and Flight+ neurons map to these two populations and 2) whether predator, social, and prey threats recruit the same neurons in dPAG. In parallel, we used volume electron microscopy (FIBSEM) combined with multiplexed dAPEX2 labeling to identify the dPAG cell-type receiving long-range excitatory ACC inputs. FIBSEM showed that ACC inputs synapse on Vglut2+ and non-Vglut2+/non-Vgat+ neurons, but not onto Vgat+ targets, leaving open the possibility that ACC inputs inhibit dPAG function via an unknown neuromodulatory cell class in dPAG. Ongoing experiments are aimed at combining anterograde trans-synaptic viral labeling from ACC inputs and spatial transcriptomics profiling to identify the gene expression signatures of ACC target cells in dPAG.

Disclosures: I.P. Ayuso-Jimeno: None. **P. Ronchi:** None. **F. Torrao:** None. **T. Salemi:** None. **C. Gallori:** None. **T. Wang:** None. **C.T. Gross:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.01/NN2

Topic: F.03. Stress and the Brain

Support: MH002386

Title: Brain immediate-early gene regulation after stress: Differential activation by parcellated signaling pathways

Authors: S. Z. JIANG[1], W. BRANCALEONE[2], H.-Y. ZHANG[2], ***L. E. EIDEN**[1];
[1]Section on Mol. Neuroscience, Lab. of Cell. and Mol. Regulation, NIMH-Intramural Res. Program, Bethesda, MD; [2]Section on Mol. Neuroscience, Lab. of Cell. and Mol. Regulation, NIMH-IRP, Bethesda, MD

Abstract: Immediate-early genes (IEGs) such as *egr1* and *fos* are up-regulated in response to neuronal activation following stress, psychomotor stimulants, and other stimuli. The neuronal signaling pathways causing IEG upregulation can be initiated by first messengers, including neurotrophins, glutamate, and metabotropic (GPCR) ligands such as dopamine, norepinephrine, and neuropeptides; and propagated by second messengers, principally calcium and cyclic AMP, and third messengers, such as calcium- and cAMP-activated intracellular protein kinases. The requirement for various first messengers in mediating behavioral responses to environmental

stimuli, including drugs, threats and stress has been extensively investigated. We examined up-regulation of the IEGs *egr1* and *fos,* correlating these with biochemical and behavior responses to stress. Activation of *fos* and *egr1* occurs in nucleus accumbens following cocaine administration. Knockout of the guanine nucleotide exchange factor RapGEF2 from D1 neurons in D1R-Cre::Cre amplifier::RapGEF2$^{fl/fl}$ mice causes loss of pERK and *egr1* induction, with preservation of *fos* induction, after cocaine treatment. During fear conditioning augmented by prior restraint stress, induction of *egr1* and pERK, but not *fos* requires RapGEF2 expression, as does fear learning (freezing to cue) under these conditions. Finally, acute restraint stress (for 2 hours) causes *fos*, pERK and *egr1* induction in paraventricular nucleus (PVN) of hypothalamus. Ablation of PACAPergic input to PVN results in loss of induction of *fos* following restraint, but with preservation of pERK elevation and *egr1* induction. In contrast, hypothalamic deletion of RapGEF2 in Sim1-Cre::RapGEF2$^{fl/fl}$ mice causes loss of *egr1* induction with preservation of *fos* induction in PVN following restraint. These results, *in toto,* suggest that IEG up-regulation in neurons responding to stress and other stimuli can be activated by two distinct signaling pathways, one dependent on the cAMP-activated guanine nucleotide exchange factor and MAP kinase activator RapGEF2, leading to *egr1* induction, and one functioning independently of RapGEF2, leading to induction of *fos.* Separate modulation of these pathways may help identify the functional effects of IEG induction during stress circuit activation under various conditions.

Disclosures: S.Z. Jiang: None. **W. Brancaleone:** None. **H. Zhang:** None. **L.E. Eiden:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.02/NN3

Topic: F.03. Stress and the Brain

Title: Effect of embryonic cortisol exposure on neuronal marker gene expression in zebrafish larvae

Authors: C. DUNNUM, L. STAEBELL, G. DAVIS, *B. CARTER;
Biol., Univ. of Wisconsin Eau Claire, Eau Claire, WI

Abstract: Exposure to prenatal stress is known to correlate with neurodevelopmental disorders and negative health outcomes later in life. Cortisol is the primary hormone mediating the impacts of prenatal stress. Cortisol has been reported to regulate gene expression of specific neuronal marker genes in adult tissues. We tested how early prenatal cortisol exposure affects mRNA gene expression of different neuronal marker genes in zebrafish using reverse transcription quantitative polymerase chain reaction (RT-qPCR). At 3 hours post fertilization (hpf), zebrafish embryos were treated with 5 uM cortisol solution or vehicle solution. Treatment solutions were fully refreshed daily until 5 days post fertilization (dpf). RNA was then extracted from pooled samples of larvae and converted to cDNA. Gene expression was measured using gene-specific primers with RT-qPCR. Tested genes included markers for different types of neurons (e.g.

glutamatergic, GABAergic, glycinergic) and glia. Differential gene expression analysis was conducted by comparing cortisol-treated samples with vehicle-treated samples using standard methods incorporating primer amplification efficiency values. No significant differences in neuronal marker gene expression based on cortisol treatment were found. These data indicate that this level of embryonic cortisol exposure does not regulate these specific genes in 5dpf zebrafish, suggesting cortisol may not directly affect early development of these types of neurons and glia.

Disclosures: C. Dunnum: None. **L. Staebell:** None. **G. Davis:** None. **B. Carter:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.03/NN4

Topic: F.03. Stress and the Brain

Support: R01DK047320
F32DK124963

Title: Spatial transcriptomic profiling (Molecular Cartography™) of the murine hypothalamus following chronic glucocorticoid administration

Authors: *D. J. TORRES[1], J. NICHOLSON[3], P. TOH[2], M. W. PITTS[4], L. SEALE[3], K. AN[3], A. HANATO[3], M. J. BERRY[3];
[2]Pacific Biosci. Res. Ctr., [1]Univ. of Hawai'i at Manoa, Honolulu, HI; [3]Univ. of Hawaii at Manoa, Honolulu, HI; [4]Dept. of Cell and Mol. Biol., Univ. of Hawaii, Honolulu, HI

Abstract: The role of chronic stress in human disease pathology has become a topic of major interest spanning multiple fields. While the stress response serves an important survival role, high levels of stress can have deleterious effects on an organism, including neurological impairments and disrupted energy homeostasis. Glucocorticoid stress hormones are commonly prescribed as anti-inflammatories but long-term consumption can cause over-eating and excess weight gain in humans and rodents. The micronutrient selenium is essential for proper brain function and is used to synthesize a family of proteins participating in redox reactions called selenoproteins. The ability of selenium to counteract the neurological detriments caused by stress has recently begun to come to light. Conversely, preliminary data suggests that long-term administration of corticosterone negatively regulates the expression of selenoproteins in mice. We hypothesize that this modification of selenoprotein expression contributes to the neurological and metabolic impairments caused by stress. The aim of this study was to assess the transcriptional effects of chronic glucocorticoid exposure in the hypothalamus of the mouse, with a focus on the selenoprotein family and selenium metabolism genes. C57 wild-type mice were administered either corticosterone or vehicle (ethanol) for 4 weeks via drinking water, after which the brain was collected. The smFISH-based Molecular Cartography™ platform (Resolve

Biosciences) was used to detect individual mRNA transcripts of target genes with sub-cellular resolution in sections from the hypothalamus and hippocampus. These data reveal the spatial and cell type-specific interactions between selenium and glucocorticoids in the brain.

Disclosures: D.J. Torres: None. **J. Nicholson:** None. **P. Toh:** None. **M.W. Pitts:** None. **L. Seale:** None. **K. An:** None. **A. Hanato:** None. **M.J. Berry:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.04/Web Only

Topic: F.03. Stress and the Brain

Support: Title III of the Higher Education Act
United Negro College Fund Henry McBay Research Fellowship

Title: Temporal profile of phosphorylated extracellular signal-regulated kinase 1/2 immunoreactivity in the mesolimbocortical stress-responsive circuit nuclei following acute restraint in male rats

Authors: *I. S. JONES[1,2], I. S. NICHOLS-OBANDE[3], C. O. OKERE[1], *I. S. JONES[2];
[1]Biol., Clark Atlanta, Atlanta, GA; [2]Dept. of Natural Sci., Gordon State Col., Barnesville, GA; [3]Biol., Spelman Col., Atlanta, GA

Abstract: Background: Prolonged acute stress has been shown to activate a discrete network of brain areas within the mesolimbocortical neural signal-response circuit. However, it is not clearly understood how the balance between cell proliferation/differentiation and apoptosis is affected by a prolonged, single episode of stress exposure.
Methodology/Principal Findings: To further understand the cellular mechanisms underlying stress-induced cellular activation, this study has compared immunohistochemical mapping of differential expression of caspase activated DNase (CAD, an enzyme that cleaves chromosomal DNA in apoptotic cells) and phosphorylated extracellular signal-regulated kinase (pERK1/2; a signaling pathway preferentially activated in response to growth factors and that regulates cell proliferation and differentiation) in male Wistar rats restrained for 1-, 3-, or 6h versus control (unstrained rats). Significant pERK1/2 immunostaining was observed in the central and medial amygdala, paraventricular nucleus of the hypothalamus, reticular thalamic nuclei, ventral posteromedial thalamus, hippocampus, and retrosplenial and sensory (but not motor) cortical areas. The pERK1/2 expression in most of the brain areas assayed was observed to be the highest within 3h of restraint compared to 1h, 6h, and unrestrained, respectively. Within the dorsal raphe nucleus (DRN), the ventromedial and dorsomedial subregions showed higher pERK1/2 expression compared with equivalent caudal subdivisions. Although low CAD staining was observed within the DRN, determination of complete apoptosis was compounded by colocalization with the nuclear marker DAPI.

Conclusions/Significance: Taken together, these observations suggest a specific stimulus activation of pERK1/2 in a discrete network of brain areas within the mesolimbocortical region that is involved with the processing of stress and sensory stimuli.

Disclosures: I.S. Jones: None. **I.S. Nichols-Obande:** None. **C.O. Okere:** None. **I.S. Jones:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.05/NN5

Topic: F.03. Stress and the Brain

Support: ORWH-U54-MH118919

Title: Transcriptional regulator PLZF/ZBTB16 is induced by stress or glucocorticoids selectively within the murine hypothalamus

Authors: *M. ROUEINFAR[1], *M. ROUEINFAR[2], P. MOHAMMADZADEH[2], R. J. HANDA[2], S. A. TOBET[2];
[2]Biomed. Sci., [1]Colorado State Univ., FORT COLLINS, CO

Abstract: Stress leads to activation of the hypothalamic-pituitary-adrenal (HPA) axis, a major neuroendocrine system that maintain homeostasis in mammals. The hypothalamic paraventricular nucleus (PVN) plays a key role in stress-induced activation of the HPA axis. Stress is also associated with increased inflammatory responses which have been linked to the development of numerous physical and mental health conditions, including depression, metabolic disorders, and immune disorders. While it is known that stress alters gene expression across the HPA axis, frequently in a sex-specific manner, the molecular mechanisms underlying these changes call for additional studies. To assess stress effects on gene expression and identify genes that are essential in regulation of homeostasis, adult wildtype C57BL/6J female and male mice (3-6 mo) were divided into control and stress groups. PVNs were dissected and analyzed via RNA sequencing (RNA-seq). We identified a novel stress-induced and glucocorticoid receptor target transcription factor, called promyelocytic leukemia zinc finger (Plzf), also known as Zbtb16. It has previously been reported that Plzf is induced under various stress conditions via GR signaling in PVN (Cheng et al., *Front. Neurosci. 2020; 14:592947)*. Our data indicates 2-fold induction of Plzf mRNA expression in the PVN of both male and female mice within 2h of either an injection of 1.2 mg/kg dexamethasone or restraint stress. Using immunohistochemistry (IHC), protein expression and localization of Plzf was observed in the PVN after 4h. By 8h the immunoreactive proteins levels appeared to decrease more so in females. Loss of Plzf has been shown to increase the expression in inflammatory cytokines such as Tumor necrosis factor (TNF-α) and interleukin 6 (IL-6) (Sadler et al., 2015: PNAS; 112:1535). Future studies will determine the role of Plzf in the regulation of the HPA axis during development and adulthood

and potential bases for sex differences. We hypothesize that up-regulation of Plzf provides a mechanism for anti-inflammatory functions of glucocorticoids after stress exposure.

Disclosures: M. Roueinfar: None. **M. Roueinfar:** None. **P. Mohammadzadeh:** None. **R.J. Handa:** None. **S.A. Tobet:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.06/NN6

Topic: F.03. Stress and the Brain

Support: National Science Foundation Graduate Research Fellowship under Grant No. DGE-1845298
NIDA Research Project Grant R01DA052465
NIDA Research Project Grant R01MH126027
NIH NIDA Avenir Director's Pioneer Award DP1DA044250

Title: Role of Cyclin dependent kinase 5 in stress

Authors: *K. L. RODRIGUEZ[1], K. CZARNECKI[2], E. A. HELLER[3];
[2]Pharmacol., [3]Dept. of Systems Pharmacol. and Translational Therapeut., [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Acute stress affects learning and memory through changes in both gene expression and protein signaling. Our research focuses on Cyclin dependent kinase 5 (Cdk5), a neuronally expressed protein kinase implicated in key neurobiological processes including negative valence state, synaptic plasticity, and brain development. In male mice, acute and chronic stress differentially regulate Cdk5 protein expression and activity via distinct Cdk5 target phosphorylation that regulate learning and memory. The role of Cdk5 in stress has been scarcely explored in female animal models, yet women exhibit increased risk of psychiatric disorders, including stress and drug use disorders. In our previous work we find that targeted *Cdk5* histone acetylation in mouse hippocampus sex-specifically regulates long term memory in response to fear conditioning. The current project aims to determine the sex and region-specific functional role of Cdk5 protein and gene after stress. To this end, we subjected male and female mice to acute and chronic stressors and characterized Cdk5 protein expression and downstream target phosphorylation. We find activation of Cdk5 protein in stressed animals and dynamic Cdk5-mediated phosphorylation activity.

Disclosures: K.L. Rodriguez: None. **K. Czarnecki:** None. **E.A. Heller:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.07/NN7

Topic: F.03. Stress and the Brain

Support: Research Budget Commission at Oregon Institute of Technology

Title: Neuroanatomical studies of cannabinoid receptor type 1 (CB1R) expression in rat brain

Authors: *B. EICHELKRAUT, J. MORNING, R. PEARSON, J. VALENCIA-MENDEZ, H.-Y. LI;
Oregon Inst. of Technol., Klamath Falls, OR

Abstract: Endocannabinoid (EC) is suggested to play a role in attenuating behavioral and endocrine responses to stress. Cannabinoid Receptor Type 1 (CB1R), the major cannabinoid receptor in the nervous system, expression is widely found in the brain, including the cortex, striatum, amygdala, and hippocampus. Despite its wide distribution, the neuroanatomical evidence underlying the functional implications of CB1R remains unclear. The catecholaminergic system is one of the key neurotransmitter systems involved in the central stress responses. We hypothesized CB1R activation could modulate catecholaminergic system via direct synaptic interaction. Brain sections of adult male Sprague-Dawley rats were processed for immunocytochemical staining of CB1R and tyrosine hydroxylase (TH). Our findings show that CB1R immunoreactivity colocalizes with TH-immunoreactive (ir) terminals, providing anatomical evidence to support the notion that EC can serve as a retrograde messenger in modulating presynaptic neurotransmission. Interestingly, we also discovered colocalization of CB1R-ir cell bodies and TH-ir terminals, indicating TH could possibly regulate the function of CB1R-containing neurons. The infralimbic cortex shows the most concentrated expression of both CB1R and TH, implicating both endocannabinoid and catecholamine exerting their functions collaboratively in regulating cortical functions. The present studies provide important neuroanatomical information regarding the potential mechanisms governing the EC effects in the central stress regulation.

Disclosures: B. Eichelkraut: None. **J. Morning:** None. **R. Pearson:** None. **J. Valencia-Mendez:** None. **H. Li:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.08/NN8

Topic: F.03. Stress and the Brain

Support: Veterans Affairs Office of Academic Affiliations
NIH Grant R01MH108665
NIH Grant K08MH130802

Title: Transcriptional regulation of Wnt signaling and Foxp2 in the amygdala during mouse fear learning

Authors: *O. PONOMAREVA[1,2], E. CATT[1,3], K. J. RESSLER[1,4];
[1]McLean Hosp., Belmont, MA; [2]Boston VA Healthcare Syst., Boston, MA; [3]Northeastern Univ., Boston, MA; [4]Harvard Med. Sch., Boston, MA

Abstract: Post-Traumatic Stress Disorder is a common and debilitating mental illness with limited available personalized treatments. Recent Genome-Wide Association Studies (GWAS) have begun to elucidate significant target genes implicated in this disease. One of these recently identified targets is the transcription factor Forkhead box P2 (*FOXP2*), which is implicated in language learning and neuropsychiatric disorders. In adult mice, *Foxp2* mRNA is expressed in clusters of Intercalated Cells surrounding the basolateral amygdala. Intercalated Cells are functionally important for conditioned fear response and fear extinction; however, the role of the Foxp2 transcription factor, its upstream mediators and downstream targets, in modulating fear-related behaviors in the amygdala remains unknown. Recent evidence suggests that Foxp2 is regulated by multiple components of the Wnt signaling pathway and, in turn, acts in a feedback loop to modulate regulation of Wnt pathway members. Our lab previously demonstrated the role of Wnt signaling pathway components in regulation of adult learning and memory, including the requirement of β-catenin for fear memory consolidation, and dynamic regulation of Wnt components at multiple timepoints after fear learning in adult male mice (Maguschak and Ressler, 2008; Maguschak and Ressler, 2011). The aim of the current study is to expand upon this work and test the hypothesis that Foxp2 regulates specific components of fear learning through its interaction with the Wnt signaling pathway in the Intercalated Cells of the amygdala. Here we show that Wnt transcription is active in the Intercalated Cells of adult male and female mice during fear learning with neutral Conditional stimulus (tone) followed by an aversive Unconditional stimulus (foot shock). We replicate our previously reported results of learning-dependent dynamic regulation of Wnt components in the amygdala with bulk RNA sequencing after fear learning in male and female C57Bl/6 mice. We find previously unidentified Wnt signaling genes (including *Tle6*, *Rfx4*, *Sox4*) to be involved in adult learning and memory. Finally, we present results suggesting that *Foxp2* mRNA is also dynamically regulated after fear learning in mice. The ongoing goal of this study is to further describe the functional role of Foxp2 and its modulators in adult fear learning and memory and identify pharmacological targets for a more personalized treatment of trauma and stressor-related disorders.

Disclosures: O. Ponomareva: None. **E. Catt:** None. **K.J. Ressler:** F. Consulting Fees (e.g., advisory boards); Acer, Bionomics, Jazz Pharma, Sage, Boehringer Ingelheim, Senseye, Brain and Behavior Research Foundation, Brain Research Foundation. Other; Alto Neuroscience.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.09/NN9

Topic: F.03. Stress and the Brain

Support: NIH Grant P50 MH096889
NIH Grant NS28912

Title: Characterization of novel corticotropin-releasing hormone projection cells in the basolateral amygdala

Authors: *Y. CHEN, M. BIRNIE, L. TANIGUCHI, J. DAGLIAN, T. BARAM;
Univ. of California-Irvine, Irvine, CA

Abstract: Background: The basolateral amygdala (BLA) is a heterogenous nuclear complex and projects to brain regions involving in reward, fear, and stress responses. Projections connecting brain regions are generally composed of several cell types defined by their neurotransmitter and often by a co-expressed neuropeptide. We have reported a GABAergic projection from BLA to the nucleus of accumbens (NAc) that expresses the neuropeptide corticotropin-releasing hormone (CRH) (Birnie et al., Nat Commun 2023). Specifically, this GABAergic CRH^+ projection from the BLA to the NAc represses reward behavior. Here, we characterize the CRH projection cells in the BLA and map their projections in mouse brain.
Methods: The identity of BLA CRH cells and the scope of BLA CRH projections were investigated by employing transgenic mice, anterograde and retrograde viral-genetic tracing, coupled with in situ hybridization, 3D imaging, and optogenetics.
Results: CRH cells in the BLA co-localize with GAD mRNA and protein, but not CaMK2, suggesting they are GABAergic. Anterograde tracing via injection of pAAV-FLEX-tdTomato into the BLA of Crh-IRES-Cre mice identified CRH expressing projections from the BLA to the NAc, bed nucleus of the stria terminalis (BNST), prefrontal cortex (PFC), and ventral hippocampus (vHip). The use of rAAV2-retro DIO-CAG-tdTomato injected into above projection targets ascertained distinct subgroups of CRH projection cells in the BLA with distinct neuroanatomical segregation within the BLA. Quantitative analyses identified ~1,200 CRH neurons in each left and right BLA.
Conclusions: GABAergic CRH expressing cells across the BLA are projection cells, targeting brain regions important for stress, reward, and cognition. These cells can be classified as distinct subgroups and each subset of cells sends axonal projection, contacting with its specific target cells.

Disclosures: Y. Chen: None. **M. Birnie:** None. **L. Taniguchi:** None. **J. Daglian:** None. **T. Baram:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.10/NN10

Topic: F.03. Stress and the Brain

Support: NIH Grant AT010984
NIH Grant DA041208
NIH Grant AG065168
NIH Grant MH094268
NIH Grant MH128765
NIH Grant AT008547
NIH Grant NS041435
NIH Grant MH113645
JHU catalyst award
JSCNP
Kanae
JST ERATO JPMJER1902
AMED-CREST JP22gm1010009
JSPS KAKENHI 22H03541
The Japan Dairy Association (J-milk)

Title: Dectin-1 Signaling in Colonic γδ T Cells: Influence on Psychosocial Stress Responses

Authors: V. TRAN[1], *X. ZHU[1], S. SAKAMOTO[3], C. ISHII[4], M. D. SMITH[2], K. ITO[5], M. OBAYASHI[1], L. UNGER[6], Y. HASEGAWA[1], S. KUROKAWA[7], T. KISHIMOTO[7], H. LI[8], S. HATANO[9], T.-H. WANG[10], Y. YOSHIKAI[9], S.-I. KANO[11], S. FUKUDA[4], K. SANADA[12], P. A. CALABRESI[2], **A. KAMIYA**[1];
[1]Dept. of Psychiatry and Behavioral Sci., [2]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Neuropsychiatry, Okayama Univ. Grad. Sch. of Medicine, Dentistry, and Pharmaceut. Sci., Okayama, Japan; [4]Inst. for Advanced Biosci., Keio Univ., Yamagata, Japan; [5]Dept. of Psychiatry, Hokkaido Univ. Grad, Sapporo, Hokkaido, Japan; [6]Inst. of Neuroimmunology and Multiple Sclerosis, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [7]Dept. of Neuropsychiatry, Keio Univ. Sch. of Med., Tokyo, Japan; [8]Sch. of Electrical, Computer and Biomed. Engin., Southern Illinois Univ., Carbondale, IL; [9]Med. Inst. of Bioregulation, Kyushu Univ., Fukuoka, Japan; [10]Departments of Mechanical Engin. & Biomed. Engin., Johns Hopkins Univ., BALTIMORE, MD; [11]Dept. of Psychiatry and Behavioral Neurobio., Univ. of Alabama at Birmingham Heersink Sch. of Med., Birmingham, AL; [12]Dept. of Psychiatry, Showa Univ. Sch. of Med., Tokyo, Japan

Abstract: The intricate crosstalk between the intestinal immune system and commensal microbiota is vital in maintaining gut homeostasis. Recent evidence suggests that stress can disrupt the delicate balance of the microbiome, leading to impaired brain function. However, the underlying mechanisms by which the intestinal immune system mediates these effects remain elusive. Here, we investigate the involvement of colonic γδ T cells and their dectin-1 signaling in shaping behavioral responses to chronic social stress. Our findings highlight a remarkable association between a decline in specific Lactobacillus species, critical for T cell differentiation and immune protection, and stress-induced social avoidance behavior. Importantly, these observations are consistent with our clinical investigations in patients with depression. We demonstrate that susceptibility to stress stems from enhanced differentiation of colonic

interleukin (IL)-17-producing γδ T cells (γδ17 T cells), accompanied by their accumulation in the meninges. We establish a causal link between these stress-prone cellular and behavioral phenotypes and dectin-1, an innate immune receptor prominently expressed in γδ T cells. Our study unveils the previously unrecognized involvement of intestinal γδ17 T cells in modulating psychological stress responses, shedding light on their potential as promising therapeutic targets. Notably, targeting dectin-1 emerges as a compelling strategy for alleviating stress-induced behaviors. These findings deepen our understanding of the intricate gut-brain axis and offer valuable insights into potential avenues for ameliorating the adverse effects of stress on mental health.

Disclosures: **V. Tran:** None. **X. Zhu:** None. **S. Sakamoto:** None. **C. Ishii:** None. **M.D. Smith:** None. **K. Ito:** None. **M. Obayashi:** None. **L. Unger:** None. **Y. Hasegawa:** None. **S. Kurokawa:** None. **T. Kishimoto:** None. **H. Li:** None. **S. Hatano:** None. **T. Wang:** None. **Y. Yoshikai:** None. **S. Kano:** None. **S. Fukuda:** None. **K. Sanada:** None. **P.A. Calabresi:** None. **A. Kamiya:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.11/NN12

Topic: F.03. Stress and the Brain

Support: R01MH111719
R01NS081054

Title: Understanding stress sensitivity: The role of Yin Yang 1 in stress-induced maladaptive behaviors

Authors: *J. MEDINA[1,2], B. XU[2], Z. XIA[1,2], Y. ZHANG[1,2], Y. HO[1,2], Z. ZHOU[1,2];
[1]Genet., Univ. of Pennsylvania, Philadelphia, PA; [2]Penn Epigenetics Inst., Philadelphia, PA

Abstract: Major depressive disorder (MDD) is the leading cause of disability in the U.S. and women are twice as likely to develop MDD compared to men. Despite stress being a major risk factor for MDD, the underlying molecular mechanisms remain poorly understood. Through transcriptome profiling at single-cell resolution, we previously found that layer 2/3 cortical excitatory neurons are particularly vulnerable to chronic stress, showing signatures of reduced neuronal activity and Yin Yang 1 (YY1) regulon. To determine the role of YY1 in mediating stress response, we genetically ablated one copy of Yy1 in forebrain excitatory neurons and found that female conditional knockout mice, but not males, selectively exhibited increased anhedonia, immobility, and anxiety-related behaviors, and only upon mild stress exposure. Via cell type-specific CUT&RUN, we also find YY1 occupancies at the promoter regions of several depression-implicated genes, including Nr3c1 (Glucocorticoid receptor) and Cnnm2 (Cyclin and CBS Domain Divalent Metal Cation Transport Mediator 2), both of which are downregulated in

female conditional knockout mice following stress exposure. We are currently assessing the functional contribution of GR and CNNM2 to stress vulnerability in female mice using CRISPR/dCas9-mediated genomic editing. Together, our findings implicate YY1 as a new regulator of stress response and stress sensitivity, particularly in female mice.

Disclosures: **J. Medina:** None. **B. Xu:** None. **Z. Xia:** None. **Y. Zhang:** None. **Y. Ho:** None. **Z. Zhou:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.12/NN13

Topic: F.03. Stress and the Brain

Support: CIHR project scheme grant awarded to N.S. and G.C.

Title: Sex differences in a stress induced depressive phenotype: a time course of behavioural and central effects

Authors: ***M. RAMLJAK**[1], S. F. SIMARD[1], S. A. SIMARD[1], A. ABOU-FALAH[1], K. LAWRENCE[1], L. BARKOVICH[1], G. COPPOLA[2], N. SALMASO[1];
[1]Dept. of Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Dept. of Pathology, Yale Univ., New Haven, CT

Abstract: One in four people in North America will be diagnosed with an anxiety or mood disorder, making these the most prevalent psychiatric illnesses. Females show a two-fold increase in the prevalence of mood and anxiety disorders and there are similar sex differences in cognitive, depressive, and anxiety behaviours following exposure to acute and chronic stressors in rodents. The relationship between stress and depressive-like phenotypes in rodents has been documented across various stress models. Furthermore, sex differences have been observed in depressive-like phenotypes in a number of these stress models. However, many of these studies show inconsistent results and have employed different stress models for varying durations and therefore a systematic study of sex differences in the response to chronic stress over time is lacking. Furthermore, it is clear that every cell type in the prefrontal cortex (PFC) is involved both in depression and its treatment, including astrocytes, microglia, oligodendrocytes, interneurons and excitatory cells, however the trajectory or onset of these perturbations by cell type remains largely unknown. Indeed, we have previously shown that astroglial cells respond to five weeks of chronic stress by upregulating perineuronal net components, accompanied by an upregulation of perineuronal nets themselves surrounding interneurons in the PFC. However, it is unknown what duration of chronic stress is needed to induce these changes or whether there are sex differences in the onset or trajectory of these changes. As such, in the current work we employed a rodent model of chronic variable stress (CVS) whereby male and female mice were exposed to a time course of chronic stress ranging from one to five weeks in duration followed

by a battery of behavioural testing to assess depressive and anxiety-like behaviours. Samples of dissected PFC underwent flex single-cell sequencing to build a complete phenotypic gene expression profile of every cell type in response to stress in males and females over time. As expected, we found a significant effect of stress on emotive behaviours as early as one-week post-stress onset in males and by two weeks both sexes showed similar stress-induced phenotypes compared to controls. Preliminary cell-specific results suggest that microglia show activation as early as one-week post-stress in males and that interneuron expression of perineuronal nets initially decreases and later increases with continued stress exposure. Altogether, these data show sex differences in behavioural and cellular patterns of response to chronic stress over time.

Disclosures: M. Ramljak: None. **S.F. Simard:** None. **S.A. Simard:** None. **A. Abou-Falah:** None. **K. Lawrence:** None. **L. Barkovich:** None. **G. Coppola:** None. **N. Salmaso:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.13/NN14

Topic: F.03. Stress and the Brain

Support: Intramural Research Program of the NIEHS, NIH (ES 100221)

Title: Revisiting the subcellular localization of hippocampal mineralocorticoid receptors using different antibodies

Authors: *M. R. ROSS, A. GRUZDEV, S. M. JONES, G. M. ALEXANDER, S. M. DUDEK; Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: Within the hippocampus, the mineralocorticoid receptor (MR; *Nr3c2*) operates as a steroid hormone receptor and transcription factor that informs baseline stress responses, learning and memory, and social behaviors. Expression of *Nr3c2* is particularly enriched in CA2 pyramidal cells where it regulates the region's distinctive molecular profile. A present curiosity is how MR's functional state relates to its subcellular localization--which has been shown in both the nucleus and cytoplasm--and how localization within CA2 might differ. Thus, this project explores whether antibodies raised against MR can first identify localization patterns in mouse hippocampal neurons under baseline conditions. Using immunohistochemistry, we assessed antibody specificity by observing fluorescence in mice exhibiting a forebrain knockout (KO) of MR as well as in their control littermates. Of note, this KO was generated from an *Emx1* promotor-driven Cre recombinase paired with loxP sites flanking *Nr3c2*'s hinge region (HR) and a portion of the ligand binding domain (LBD). A panel of monoclonal antibodies—provided by the Gomez-Sanchez group—with epitopes targeting the amino-acid terminal (NTD) presented clear nuclear staining candidates in which control expression was highest in CA2 and expression in KOs was absent in all hippocampal regions. Additional monoclonal antibodies targeting the

NTD exhibited cytosolic staining that appeared to be within the dendrites; however, such staining was still present in KOs. A panel of commercially-available polyclonal antibodies targeting the DBD or LBD regions also exhibited cytosolic staining in controls which remained present in KOs. Moving forward, verification of the presence or absence of truncated translation products in the KOs will be necessary to determine if the antibodies targeting the NTD or DBD are specific. Lastly, we sought an additional localization strategy and thus created a mouse strain in which the C-terminus of *Nr3c2* was HA-tagged via single cell C57BL/6J embryo CRISPR/Cas9 microinjection. Preliminary staining with anti-HA antibodies exhibited primarily nuclear staining that was enriched in CA2. Overall, results so far support a model in which hippocampal MR is localized within the nucleus under baseline conditions, suggesting an activated state.

Disclosures: M.R. Ross: None. **A. Gruzdev:** None. **S.M. Jones:** None. **G.M. Alexander:** None. **S.M. Dudek:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.14/NN15

Topic: F.03. Stress and the Brain

Support: R01MH123993
R01MH108665
P50MH115874

Title: The role of mPFC projections to CRF-positive neurons in BLA in stress-associated effects on fear-related behaviors in mice

Authors: X. DENG, Y. LI, B. PENG, S. SHRESTHA, *V. Y. BOLSHAKOV;
McLean Hosp- Harvard Med. Sch., Belmont, MA

Abstract: Anxiety-related disorders, such as Post-Traumatic Stress Disorder (PTSD) and generalized anxiety, are characterized by dysregulation of fear system in the brain, including inability to extinguish fear memories. Neural circuits underlying anxiety and fear regulation involve projections from the medial prefrontal cortex (mPFC) to amygdala, where fear responses are promoted or suppressed by distinct cell types, and the function of these circuits can be affected by repeated stress. As neurons expressing neuropeptide corticotropin-releasing factor (CRF) are known to play key roles in control of anxiety and fear mechanisms, we aimed to understand how specific projections from mPFC to CRF-positive neurons in the basolateral amygdala (BLA) may contribute to stress-associated dysregulation of fear and anxiety-related behaviors. Consistent with the previous reports, the repeated stress protocol, consisting of repeated footshocks for 7 days (RFS), resulted in enhanced anxiety levels in our experiments, as assessed with elevated plus maze (EPM) and open field (OF) behavioral tests in CRF-tdTomato

reporter mice expressing ChR2 in PL division of the mPFC. To assess the effects of RFS on neurotransmission in projections from mFC to CRF-BLA neurons, we examined synaptic responses in these cells triggered by photostimulation of ChR2-expressing mPFC fibers. The light-induced EPSCs recorded in CRF-BLA neurons under these conditions were monosynaptic and blocked by glutamate receptors' antagonists. Performing recordings in slices from control and repeatedly stressed mice, we found that the efficacy of glutamatergic synaptic transmission in mPFC inputs to CRF-BLA neurons, as assessed with the input-output curves for photostimulation-induced EPSCs, was enhanced in the RFS group compared to control mice, possibly contributing to anxiety enhancing effects of RFS. The observed synaptic strengthening was likely due to presynaptic modifications as the paired-pulse ratio (PPR; an index of presynaptic function) at short interpulse intervals was decreased in repeatedly stressed mice, whereas the AMPAR/NMDAR EPSC amplitude ratio remained unchanged. Notably, we found that fear extinction memory was suppressed in repeatedly-stressed mice, suggesting a possibility, which will be systematically tested in future studies, that RFS-associated synaptic enhancements in projections from mPFC to CRF-BLA cells could contribute to both stress-induced anxiety and fear extinction impairments in repeatedly-stressed subjects.

Disclosures: **X. Deng:** None. **Y. Li:** None. **B. Peng:** None. **S. Shrestha:** None. **V.Y. Bolshakov:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.15/NN16

Topic: F.03. Stress and the Brain

Support: NIH R01 Grant MH123545

Title: Depletion of glucocorticoid receptors in prefrontal cortex pyramidal neurons mitigates stress-induced synaptic and behavioral deficits

Authors: *D. DADOSKY[1,2], H. ASRAT[2], L. VOLLMER[2], S. DAVIDSON[3], E. WOHLEB[2]; [2]Pharmacol. and Systems Physiol., [3]Anesthesiol., [1]Univ. of Cincinnati, Cincinnati, OH

Abstract: Neuroendocrine responses to chronic stress can promote physiological and behavioral adaptations through glucocorticoid receptor (GR) signaling. Specifically, GR signaling has been implicated in stress-induced structural remodeling on pyramidal neurons in the prefrontal cortex (PFC). Significantly, stress-induced synaptic loss in the PFC underlies behavioral deficits and working memory impairments. Our recent studies indicate that GR signaling engages neuron-microglia interactions and this contributes to synapse loss on neurons in the PFC. Despite this work, the cell type-specific role of GR signaling in stress has not been studied. Thus, we aimed to determine how GR depletion in PFC pyramidal neurons influenced neurobiological and behavioral responses to chronic unpredictable stress (CUS). Transgenic mice targeting the *Nr3c1*

gene (GR-flox) or wild-type conspecifics received bilateral infusion of AAV5-Camk2a-Cre-mCherry in the medial PFC to selectively deplete GR in pyramidal neurons. Immunofluorescence (IF) showed that GR-flox mice had a significant reduction in GR levels in mCherry+ neurons compared to wild-type mice. Additionally, all mice had elevated plasma corticosterone levels and reduced weight gain after exposure to CUS, suggesting that GR depletion did not influence stress responses. We found that wild-type mice exposed to CUS had impaired discrimination in temporal object recognition and that this deficit was mitigated in GR-flox mice. Patch-clamp electrophysiology was performed on mCherry+ pyramidal neurons in layer 5 of the PFC. We observed that GR-flox cells have an increased rising slope of action potentials and have a greater frequency of 5-HT-evoked EPSCs, compared to wild-type cells. Current studies are also examining the effects of CUS and GR depletion on neurophysiology and excitability. Together, these findings suggest the GR depletion increases neuronal excitability. Ongoing studies are using histological approaches to quantify dendritic spine density and glial morphology in the PFC as well as fluorescence-activated nuclei sorting to sort neuronal and non-neuronal nuclei from the PFC to explore gene pathways influenced by GR depletion and CUS exposure. Altogether, our results provide strong evidence that GR depletion specifically in PFC pyramidal neurons attenuates neurobiological and behavioral consequences of CUS.

Disclosures: D. Dadosky: None. **H. Asrat:** None. **L. Vollmer:** None. **S. Davidson:** None. **E. Wohleb:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.16/NN17

Topic: F.03. Stress and the Brain

Support: MH108286
MH129495
HD097093
HD105771

Title: Cellular mechanisms of stress-mediated allostasis regulate intercellular communication by extracellular vesicles

Authors: *N. R. MOON[1], C. P. MORGAN[3], N. EPPERSON[2], T. BALE[1];
[2]Psychiatry, [1]Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [3]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Stress drives cellular allostasis, however, the molecular mechanisms and intra- and intercellular signaling important for changes in cellular communication are unclear. In males, mechanistic studies identified lasting changes following chronic stress at epididymal epithelial cells (EECs) that provide sperm with essential maturation signals. As stress-responsive

modulators of cellular energy, mitochondria are likely mediators of allostasis. Therefore, we utilized this system to examine the hypothesis that prior stress initiates allostatic mechanisms to establish a new metabolic set point. Using cellular respirometry and transmission electron microscopy, we found that prior stress decreased EEC energy requirements, oxidative respiration, and altered mitochondrial ultrastructure. Mechanistically, mitochondrial complex I was identified as a key regulator of this new allostatic state by weighted gene co-expression network analysis, respirometry, and enzyme function assays. Furthermore, using CUT&RUN sequencing, a high-efficiency epigenetic profiling approach, we revealed that stress significantly increased binding by the ubiquitous transcriptional repressor, H3K27me3, at 7283 regions of the EEC genome. Interestingly, differentially H3K27me3 bound regions were associated with mitochondrial processes by Gene Set Enrichment Analysis revealing that allostasis is balanced by regulation at both the nucleus and the mitochondria. As extracellular vesicles (EVs) secreted by EECs convey cargo altered by stress and necessary for sperm maturation, we assessed the role of EVs as intercellular communicators of energy regulation. Amazingly, stress-EV exposure increased sperm mitochondrial respiration, supporting a signaling pathway by which a new allostatic state can be communicated to other cells. As stress-mediated allostatic changes in EECs influence sperm physiology via EV cargo, ongoing longitudinal human subjects studies will use a multilevel modeling approach to assess the impact of ACEs and perceived stress on sperm motility, sncRNAs, and critical EV cargo and characteristics important for offspring neurodevelopment within and between subjects. Together, these studies identify cellular mechanisms of allostasis following stress that regulate somatic to germ cell signaling. These regulatory mechanisms broadly apply to other stress-vulnerable cells, including neurons and glia, and are important to better understand the enduring pathophysiology of trauma. Further, by identifying the functional roles of EVs following stress, we establish a foundation for the development of future therapeutic interventions.

Disclosures: **N.R. Moon:** None. **C.P. Morgan:** None. **N. Epperson:** None. **T. Bale:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.17/NN18

Topic: F.03. Stress and the Brain

Support: NIMH 129495
NIMH 108286
NICHD 105771
NICHD 097093

Title: DREADD-ing stress: using chemogenetics to examine lasting cellular and physiological outcomes of chronic CRF neuron activation

Authors: *K. MONTGOMERY, A. JENG, I. SIBLEY, T. BALE;
Psychiatry, Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Chronic lifetime adversity is one of the strongest predictors of neuropsychiatric disease. Further understanding of the biological mechanisms underlying disease risk requires consideration of additional factors that interact with chronic stress, such as biological sex, and is essential for developing novel therapeutic interventions. Incoming stress signals are integrated by corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN). These neurons project to the median eminence and initiate the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis response that ultimately results in glucocorticoid release, but also project to the locus coeruleus to modulate the autonomic arm of the stress response. We previously showed that chemogenetic activation of CRF neurons in mice replicates the physiological effects of traditional stress paradigms using the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) system to express the Gq-coupled receptor hM3Dq in all CRF neurons. We found that chronic administration of the DREADD ligand clozapine-N-oxide (CNO) induced sex-specific behavioral and physiological effects, where females displayed heightened freezing during auditory fear conditioning and males showed classic physiological stress phenotypes and elevated HPA axis reactivity. To examine the biological mechanisms underlying these sex-specific outcomes, ongoing studies using our novel DREADD stress model are utilizing single-nucleus RNA sequencing to determine how chronic activation differentially affects the median eminence-projecting and locus coeruleus-projecting populations of PVN CRF neurons in males and females. Additional investigations using implantable radio telemetry will assess the lasting physiological effects of chronic stress driven by the autonomic stress response. We are also using pharmacological manipulations of the CRF and vasopressin systems to further examine the well-established sex-differences in adult rodent HPA axis reactivity and determine how these underlying differences may interact with chronic stress. Together, results from these studies will provide novel insight into the lasting cellular and physiological changes resulting from chronic stress that may underlie sex-specific disease risk.

Disclosures: K. Montgomery: None. **A. Jeng:** None. **I. Sibley:** None. **T. Bale:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.18/NN19

Topic: F.03. Stress and the Brain

Support: MH129495

Title: Functional and transcriptomic changes of Merkel cells in response to pubertal sensory stress in adult mice

Authors: *A. C. KORGAN[1,1], L. M. FOLTS[2], K. R. MONTGOMERY[1,1], I. SIBLEY[1,1], T. L. BALE[1];
[1]Psychiatry, Univ. of Colorado Anschutz Med. Sch., Aurora, CO; [2]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Physiological stress responses trigger cascades of tissue and cell-specific reactions. Chronic or severe stressors increase susceptibility to disease throughout the lifespan, especially during critical developmental windows. We previously identified a pubertal developmental window when sexual trauma in women or chronic sensory stress in mice caused significant changes in stress reactivity and lifelong stress-related disease. Utilizing an unbiased proteomics approach in circulating extracellular vesicles (EV) isolated from adult women and mice, we found a robust and novel signal from several keratin-related proteins from the 17q21 (human) and 11gD (mouse) cluster of genes; these are unique to Piezo2 Merkel cells, responsible for mechanosensory response to light touch. Critically, Piezo2 Merkel cells are the only neuron-like cells in the skin and reach full maturation during the same stage of development as the pubertal window that resulted in trauma and stress-induced changes to EV protein signatures. Increased tactile sensitivity is common in many neuropsychiatric disorders (ADHD, ASD, PTSD). The current study examines the novel role of Merkel cells in stress-induced dysfunction in a mouse model of chronic sensory stress exposure during the critical pubertal window (PN21-35). We utilized Piezo2-cre Ribotag mice to examine the adult transcriptome of Merkel cells following pubertal stress. Differential gene expression analysis found differences in dorsal neck/back, paw pad, and lip between stressed and unstressed male and female mice. We also utilized DREADD inhibition or activation in $Krt14^+$ Merkel cells to test their unique contribution to adult stress and sensory sensitivity. First, we identified the *necessity* of Merkel cell activation in programming stress and sensory sensitivity by inhibiting Merkel cells in Krt14-EGFP-Cre DREADD Gi (hM4Di) mice during pubertal chronic sensory stress and testing adult stress reactivity and assessing Merkel cell gene expression and function. Second, we identified the *sufficiency* of Merkel cell activation in programming stress and sensory sensitivity by activating Merkel cells using Krt14-EGFP-Cre DREADD Gq (hM3Dq) mice during the pubertal window and testing adult stress reactivity and assessing Merkel cell gene expression and function. These models allowed us to assess causal mechanisms by testing for *necessity* and *sufficiency* of these cells in defined stress-relevant behavioral and cellular outcomes. Results from these studies provide new therapeutic interventions and identify novel mechanisms involved in stress-related diseases.

Disclosures: A.C. Korgan: None. **L.M. Folts:** None. **K.R. Montgomery:** None. **I. Sibley:** None. **T.L. Bale:** None.

Poster

PSTR492. Cellular Actions of Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR492.19/NN20

Topic: F.03. Stress and the Brain

Support: FAPERJ Grant 202.944/2015
FAPERJ Grant 202.744/2019
FAPERJ Grant 201.432/2014
CNPq Grant 473324/2013-0
CNPq Grant 434093/2018-1
CNPq Grant 311487/2019-0
CNPq Grant 406436/2016-9
Alzheimer's Association Grant AARG-D-615714
Serrapilheira Institute Grant R-2012-37967

Title: Brain FNDC5/irisin expression in patients and mouse models of major depression

Authors: *R. A. S. LIMA-FILHO[1], J. S. FORTUNA[2], D. COZACHENCO[1], A. R. ISAAC[1], N. LYRA E SILVA[4], A. L. SALDANHA[1], L. E. SANTOS[3,5], S. T. FERREIRA[3,1,5], M. V. LOURENCO[1], F. G. DE FELICE[4,1,5];
[1]Inst. of Med. Biochem. Leopoldo De Meis, [2]Inst. of Biomed. Sci., [3]Inst. of Biophysics Carlos Chagas Filho, Federal Univ. of Rio De Janeiro, Rio de Janeiro, Brazil; [4]Queen's Univ., Queen's Univ. Ctr. For Neurosci. Studies, Kingston, ON, Canada; [5]D'Or Inst. for Res. and Educ., Rio de Janeiro, Brazil

Abstract: Major depressive disorder (MDD) is a major cause of disability in adults. MDD is both a comorbidity and a risk factor for Alzheimer's disease (AD), and regular physical exercise has been associated with reduced incidence and severity of MDD and AD. Irisin is an exercise-induced myokine derived from proteolytic processing of fibronectin type III domain-containing protein 5 (FNDC5). FNDC5/irisin is reduced in the brains of AD patients and mouse models. However, whether brain FNDC5/irisin expression is altered in depression remains elusive. Here, we investigate changes in fndc5 expression in postmortem brain tissue from MDD individuals and mouse models of depression. We found decreased fndc5 expression in the MDD prefrontal cortex, both with and without psychotic traits. We further demonstrate that the induction of depressive-like behavior in male mice by lipopolysaccharidedecreased fndc5 expression in the frontal cortex, but not in the hippocampus. Conversely, chronic corticosterone administration increased fndc5 expression in the frontal cortex, but not in the hippocampus. Social isolation in mice did not result in altered fndc5 expression in either frontal cortex or hippocampus. Finally, fluoxetine, but not other antidepressants, increased fndc5 gene expression in the mouse frontal cortex. Results indicate a region-specific modulation of fndc5 in depressive-like behavior and by antidepressant in mice. Our finding of decreased prefrontal cortex fndc5 expression in MDD individuals differs from results in mice, highlighting the importance of carefully interpreting observations in mice. The reduction in fndc5 mRNA suggests that decreased central FNDC5/irisin could comprise a shared pathologic mechanism between MDD and AD.

Disclosures: R.A.S. Lima-Filho: None. **J.S. Fortuna:** None. **D. Cozachenco:** None. **A.R. Isaac:** None. **N. Lyra e Silva:** None. **A.L. Saldanha:** None. **L.E. Santos:** None. **S.T. Ferreira:** None. **M.V. Lourenco:** None. **F.G. De Felice:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.01/OO1

Topic: F.03. Stress and the Brain

Support: CIHR

Title: Hypothalamic persistent activity state evoked by stress

Authors: *T. FUZESI[1], M. ROJAS-CARVAJAL[3], T. CHOMIAK[4], A. BISHT[1], K. SIMONE[2], N. RASIAH[5], D. G. ROSENEGGER[1], N. DAVIU[6], L. A. MOLINA[1], K. MURARI[1], W. NICOLA[1], J. BAINS[1];
[2]Schulich Sch. of Engin., [1]Univ. of Calgary, Calgary, AB, Canada; [3]Hotchkiss Brain Inst., Hotchkiss Brain Inst., Calgary, AB, Canada; [4]Clin. Neurosci, Univ. Calgary, Calgary, AB, Canada; [5]Hotchkiss Brain Inst. - Univ. of Calgary, Calgary, AB, Canada; [6]Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: In order to survive, organisms must respond effectively to stress. In order to thrive, however, organisms must recover from the stress *and* retain salient information about the stressor. Examining neural dynamics of key cell population in the time after stress may offer important insights into how the brain processes stressful experiences, yet we know relatively little about neural dynamics immediately *after* an acute stress. By measuring local hemodynamics and imaging population calcium dynamics of hypothalamic CRH neurons that coordinate the stress response, we detected an activity pattern that is evident exclusively after a threat. Recording calcium signal from individual CRH neurons in vivo revealed a stable activity state that persists for minutes after exposure to footshock. This persistence does not reflect an increase in activity of the population that exhibits a slow recovery function. Rather, computational techniques revealed that this post stress activity represents a persistent activity state that is distinct from the resting state observed prior to the threat exposure. We propose that the post-stress persistent activity state may represent a critical period that promotes adaptive coping behaviors and allows organisms to process and retain information about the aversive experience.

Disclosures: T. Fuzesi: None. **M. Rojas-Carvajal:** None. **T. Chomiak:** None. **A. Bisht:** None. **K. Simone:** None. **N. Rasiah:** None. **D.G. Rosenegger:** None. **N. Daviu:** None. **L.A. Molina:** None. **K. Murari:** None. **W. Nicola:** None. **J. Bains:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.02/OO2

Topic: F.03. Stress and the Brain

Support: NSERC Discovery Grant
CIHR Foundation Grant
IZAAK WALTON KILLAM MEMORIAL SCHOLARSHIP
Cumming School of Medicine Graduate Scholarship

Title: Acute exercise erases stress-induced synaptic priming in CRH-PVN neurons

Authors: *M. ROJAS-CARVAJAL[1], T. FUZESI[2], D. V. BAIMOUKHAMETOVA[1], N. DAVIU[1], S. COOK[1], J. BAINS[1];
[1]Hotchkiss Brain Inst., [2]Univ. of Calgary, Calgary, AB, Canada

Abstract: Neuroendocrine and autonomic responses to stress are critical for survival. Stress imprints the brain to promote adaptations to future stressors and may contribute to maladaptive responses implicated in neuropsychiatric diseases. Multiple plasticity mechanisms have been described, but little is known about how these processes might be reversed. In humans, exercise is used to buffer stress. Here we tested the effects of exercise on synaptic metaplasticity induced by acute stress in corticotropin release hormone cells in the paraventricular nucleus of the hypothalamus (CRH^{PVN}). We examined the effects of exercise on short-term potentiation (STP) of glutamate synapses on CRH^{PVN} neurons. We obtained whole-cell patch clamp recordings from mouse CRH^{PVN} neurons in hypothalamic slices and evaluated the effects of running for 1h after acute stress on STP. Following footshock, high frequency stimulation (HFS) of glutamate synapses on CRH^{PVN} elicited STP. By contrast, STP was blunted if footshock was followed by exercise. To investigate how exercise affects the downstream neuroendocrine response after stress, we quantified circulating corticosterone (CORT). CORT levels increased 15 min after stress and further increased after exercise. Exercise promotes brain-derived neurotropic factor (BDNF) release. It acts on the tropomyosin receptor kinase B (TrkB) and modulates glutamatergic receptor subunits. We used western blot to quantify protein levels of BDNF, and pGluA1 (AMPA), pGluN2A (NMDA) subunits. We also investigated the role of TrKB receptor activation on stress induced STP and CORT. Our results suggest that exercise may be reversing metaplasticity in the CRH^{PVN} neurons through the BDNF-TrkB pathway.

Disclosures: M. Rojas-Carvajal: None. **T. Fuzesi:** None. **D.V. Baimoukhametova:** None. **N. Daviu:** None. **S. Cook:** None. **J. Bains:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.03/OO3

Topic: F.03. Stress and the Brain

Support: IA/I/14/1/501306
DST/CSRI/2017/271
(BT/INF/22/SP17358/2016

Title: Early life stress induced murine olfactory perceptual learning deficits involves somatostatin releasing inhibitory interneurons of olfactory bulb

Authors: *M. PARDASANI[1,2], A. RAMAKRISHNAN[1], S. MAHAJAN[1], M. KANTROO[1], E. MCGOWAN[1], S. DAS[1], P. SRIKANTH[1], S. PANDEY[1], N. ABRAHAM[1];
[1]Dept. of Biol., Indian Inst. of Sci. Educ. and Res., Pune, India; [2]HBI, Calgary, AB, Canada

Abstract: Adversity during early postnatal life can be detrimental. Depending on the nature and duration of the Early life stress (ELS) paradigm, it can induce a variety of behavioral abnormalities. Apart from the emotional disturbances that it can cause, ELS can also lead to alterations in sensory perception which can negatively affect daily activities of an animal. In this study, we employed early weaning paradigm as ELS. Olfaction is one of the key senses in rodents that are prone to stress-mediated changes, we were interested in assessing how early weaning can affect murine olfactory perceptual behaviors. Poor olfactory learning and memory were observed on a Go/No-go odor discrimination paradigm in the early weaned mice. Further, we found a reduction in the learning-induced c-Fos activity in the external plexiform layer of the Olfactory bulb (OB), the first pre-cortical station that underlies olfactory behaviors. We also found decreased dendritic complexity in the somatostatin-releasing inhibitory inter-neurons (SOM-INs) of the OB. A decline in the synaptic inhibitory feedback on the principal neurons of the OB, by carrying out in vitro whole-cell recordings, was suggestive of impairment within the OB circuit of ELS mice. Further, the role of SOM-INs in olfactory discrimination behaviors was facilitated by the learning-dependent refinement of calcium activity quantified using GCAMP6f signals. Upon optogenetic bi-directional modulation of SOM-INs, we could identify the changes occurring in the behavior. Photo-activating SOM-INs rescued ELS-dependent learning deficit while photo-inhibiting completely abolished learning in ELS mice. In addition, photo-inhibiting them in control mice phenocopied the ELS-induced learning deficits. Thus, by using a multi-pronged approach, we have shown that bulbar micro-circuitry involving SOM-INs play a major role in mediating ELS-mediated deficits.

Disclosures: M. Pardasani: None. **A. Ramakrishnan:** None. **S. Mahajan:** None. **M. Kantroo:** None. **E. McGowan:** None. **S. Das:** None. **P. Srikanth:** None. **S. Pandey:** None. **N. Abraham:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.04/OO4

Topic: F.03. Stress and the Brain

Support: CIHR Foundation Grant
BBRF Young Investigator Award

Title: Hypothalamic CRH neurons generate an internal danger signal to drive escape

Authors: *N. DAVIU[1], K. SIMONE[2], T. FUZESI[3], J. BAINS[3];
[1]Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada; [2]Schulich Sch. of Engin., [3]Univ. of Calgary, Calgary, AB, Canada

Abstract: Selecting the optimal defensive strategy in response to threats is critical for survival. This complex decision process is governed by multiple brain regions and can be influenced by both external and internal factors. Advancing aerial predators trigger escape behavior that relies on recruitment of several key nuclei. CRH^{PVN} neurons show an increase in activity in response to the visual threat that anticipates escape initiation. We have previously shown that uncontrollable stress decreases escape behavior by reducing CRH^{PVN} anticipatory activity in response to a looming, advancing predator. Similarly, optogenetic inhibition of CRH^{PVN} neurons prior to presentation of the visual stimulus decreases escape, indicating a key role for these cells in escape initiation. Here we sought to better understand **the activity signatures of individual CRH^{PVN} neurons during defensive escape.** We used head-mounted miniscopes in freely moving animals to evaluate single cell calcium activity during the behavioral task. We focused on two different time points, prior to and during presentation of the visual stimulus to assess changes in cell activity in response to the stimulus. We also asked whether the escape decision is governed by a hard-wired network of cells and probed the requirement for the time-specific cell activity in the execution of the proper behavioural outcome.

Disclosures: N. Daviu: None. **K. Simone:** None. **T. Fuzesi:** None. **J. Bains:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.05/OO5

Topic: F.03. Stress and the Brain

Support: NIH NIAAA R00 AA027774
Brain and Behavior Research Foundation NARSAD Young Investigator Award 30996

Title: Stress-induced recruitment of insular cortex neuronal activity is driven by upstream basolateral amygdala inputs

Authors: *A. BUCH[1,2], J. LITTLE[2,1], S. CENTANNI[2,1];
[2]Physiol. and Pharmacol., [1]Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: Stress is a regular part of our lives, yet the fundamental neurocircuitry recruited while experiencing this behavioral state is unclear. Neural circuits are the basic functional units that encode for behaviors, providing a unique insight into the functional causes and changes seen with behavior. Though stress disorders are common in our society, foundational studies are needed to uncover the networks engaged during a stressor. Identifying distinct stress circuit biomarkers has the potential to facilitate early detection and intervention of stress-related disorders.
Our previous work to characterize in vivo neuronal activity in a mouse model of stress indicate an increase in glutamate and calcium in the insula, suggesting the insula as an integral network hub for stress response. New tools emerging in recent years allow us to isolate neurons activated during specific behaviors. To better understand the insular mechanisms during a stress response, we isolated and gained access to insula stress ensembles by using FosTRAP2 knock-in mice that express Cre under a *fos* promoter, a proxy for neuronal activity. This strategy confirmed an increase in expression of *fos*-trapped cells in the insula after stress. Next, we wanted to map the route of these neurons to establish a circuitry. We used a retrograde viral tracer expressed exclusively in insula stress ensembles, and utilized whole-brain light sheet microscopy, a powerful tool for unbiased mapping for discovery of new and unique circuits. This experiment revealed that insular stress ensembles receive inputs from several diverse brain areas, the densest projections coming from the anterior basolateral amygdala (aBLA), a brain region implicated in emotional processing and learning. We hypothesize that the BLA will project hyperexcitatory glutamatergic signals to the insula, causing the overall insular glutamate increase we have observed in a stressed state. It is possible that the hyperexcited insula then sends downstream signals to the BNST, which we and others have determined regulates negative emotional states. To establish a functional relationship of BLA-insula, current studies are using viral retrograde tracing and channelrhodopsin-assisted circuit mapping with patch clamp electrophysiology. The current line of studies will help us dissect the BLA-insula stress circuitry and potentially identify a unique BLA-insula-BNST pathway involved in regulating affective state. Using these novel technologies to understand the components of stress circuitry will help us better identify diagnosis and treatment options of many disease states that are often comorbid with stress.

Disclosures: **A. Buch:** None. **J. Little:** None. **S. Centanni:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.06/OO6

Topic: F.03. Stress and the Brain

Support: NIH NIAA ROO-41000000054
NARSAD/BBRF - 110000001515

Title: The mid-insula-BNST pathway drives stress-induced susceptibility to negative affect-like behaviors in alcohol abstinence

Authors: *B. M. WILLIAMS, J. R. LITTLE, S. W. CENTANNI;
Physiol. and Pharmacol., Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: It is well known that negative affect and alcohol use disorder (AUD) are highly correlated, however the underlying brain circuits involved are poorly understood. We have previously shown that a mid-insular-BNST circuit drives negative affect-like behavior associated with alcohol (EtOH) abstinence in female mice. In a follow up study, we demonstrated that this same circuit is recruited while mice are undergoing restraint stress. Moreover, chemogenetically enhancing activity specifically in this pathway was sufficient to induce a strong negative affect-like phenotype in a novel suppressed feeding test (NSFT) model. Taken together, this stress- and alcohol-sensitive insula-BNST pathway could serve as a circuit biomarker for susceptibility to stress-induced relapse in alcohol abstinence. We hypothesized that activity in the insula-BNST pathway and coping behavior during 5 consecutive days of stress could predict subsequent EtOH drinking patterns and negative affect-like state during abstinence in a two-bottle choice continuous access drinking model. Then, mice underwent six weeks of EtOH drinking and two weeks of forced abstinence. We found that stress exposure increased negative affect-like behavior in protracted abstinence relative to non-stressed female mice, but only in ~50% of the mice, indicating the existence of a subpopulation that is vulnerable to stress. In the subpopulation of mice that were vulnerable to stress, EtOH consumption was positively correlated with negative affect-like behavior (NSFT latency), however in the mice that were resilient to stress, EtOH consumption was negatively correlated with NSFT latency. Moreover, the same correlation exists between NSFT latency and the number of escape bouts during stress. Interestingly, inhibiting the insula-BNST circuit prior to stress exposure significantly reduced EtOH consumption in male mice, but not in females. To assess the role of the insula-BNST pathway in stress-induced susceptibility to negative affect in abstinence, we chemogenetically decreased activity specifically in insula-BNST neurons prior to restraint stress exposure for five consecutive days. Surprisingly, this had no effect on subsequent ethanol drinking patterns, suggesting this pathway may not drive stress-induced drinking. However, chemogenetically inhibiting the insula-BNST pathway attenuated the stress induced increases in NSFT latency, suggesting that this circuit is involved in susceptibility to stress during protracted ethanol abstinence. These results indicate that there are potential differences in insular-BNST signaling that may predict stress vulnerability during forced abstinence.

Disclosures: B.M. Williams: None. **J.R. Little:** None. **S.W. Centanni:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.07/OO7

Topic: F.03. Stress and the Brain

Support: NIMH Grant RO1MH115016

Title: Amygdala afferent influences on primate ventral midbrain dopamine (DA) systems: A view from soma and dendrites.

Authors: *E. KELLY[1], J. L. FUDGE[2];
[1]Univ. of Rochester SMD, Rochester, NY; [2]Dept of Neurosci. and Psychiatry, Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: In primates, the DA neurons are physiologically heterogeneous with respect to both intrinsic firing and coding properties across the medial to lateral expanse of the ventral midbrain. The central nucleus (CeN) of the amygdala innervates the midbrain and is a direct conduit by which emotional cues can affect DA activity. This projection to the ventral midbrain largely avoids the midline subnuclei of the A10 (VTA), instead terminating over the laterally placed, elongated **PBP (A10)**, as well as **A8**. A key question is whether the CeN path terminates onto DA, GABAergic, or both neuronal types. We used anterograde injections (n=5) into the CeN to examine amygdalo-nigral path terminals onto 1) proximal dendrites and soma and 2) more distal dendritic sites, of both DA and GABAergic neurons. After light microscopic mapping of labeled fibers, we first examined terminal contacts onto TH immunoreactive (IR) or GAD67-IR cell soma/proximal dendrites using double immunofluorescent processing and confocal microscopy. Terminal overlap with DAergic and GABAergic neuronal soma/proximal dendrites was assessed using Imaris surface/spot rendering. Similar proportions of TH+ and GAD+ cells received at least one contact from CeN-originating afferents across both subregions, with the number of axonal contacts per neuron ranging from 1-10. However, most postsynaptic neurons had 1-2 contacts, regardless of cell type or region, suggesting that neuronal phenotype did not predict the chance of a CeN afferent contact or the number of contacts per cell soma. Next, using dual immuno-peroxidase electron microscopy for tracer (DAB) and TH or GAD (gold), we found that tracer-IR axon terminals made more inhibitory (symmetric) synaptic profiles compared to excitatory type (asymmetric) synaptic profiles overall (57% versus 43%, $p = 0.04$), with similar ratios in PBP versus A8 regions. DAB-IR axon terminal synapses showed no significant difference in the proportion contacting gold-tagged TH+ and GAD+ dendrites (20.25 versus 27.50, $p=0.30$) in either PBP or A8 [$F(3,4)=5.987$, $p=0.06$], paralleling the pattern onto TH+ and GAD+ neuronal soma/proximal dendrites seen in confocal studies. The inhibitory/excitatory ratio of synaptic profiles onto both TH+ and GAD+ dendrites was similar at 1.3 in both in the PBP and A8. Our findings indicate that CeN inputs to the ventral midbrain (PBP and A8) are similar across regions in terms of inhibitory/excitatory contacts. Furthermore, confocal and EM methods revealed that across the PBP/A8, DA and GABA phenotypes do not predict either the number of CeN contacts or the ratio of inhibitory/excitatory terminals innervating them.

Disclosures: E. Kelly: None. **J.L. Fudge:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.08/OO8

Topic: F.03. Stress and the Brain

Title: Unraveling the Interplay Between Central and Peripheral Mechanisms in Acute Restraint Stress: Insights from Basolateral Amygdala Activity

Authors: *F. S. POLLI[1], I. F. NALEPA[2], T. BRUUN[1], R. CHRISTENSEN[2], V. NIELSEN[2], C. HOUGAARD[1], B. HALL[1], M. GRUPE[3], P. BOTTA[1], C. RATNER[2];
[1]Circuit Biol., [2]Preclinical Fluid Biomarkers & Occupancy, [3]Symptom Biol., H. Lundbeck A/S, Copenhagen, Denmark

Abstract: Acute restraint stress (ARS) in rodents elicits a cascade of physiological responses, involving the activation of the hypothalamic-pituitary-adrenal axis and alterations in neurotransmitter systems. Despite its usefulness as a tool for studying therapeutic treatments, the interplay between central mechanisms and the effects of corticosterone feedback in the brain during stress responses constitute challenges in data interpretation. Here, we provide valuable insights into stress physiology by monitoring the activity of the basolateral amygdala (BLA), a critical brain region orchestrating adaptive behavioral and physiological responses to stressors. We aimed to bridge the understanding of how stress-related peripheral changes can impact activity at the circuit level by leveraging pharmacokinetic analysis of corticosterone as the main stress-related fluid biomarker, behavior, *ex vivo* electrophysiology, and *in vivo* single-cell calcium imaging in freely moving animals using an ARS model. All experiments were performed with male mice to avoid sex- and estrous cycle-dependent biases. Results showed that a 30-minute ARS exposure was sufficient to elevate corticosterone plasma levels and to reduce the number of entries in the center of an Open Field arena, without affecting basal locomotor activity. Using slice electrophysiology, we found that BLA pyramidal neurons (PNs) in mice following ARS exhibited a significantly lower action potential threshold compared to control mice. Interestingly, while *in vivo* single-cell activity of BLA PNs was decreased in ARS-exposed mice, no correlation was found between corticosterone plasma levels and neuronal activity. However, further analysis among control mice indicated a positive correlation in this regard. These findings suggest a complex interplay between central and peripheral stress-related mechanisms that likely occur in parallel and in a state-dependent manner. Given that manipulations targeting the BLA have been shown to impact stress resilience, modulating its activity holds therapeutic potential for stress-related disorders. This study contributes to our understanding of the intricate neurobiological processes underlying stress response and highlights the importance of investigating the BLA as a potential target for stress-related therapeutic interventions.

Disclosures: F.S. Polli: None. **I.F. Nalepa:** None. **T. Bruun:** None. **R. Christensen:** None. **V. Nielsen:** None. **C. Hougaard:** None. **B. Hall:** None. **M. Grupe:** None. **P. Botta:** None. **C. Ratner:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.09/OO9

Topic: F.03. Stress and the Brain

Support: NIMH R01 Grant MH043454 in part by a core grant from NICHD P50-HD105353

Title: Associations among lifetime stressor exposure, amygdala and hippocampal volumes, and the acute stress response.

Authors: *C. E. HAEFFNER[1], E. T. HIGGINS[1], C. M. LAUBACHER[1], L. K. GRESHAM[1], E. C. NORD[1], A. L. BARNES[1], H. C. ABERCROMBIE[1], M. A. ROSENKRANZ[1], R. J. DAVIDSON[1], G. M. SLAVICH[2], S. M. SCHAEFER[1];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]UCLA, Los Angeles, CA

Abstract: The hypothalamic-pituitary-adrenal (HPA) neuroendocrine pathway mediates aspects of the human stress response by releasing cortisol. Chronic cortisol elevation impacts HPA axis-regulatory structures, including reductions in hippocampal volume and differentially impacting the volume of multiple amygdala nuclei. Given cortisol's regulatory role in the HPA axis, individuals' stressor exposure history may impact their acute stress response. To investigate, we examined interactions among lifetime stressor history, amygdala and hippocampal volume, and cortisol reactivity and recovery in response to acute psychosocial stress. Fifty-nine adults (ages 25-65, mean age = 40.4, 66% female, 86% White) completed the Stress and Adversity Inventory (STRAIN), underwent structural MRI scanning, and provided salivary cortisol samples during the Trier Social Stress Test (TSST). Results indicated that greater lifetime stressor severity was significantly associated with smaller hippocampal and amygdala volumes. A piecewise analysis distinguishing TSST reactivity and recovery showed that a flatter cortisol recovery slope was significantly associated with smaller hippocampal and amygdala volumes and marginally associated with greater lifetime stressor severity. After adjusting for intracranial volume, the direction of these associations persisted, though no longer significant. These findings highlight the complex interplay among lifetime stressor history, acute stress response, and HPA axis regulatory structures.

Disclosures: C.E. Haeffner: None. **E.T. Higgins:** None. **C.M. Laubacher:** None. **L.K. Gresham:** None. **E.C. Nord:** None. **A.L. Barnes:** None. **H.C. Abercrombie:** None. **M.A. Rosenkranz:** None. **R.J. Davidson:** None. **G.M. Slavich:** None. **S.M. Schaefer:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.10/OO10

Topic: F.03. Stress and the Brain

Support: NCRR P20RR16435

Title: Pac1 expressing neurons in the bed nucleus of the stria terminalis (bnst) and their projections

Authors: *M. AKTAR, S. E. HAMMACK, V. MAY;
The Univ. Of Vermont, Burlington, VT

Abstract: PAC1 expressing neurons in the bed nucleus of the stria terminalis (BNST) and their projections Mahafuza Aktar, Victor May and Sayamwong E. Hammack
Abstract
Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide playing essential roles in numerous physiological functions, and we and others have implicated central PACAP neurocircuits in mechanisms by which stressor exposure increases anxiety. PACAP binds to several receptor subtypes, including PAC1, VPAC1 and VPAC2, to activate several signaling cascades that can alter neuronal excitability as well as enhance indices of neuroplasticity. We have demonstrated that PACAP release in the bed nucleus of the stria terminalis (BNST) is critical for many of the behavioral and physiological consequences of stressor exposure, and much of our prior work has suggested that these effects of BNST PACAP anxiogenic behavior depend on the activation of PAC1 receptors. Moreover, we have shown that chemogenetic inhibition of BNST PAC1-expressing neurons reduces anxiety-related behaviors in mice. Here, we use PAC1-ires-Cre mice to examine the projection targets of BNST PAC1 neurons. We infused AAV2-EF1a-DIO-mCherry reporter vector into the BNST region of PAC1-ires-Cre mice to identify BNST PAC1expressing neurons, and examined mCherry expression in several downstream targets of the BNST. We observed robust PAC1 expressing neurons in BNST. PAC1 neurons were observed in anterior, posterior and ventral regions of the BNST. We also observed mCherry expression in the nucleus accambens (NAc), paraventricular nucleus of thalamus (PVT), lateral habenula (LHb), lateral hypothalamus (LH) and substantia nigra (SNR), consistent with other reports demonstrating that these brain regions receive BNST projections. These observations help to clarify the neural circuits involved in anxiety like behavior.

Disclosures: M. Aktar: None. **S.E. Hammack:** None. **V. May:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.11/OO11

Topic: F.03. Stress and the Brain

Title: Oxytocin receptor neurons in the posterior hypothalamus

Authors: *R. OYAMA[1], I. CARCEA[2];
[1]Rutgers Brain Hlth. Inst., Newark, NJ; [2]Rutgers, Newark, NJ

Abstract: Oxytocin plays an important role in attenuating the behavioral manifestations of stress and anxiety, via its actions in the central amygdala. Whether and how oxytocin signaling also

modulates autonomic responses to stress remains unclear. The posterior hypothalamus (PH) is a brain structure primarily implicated in the autonomic control of cardiac activity, body temperature, and blood pressure. We identified a population of neurons in the PH expressing the oxytocin receptor (OTR). Using fiber photometry, we recorded the calcium activity of these neurons in behaving animals. We find that an escapable threat, induced with an experimentally controlled spider toy, leads to increased activity of PH-OTR+ cells in the posterior hypothalamus. An inescapable threat induced with a looming stimulus does not change the activity of PH-OTR+ cells. Social interaction, on the other hand, suppresses PH-OTR+ calcium activity. We used trans-synaptic anterograde tracing and determined that PH-OTR+ send inputs to pre-autonomic nuclei in the brain stem, suggesting that they could be implicated in modulating autonomic manifestations of stress. Indeed, chemogenetic activation of PH-OTR+ neurons increased both heart rate and core body temperature. Taken together, our findings indicate that oxytocin signaling in PH plays an important role in adjusting autonomic functions to rapidly changing environmental conditions.

Disclosures: **R. Oyama:** None. **I. Carcea:** None.

Poster

PSTR493. Stress-Modulated Pathways: Amygdala, BNST, and Hypothalamus

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR493.12/OO12

Topic: F.03. Stress and the Brain

Support: R01 HL150559

Title: Intrinsic and synaptic properties of posterior hypothalamus neurons in male and female rats

Authors: *C. A. BOUCHET, C. E. VAAGA, B. N. SMITH, B. MYERS;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Cardiovascular diseases, the leading cause of death globally, are exacerbated by stress. Additionally, stress-cardiovascular comorbidities are more prevalent in females. However, the neurobiological mechanisms linking stress to cardiovascular outcomes are not well understood. Neurons within the posterior hypothalamus (PH) are sensitive to stress and project to brain regions that regulate endocrine stress responding and cardiovascular physiology. Previous work indicates that PH pharmacological inactivation *in vivo* restrains acute hypothalamic-pituitary-adrenal (HPA) axis stress responses, while pharmacologically activating the PH exacerbates acute HPA axis responses. Furthermore, pharmacologically activating the PH increases blood pressure and heart rate while pharmacological inactivation robustly blocks stress-induced increases in heart rate. Although these data support the role of the PH in both neuroendocrine stress responding and cardiovascular function, investigations of the PH to this point have been limited to males. Further, physiological properties of these neurons have not

been reported. Thus, understanding the physiological features and activity of the female and male PH is of the upmost importance. Therefore, this project aims to determine intrinsic and synaptic properties of neurons within the PH of male and female Long-Evans rats (n = 5 per sex, PND 102-136). Using whole-cell patch clamp electrophysiology, action potential firing at rest was used to determine the proportion of quiescent cells at rest, basal firing frequency, and action potential properties including half-width and phase-plane analysis. Intrinsic excitability was further evaluated by constructing a frequency-current (FI) curve to evaluate action potential dynamics evoked by current injection. A current ramp was injected to determine rheobase of each neuron. Additionally, spontaneous excitatory and inhibitory synaptic input onto each neuron was evaluated in voltage clamp. Results indicate that 43% of PH neurons in male rats fire at rest while 54% of neurons in female PH fire at rest. Overall, the data presented indicate differences in intrinsic and synaptic properties of neuronal populations within the male and female PH. Future experiments will investigate alterations in firing properties induced by chronic variable stress and could identify factors underlying cardiovascular susceptibility and resilience.

Disclosures: C.A. Bouchet: None. **C.E. Vaaga:** None. **B.N. Smith:** None. **B. Myers:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.01/OO13

Topic: F.04. Neuroimmunology

Support: NIH Grant RF1NS130481
NIH Grant R01 NS129191
Mary Hoffman Shaw Travel Award

Title: Immune regulation by small extracellular vesicles in attenuating inflammatory pain

Authors: *X. LUO[1], A. SACAN[2], S. K. AJIT[1];
[1]Pharmacol. & Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Sch. of Biomed. Engineering, Sci. & Hlth. Systems, Drexel Univ., Philadelphia, PA

Abstract: Chronic pain affects millions in the United States. Pain is not generated by a hardwired system, but rather by highly plastic circuits and molecules. Such molecules can be small extracellular vesicles (sEVs) released by cells that transport RNAs, proteins, and lipids. Uptake of sEV cargo by recipient cells can alter gene expression, and this process can serve as a novel mechanism of cellular communication. We showed that sEVs from RAW 264.7 macrophages are immunomodulatory, and prophylactic intrathecal injection of sEVs two weeks prior, attenuated inflammatory pain induced by intraplantar injection of complete Freund's adjuvant (CFA). How this long-term memory develops and how sEVs regulate immune responses are unknown. Recent studies show that microglia that were primed with inflammatory stimuli can enhance or suppress responses to a delayed secondary insult via epigenetic

modifications. We hypothesize that prophylactic macrophage-derived sEVs, when administered intrathecally, confer pain resolution by reprogramming epigenetic immune memory in spinal microglia and by promoting anti-inflammatory immune responses in recipient mice. Using a microglial ablation strategy, our preliminary behavioral data showed that microglia may be required for the later phase of the sEV-induced pain attenuation. To study immune cell dynamics in spinal cord of CFA recipient mice, a high-plex spatial assay, ChipCytometry, was used. We observed a decrease in natural killer (NK) cells in spinal cord and an increase in M2 macrophages in dorsal root ganglion (DRG) in CFA mice prophylactically treated with sEVs compared to control mice. Studies are ongoing to investigate if this sEV-conferred prophylaxis is mediated by epigenetic alterations in spinal microglia, and how sEVs regulate immune cells in spinal cord to induce pain resolution.

Disclosures: X. Luo: None. **A. Sacan:** None. **S.K. Ajit:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.02/OO14

Topic: F.04. Neuroimmunology

Support: NIH Grant R01GM132672
NIGMS 1R35 GM118182

Title: Distinct neuronal populations in the BNST control IL1-mediated inflammatory responses.

Authors: *O. HASHIMOTO, T. HEPLER, A. TYNAN, K. J. TRACEY, S. S. CHAVAN; Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: Interleukin-1 β (IL-1β) is a prototypic proinflammatory cytokine that mediates a number of physiological effects such as thermoregulation, cardiac function, and induction of cytokines. Although these responses are regulated by the central nervous system to maintain homeostasis, the underlying neural mechanisms are yet unclear. Here we show that a specific neuronal population in the brain can control diverse responses of IL-1β. We utilized the targeted-recombination-in-active-populations (TRAP2) mice crossed with a tdTomato reporter line to produce double transgenic TRAP2/tdTomato mice. Using activity-dependent cell labeling, we captured IL-1β-responsive neuronal ensembles. Increased tdTomato expression (indicative of neuronal activity) in response to IL-1β administration is observed in the paraventricular nucleus (PVN), the bed nuclei of the stria terminalis (BNST) and the nucleus of the solitary tract (NTS). Specific subpopulation of neurons in the BNST shows IL-1β -specific activation. Chemogenetic reactivation of these IL-1β-responsive neuronal subsets in the BNST is sufficient to broadly retrieve the IL-1β -induced responses under which these neurons were captured. Reactivation of these IL-1β -responsive neurons in the BNST recapitulates tachycardia and systemic inflammation induced by IL-1β administration. Specific ablation of IL-1β-responsive neurons in

the BNST abrogates IL-1β-induced tachycardia and stress-induced increase in circulating IL-6. Together, these findings suggest the brain encodes the IL-1β specific information in specific neuronal population, extending the classical concept of immunological memory to neuronal representations of inflammatory information.

Disclosures: O. Hashimoto: None. **T. Hepler:** None. **A. Tynan:** None. **K.J. Tracey:** None. **S.S. Chavan:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.03/OO15

Topic: F.04. Neuroimmunology

Title: Sympathetic modulation of the immune microenvironment in lung tumors

Authors: *J. YANG;
Peking Univ., Beijing, China

Abstract: Lung cancer is a significant threat to human health despite recent developments in therapeutic strategies. Notably, clinical evidence has implicated that psychological stresses such as anxiety and depression, which cause chronic activation of the body's sympathetic signals, may result in a poor prognosis of lung cancer. However, cellular and molecular mechanisms underlying this sympathetic modulation remain mostly unknown. Here, we exploit advanced whole-tissue 3D imaging to reveal the frequent presence of sympathetic innervations in human lung tumors. Also, similar spatial engagement of sympathetic inputs and tumors is observed with lung metastases in the mouse PyMT model. We then show that the ablation of local sympathetic innervations inhibits tumor growth in the lungs of PyMT mice. Mechanistically, the sympathetic neurotransmitter norepinephrine directly stimulates the cancer cell expression of CXCL1 and CXCL2, the central chemokines recruiting neutrophils into lung tumors. At the same time, norepinephrine acts via the β2-adrenergic receptor to trigger the immunosuppressive function of those recruited neutrophils. Moreover, we demonstrate that the β2-adrenergic receptor antagonist can potentiate the anti-PD-L1 immunotherapy of lung cancer in mice. Together, these results have elucidated the sympathetic modulation of the immune microenvironment in lung cancer, providing a novel target for effective treatments.

Disclosures: J. Yang: None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.04/OO16

Topic: F.04. Neuroimmunology

Title: Activation of lateral habenula modulates peripheral and central immune responses in sex dependent manner

Authors: *S. CASTANY, O. BARBA, D. ENGBLOM;
Linkoping Univ., Linköping, Sweden

Abstract: Depression is a mood disorder that is currently the second leading cause of disabilities worldwide being twice as prevalent in women. Depressed patients are more susceptible to develop diseases than healthy population indicating an underlying dysfunctional immune system and evidencing a brain-to-immune communication. Understanding the sex differences on how the brain directly modulates the immune system in the context of depression is scarcely explored. Lateral habenula (LHb) has been postulated to be the “anti-reward” center and its hyperactivity has a key role in the pathophysiology of depression. Therefore, the aim of this study is to investigate the role of LHb in modulating the immune system under aversive states in males and females. To do that, we used optogenetics to selectively activate the neurons in the Lhb and we checked whether this was enough to trigger an aversive state in C57/bl6 WT males and female mice. Then, we subjected them to a chronic protocol of stimulation (Day 1: 20min, Day2 :40 min, Day 3: 70min at 20Hz-10λ 3s ON7 min OFF). At the end of the protocol, we challenged them with LPS to study the innate immune reactivity in the periphery (eg, spleen, liver, plasma) and centrally (eg brain). Lhb activation induced a robust aversive state in both male and female as they spent less time in the light paired chamber during the real time place preference test. The aversion induced by Lhb activation was associated with sex-specific changes in cytokine expression in the spleen. Females with Lhb activation showed a potentiated IL6 an COX2 expression after LPS challenge and males with Lhb activation showed a decreased COX2 and IL1B expression after the same immune challenge. We also studied microglia reactivity in the insular cortex, an area that encodes interoceptive information and it is known to sense peripheral immunity changes. Lhb activation increased microglia reactivity to LPS only in females. Altogether, this data indicates that the activation of lateral habenula induced a sex-specific dysregulation of the peripheral and central immune system response to an immune challenge. These findings may contribute to elucidate why depressive states lead so often to increased risk of comorbidities sex (e.g. cardiovascular problems, autoimmune diseases, or infections).and the sex/gender influence.

Disclosures: **S. Castany:** None. **O. Barba:** None. **D. Engblom:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.05/OO17

Topic: F.04. Neuroimmunology

Support: Grant-in-Aid for Scientific Research (B) 23H03307
Grant-in-Aid for Challenging Exploratory Research 22K19617

Title: The role of adrenal gland adrenergic response on pulmonary protection via C1 neurons in the medulla oblongata

Authors: *C. ABE[1], M. TANIDA[2], Y. IWASAKI[3];
[1]Gifu Univ. Grad. Sch. of Med., Gifu, Japan; [2]Kanazawa Med. Univ., Uchinada, Japan; [3]Kyoto Prefectural Univ., Kyoto, Japan

Abstract: The autonomic nervous system plays a crucial role in the neuro-immune system. Previous studies have demonstrated that stimulating C1 neurons in the medulla oblongata, a component of the autonomic nervous system, provides protection against acute kidney or lung injury in mice. The protective effect relies on the activation of CD4 T cells in the spleen through β2 adrenergic receptors. Considering the receptor's ligand affinity, adrenaline, rather than noradrenaline, may be more suitable for immune cell activation. While adrenaline is known to be released from the medulla in the adrenal gland, its involvement in C1 neuron stimulation-induced protection against acute lung injury remains uncertain. Thus, this study aimed to investigate the impact of adrenal gland manipulation on the pulmonary protective effect mediated by C1 neurons. DβH$^{cre/0}$ mice were utilized to manipulate both C1 neurons and catecholaminergic cells in the adrenal gland. Chemogenetic activation of C1 neurons using viral vector injection (AAV2-hSyn-DIO-hM3D(Gq)-mCherry) increased adrenal sympathetic nerve activity and plasma catecholamine levels, including adrenaline and noradrenaline. Deletion of catecholaminergic cells in the adrenal gland through local viral vector injections (AAV2-DIO-taCasp3-TEVp) abolished the protective effect against acute lung injury when C1 neurons were stimulated using either chemogenetic or optogenetic methods. These findings indicate that the adrenal gland adrenergic system is indispensable for the protective effect of C1 neuron stimulation against acute lung injury. Co-activation of both the splenic and adrenal sympathetic nervous systems may be crucial, as a previous study demonstrated that splenic sympathetic denervation also eliminates the pulmonary protective effect mediated by C1 neurons.

Disclosures: C. Abe: None. **M. Tanida:** None. **Y. Iwasaki:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.06/OO18

Topic: F.04. Neuroimmunology

Title: 3d anatomy of autonomic innervations in immune organs of a non-human primate and the human

Authors: *Y. CAO;
Peking Univ., Beijing, China

Abstract: Direct neural inputs to immune organs have been observed for decades, with their functions in neuroimmune regulation being increasingly appreciated. However, the current knowledge of such neural structures, particularly those in primate immune organs, remains incomplete. In this study, we comprehensively assessed the 3D anatomy of autonomic (i.e., sympathetic and parasympathetic) innervations in the immune organs of the rhesus macaque monkey and the human for the first time. Aided with the advanced technique of whole-tissue immunolabeling and lightsheet fluorescence imaging, we revealed the densely organized sympathetic architecture in the parenchyma of the adult monkey and human spleens. On the other hand, only sparse, if any, sympathetic inputs were observed inside the lymph nodes, Peyer's patches, or thymus. In contrast, there were minimal parasympathetic innervations in the parenchyma of these examined immune organs. Together, this work has documented the unique patterns of autonomic innervations in different immune organs of a non-human primate and the human, serving as an essential reference for future research on neuroimmune regulation in the field.

Disclosures: Y. Cao: None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.07/OO19

Topic: F.04. Neuroimmunology

Support: NIH/NIAAA R01 AA022460
Azrieli Foundation

Title: Differential neuroinflammatory responses to TLR7/8 and TLR4 activation in male and female rats prenatally exposed to alcohol

Authors: *C. LUFT[1], K. WONG[1], V. VELLA[1], P. J. HOLMAN[1], T. S. BODNAR[3], C. RAINEKI[2];
[1]Brock Univ., St Catharines, ON, Canada; [2]Brock Univ., Brock Univ., St. Catharines, ON, Canada; [3]The Univ. of British Columbia, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Cumulative experimental and clinical evidence demonstrates that prenatal alcohol exposure (PAE) has significant long-term effects on the immune system, such as increased susceptibility to infections. Studies have also shown that individuals with PAE are also more vulnerable to autoimmune diseases. Importantly, toll-like receptors (TLRs) play an important role in regulating immune function, with activation of TLR-4 inducing innate immune responses and TLR7/8 activation contributing to the pathogenesis of autoimmune diseases. To explore

potential mechanisms by which PAE-induces immune dysfunction, the current study examined the impact of PAE on both TLR4 and TLR7/8 activation. To address this question, pregnant Sprague-Dawley rats were assigned to PAE - liquid ethanol diet ad libitum and CON - pelleted control diet ad libitum. In adulthood, males and females were challenged with 40 µg/kg LPS i.p. (TLR4 activation), 1 mg/kg R848 i.p. (TLR7/8 activation) or DMSO 10% i.p. (vehicle) followed by blood and brain sample collection to measure cytokine levels (IL-1β, IL-10, IFN-y, IL-6, KC/GRO, TNF-α) and TLR4 and TLR8 protein expression. All animals regardless of sex or prenatal treatment showed increased serum IL-10, IL-1β, IL-6, KC/GRO, and TNF-α levels 90 min following LPS and R848 injection. Moreover, PAE male and female rats showed higher serum IL-1β responses to R848, compared to CON, and PAE male rats showed higher IL-6 and TNF-α responses to LPS and R848, compared to CON. Additionally, both LPS and R848 increased the serum levels of IFN-y in PAE females, but not in CON. The analysis of brain cytokines 24 hours after the injections indicates that PAE disrupts central immune function in a sex-dependent manner. PAE males and females, when challenged with R848, exhibited higher levels of IL-6 in the hypothalamus compared to CON. Additionally, PAE males displayed a greater IL-10 response to R848 in the hypothalamus compared to CON males. No significant effects of PAE were observed in the amygdala of males. However, in females, increased levels of IL-1β were detected following R848 injection. Collectively, these results indicate that PAE disrupts immune system regulation, resulting in an elevated immune response when exposed to a challenge that activates TLR7/8 receptors. This PAE-induced heightened immune response is characterized by elevated production of cytokines, which play a crucial role in the development of autoimmune diseases. Notably, both male and female rats exposed to PAE exhibit distinct responses to immune challenges, underscoring the importance of using both sexes.

Disclosures: C. Luft: None. **K. Wong:** None. **V. Vella:** None. **P.J. Holman:** None. **T.S. Bodnar:** None. **C. Raineki:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.08/OO20

Topic: F.04. Neuroimmunology

Support: NRF-2021R1A2C2014123

Title: Characterization of Double-negative T Cells during mouse brain development

Authors: *J. LEE, K. CHOI, H. KANG;
Chung Ang Univ., Seoul, Korea, Republic of

Abstract: The central nervous system (CNS) was considered to have immune privilege, however, recent studies have demonstrated that the immune system plays an important role in brain development and function. Therefore, investigating the distribution of the immune cell

population in the brain is important to understand the brain-specific immune responses that occur in various conditions. Here, in order to identify the temporal pattern of the immune cell population in the brain, we prepared single-cell suspension from the various tissues including the brain, meninges, spleen, or blood during the developmental period (E14 and P1) and adulthood, followed by an examination of the immune cell population at each period through flow cytometric analysis. As a result, the number of microglia ($CD45^{low}CD11b^{high}$) and macrophage ($CD45^{high}CD11b^{high}$) in the brain were the highest in adulthood, and the total number of T cells ($CD3^{+}$) increased in the postnatal period and maintained in adulthood. Interestingly, compared to periphery tissue, brain resident double-negative T (DNT; $CD3^{+}CD4^{-}CD8^{-}$) cells have shown the highest proportion among the total T cells both in the developmental period and in adulthood. It was indicated that DNT cells are a majority T cell subpopulation in the brain, which would be brain-specific characteristics compared to other tissues. These results will contribute to a better understanding of the immune response and the role of T cells in the immune environment of CNS.

Disclosures: **J. Lee:** None. **K. Choi:** None. **H. Kang:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.09/OO21

Topic: F.04. Neuroimmunology

Support: Anonymous Benefunder

Title: Effects of a soil Mycobacterium-derived lipid, 10(Z)-hexadecenoic acid, on murine dendritic cells: A mechanistic analysis

Authors: *I. RUTHERFORD[1], L. HUNTER[2], C. WRIGHT[3], E. HOLBROOK[4], C. A. LOWRY[5];
[2]Psychology and Neurosci., [3]Molecular, Cellular, and Developmental Biol., [4]Integrative Physiol., [5]Dept. of Integrative Physiol. and Ctr. for Neurosci., [1]Univ. of Colorado Boulder, Boulder, CO

Abstract: Inflammatory disorders and disorders in which inflammation is a risk factor, including anxiety disorders, mood disorders, and trauma and stressor-related disorders are increasingly prevalent in industrialized societies. Current interventions are limited by poor efficacy and significant side effects that impair quality of life and promote treatment non-adherence, mandating novel prevention strategies and therapeutic interventions. An explanation for the rise in inflammatory disorders is the “Old Friends” hypothesis, which proposes people living in modern societies have reduced exposure to diverse microbial communities, including “Old Friends”, i.e. commensal microbiota, pathogens associated with the “old infections” that were present in evolving human hunter-gatherer populations, and organisms from the natural environment. *Mycobacterium vaccae* NCTC 11659, a soil-derived mycobacterium with anti-

inflammatory, immunoregulatory, and stress resilience effects is one such “Old Friend”. Some of these effects may be mediated by a lipid: 10(*Z*)-hexadecenoic acid (10(*Z*)-HDA), which was isolated from *M. vaccae* NCTC 11659 and recently characterized. Previous studies have shown that 10(Z)-HDA acts as an agonist at the host peroxisome proliferator-activated receptor (PPAR)-alpha (PPARα) receptor to induce anti-inflammatory effects in murine peritoneal macrophages. To determine if 10(*Z*)-HDA acts via PPARα to induce anti-inflammatory effects in dendritic cells, we conducted studies using 10(*Z*)-HDA and the PPARα antagonist GW6471 and assessed biomarkers of inflammation in murine bone marrow-derived dendritic cells (BMDCs) in the presence or absence of subsequent immune challenge with lipopolysaccharide. Murine BMDCs were treated with two doses of GW6471 (0.24 μg/ml, 2.4 μg/ml) - or vehicle (1.5% DMSO). This was followed by 10(*Z*)-HDA at 250 μg/ml, or vehicle (13% DMSO, resulting in a final well concentration of 0.5%). Twenty-four hours later, an immune challenge with lipopolysaccharide (LPS; Escherichia coli 0111:B4; 250 ng/ml) or vehicle was added, followed, 24 hours later, by cell harvest and quantification of gene expression assessed using real time reverse transcriptase polymerase chain reaction (RT-PCR). After treatment with 2.4 μg/ml GW6471, relative to a vehicle-treated control condition, a significant decrease in *Il6* expression following 10(*Z*)-HDA and LPS treatment was seen. This suggests that *Il6* expression in murine BMDCs is controlled in part by PPARα. These results further our understanding of the mechanism by which 10(*Z*)-HDA has its effects and of understanding host mycobacteria interactions.

Disclosures: I. Rutherford: None. **L. Hunter:** None. **C. Wright:** None. **E. Holbrook:** None. **C.A. Lowry:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.A.L. is Cofounder, Board Member, and Chief Scientific Officer of Mycobacteria Therapeutics Corporation, and is a member of the faculty of the Integrative Psychiatry Institute, Boulder, Colorado, USA.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.10/OO22

Topic: F.04. Neuroimmunology

Support: NSF Grant #1933264
Georgia State University Molecular Basis of Disease PhD Fellowship

Title: Effects of a reduced, defined microbiota on early-life neuroimmune development

Authors: *H. STURGEON[1], A. CASTILLO-RUIZ[1], E. UKPONG[1], B. CHASSAING[2], N. G. FORGER[1];
[1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]INSERM, Paris, France

Abstract: Mammalian birth is characterized by a remarkable shift in conditions for the offspring, including the transition from the womb to a highly microbial world. This leads to the colonization of the newborn by trillions of bacteria, viruses, and fungi, collectively referred to as the microbiota. The most abundant population by far are bacteria in the distal gastrointestinal tract (i.e., colon), which can influence the brain via the gut-brain axis. We have previously shown that germ-free (GF) mice have altered brain development in the first days of life. This suggests that the arrival of microbes to the neonate upon birth may be essential for normal early-life brain development. However, GF mice are a highly artificial model system, with known deficits in immune and intestinal development. To explore the effects of a more realistic curtailment of bacterial exposure on brain development, we utilized a reduced, defined microbiota. Specifically, GF mouse dams were colonized with an Altered Schaedler Flora (ASF) microbiota, a defined community of exactly 8 bacterial species, previously shown to support relatively normal immune and intestinal development in adult mice. Peripheral and central measures were then compared in offspring born to ASF, GF, and conventionally colonized (CC; i.e., control) mice. Peripherally, we found that total bacterial load in the colons of ASF offspring was at least as high as in CC mice 3 days after birth. Spleen weight was also normalized in ASF mice at postnatal day (P)23. We next compared neuroimmune development in the three groups. Using immunohistochemistry for Iba1, we found that the density of microglia in the hippocampus is higher in GF than in CC neonates, confirming previous observations, and is intermediate in ASF neonates. We also assessed the expression of cytokines in the forebrain, and found higher expression of TNF-α and IL-1β in ASF mice compared to that of CC neonates, suggesting a greater inflammatory tone. We tested for an effect of sex in all analyses but did not find one. Additional, ongoing analyses assess the effects of colonization by the ASF community at birth on the levels of circulating cytokines and developmental neuronal cell death in the brain. Our findings suggest that microbiota colonization at birth plays a role in early-life neuroimmune development, one that may be driven not just by bacterial load, but by the presence or absence of specific taxa.

Disclosures: H. Sturgeon: None. **A. Castillo-Ruiz:** None. **E. Ukpong:** None. **B. Chassaing:** None. **N.G. Forger:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.11/OO23

Topic: F.04. Neuroimmunology

Title: Perinatal antiretroviral drug exposure in deer mice bolsters cytokine expression and alters compulsive-like behavioral outcomes in adulthood

Authors: *D. WOLMARANS[1], J.-A. STROEBEL[1], A. U. HAPPEL[2], H. B. JASPAN[3,4,2]; [1]North-West Univ., Potchefstroom, South Africa; [2]Univ. of Cape Town, Cape Town, South

Africa; [3]Seattle Children's Res. Inst., Seattle, WA; [4]Dept. of Pediatrics, Univ. of Washington, Seattle, WA

Abstract: Perinatal combined antiretroviral therapy (cART) prevents vertical transmission of HIV, but may be associated with adverse effects, i.e., inflammation and neurodevelopmental conditions in uninfected, but HIV-exposed infants. As the long-term study of these outcomes is problematic in clinical cohorts, where it is difficult to disentangle the effects of HIV vs. cART exposure, we investigated the effects of perinatal cART exposure on cytokine expression and behavioral outcomes in uninfected 14-week-old deer mouse *(P. maniculatus bairdii)*. Deer mice were chosen for this study since adult mice varyingly and spontaneously present with compulsive-like large nesting behavior, allowing study of the natural etiological mechanisms that contribute to both normal and dysregulated neurodevelopment. Two groups of 10 deer mouse dams were orally exposed to either normal water (n = 10) or water with cART (tenofovir 15 mg/kg/day and emtricitabine 10 mg/kg/day, n = 10; ethics approval no.: **NWU-00524-20-A5**) from 21 days prior to mating until the end of nursing. Pups were raised until the age of 14 weeks when compulsive-like behavior was tested (7-day nesting assessment), whole-brain tissues sampled, and analysis of cytokine expression (IL-1β, IL-6, IL-10, IL-17, IFN-γ, and TNF-α) performed using multiplex assays. Our results show that perinatal cART exposure had potent long-term immunogenic effects in normal, compared to compulsive-like adult mice, with significant elevations in the whole-brain expression of IL-1β (p = 0.04), IL-6 (p < 0.0001), IL-10 (p = 0.0002), IL-17 (p = 0.007), and TNF-α (p = 0.02) shown. Also, cART-exposed, compared to water-exposed compulsive-like offspring, generated lower overall total nesting scores (p = 0.05), thus presenting with an attenuated level of compulsive-like behavior. In conclusion, our data highlight unique cART-immune-brain interactions in mice that were selected for normal and compulsive-like behavioral expression. Specifically, brain processes underlying naturalistic compulsive-like expression likely blunt the long-term pro-inflammatory actions of perinatal cART exposure, providing an exciting avenue for future studies into neurodevelopmental brain-immune crosstalk mechanisms.

Disclosures: D. Wolmarans: None. **J. Stroebel:** None. **A.U. Happel:** None. **H.B. Jaspan:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.12/OO24

Topic: F.04. Neuroimmunology

Support: NRF UID 140710

Title: Naturalistic sex-related differences in the neutrophil-lymphocyte ratio of normal and compulsive-like deer mice: association in the absence of causation

Authors: *J. STRYDOM, M.-L. MEIRING, M. DE RIDDER, F. P. VILJOEN, L. BRAND, D. WOLMARANS;
North-West Univ., Potchefstroom, South Africa

Abstract: Brain-immune crosstalk influences the manifestation and prognosis of neuropsychiatric conditions, including obsessive-compulsive disorder (OCD). However, causal relationships between immune perturbations, e.g. elevations in the neutrophil-lymphocyte ratio (NLR), a non-specific marker of innate immune reactivity, and altered brain processes remain difficult to show. Deer mice *(P. maniculatus bairdii)* variably and naturalistically present with compulsive-like behavioral persistence, i.e. high motor stereotypy (HS), and thus allow studies of the natural etiological mechanisms that contribute to dysregulated neurodevelopment. Here, we aimed to (i) determine if HS is associated with an elevated NLR, and (ii) if an elevated NLR, induced throughout the neurodevelopmental window, will increase stereotypical expression in adulthood. For aim (i) sixty-two mice (12-week-old, 31 normal (NS) and HS mice, equally distributed between sexes; ethics no.: **NWU-00532-20-A5**) were used. For aim (ii), seventy offspring were exposed to weekly subcutaneous injections with either normal saline (n = 35; 15 male, 20 female) or pegfilgrastim (Peg), a recombinant granulocyte colony stimulating factor analogue (1 mg/mg/kg, n = 35; 16 male, 19 female), between the ages of 3 to 12 weeks (ethics no.: **NWU-00760-22-A5**). Our results show that male HS mice present with a robust increase in the NLR, compared to female HS (p = 0.0015), and both sexes of NS mice ($p < 0.05$). However, chronic and significant Peg-induced NLR elevation ($p < 0.0001$ vs. saline) did not associate with increased adulthood stereotypy in mice of either sex and irrespective of stereotypical cohort ($p >$ 0.05 for all comparisons). In conclusion, our data concur with clinical literature in showing increased innate immune reactivity in HS compared to NS mice. We further conclude that the bidirectional selection of NS and HS mice based on behavioral expression alone, provides a suitable framework for investigations into the role of naturalistic etiological mechanisms that may uniquely promote normal and HS behavioral development. While we present unquestionable evidence for altered immune functioning in HS mice, further study is needed to explore the nature of brain-immune relationships in this species, especially considering that NLR elevation is non-specific in its meaning and since causal conclusions relating to brain-immune crosstalk cannot be drawn from the present data.

Disclosures: **J. Strydom:** None. **M. Meiring:** None. **M. De Ridder:** None. **F.P. Viljoen:** None. **L. Brand:** None. **D. Wolmarans:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.13/OO25

Topic: F.04. Neuroimmunology

Support: Alzheimer's Association AARG-NTF-22-974072
NIH AG08168501

Title: Covid-19-like immune challenge causes lasting memory deficits: role of neuroimmune priming

Authors: H. CHOI[1], P. L. KE-LIND[1], M. VAANDRAGER[1], K. M. SCHUH[1], *N. C. TRONSON[2];
[1]Psychology, Univ. of Michigan, Ann Arbor, MI; [2]Psychology, Univ. of Mchigan, Ann Arbor, MI

Abstract: COVID-19 has affected more than 770 million individuals worldwide, and up to 40% of survivors experience post-acute covid sequelae (PASC, "long COVID"). The symptoms of long COVID include "brain fog", cognitive impairments, and mood-related symptoms such as depression or anxiety. SARS-COV-2 virus only rarely infects the brain, suggesting that other effects of COVID-19 cause changes including memory impairments. Innate immune activation, or inflammation, during illnesses is known to modulate memory, cognition, and mood. Recent studies demonstrate that effects of a peripheral immune challenge on memory can last months after resolution of the inflammatory response (Tchessalova & Tronson 2019, 2020). Whereas much of the prior work has focused on Toll-like receptor (TLR) 4 and TLR3-mediated mechanisms, single stranded RNA (ssRNA) viruses like SARS-COV2 trigger innate immune activation *via* TLR7 and TLR8. Although less well-studied than its counterparts, TLR7 has previously been linked with cognitive impairments and neurodegenerative disorders including Alzheimer's Disease (AD). In this project we examined the hypothesis that TLR7-triggered immune challenge causes lasting changes in the brain that contribute to the cognitive impairments in long-COVID, and increase risk for age-related cognitive decline and dementias including AD. We used our two week subchronic immune challenge protocol to determine whether the TLR7 agonist R848 (400-1000μg/kg) causes emerging memory impairments or anxiety- and depression-like phenotypes. Further, we identified the time course of cytokine responses over the recovery period and determined the persistence of neuroimmune priming 8 weeks after immune challenge. We observed a clear dose-response of cytokine and chemokine elevations in the hippocampus of young and mid-aged male and female to an acute dose of R848. Eight weeks after immune challenge, we observed memory impairments that were more striking in male animals. These effects on memory were not due to ongoing elevations in neuroimmune activation during behavioral tasks. Nevertheless, persistent changes in immune priming suggest that changes in neuroimmune states or activity might modulate memory processes even in the absence of elevated cytokines. This work is an initial step towards understanding how persistent changes in neuroimmune processes after viral illnesses including COVID-19 might contribute to risks for cognitive deficits, age-related cognitive decline, and Alzheimer's Disease in the decades to come.

Disclosures: H. Choi: None. **P.L. Ke-Lind:** None. **M. Vaandrager:** None. **K.M. Schuh:** None. **N.C. Tronson:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.14/PP1

Topic: F.04. Neuroimmunology

Support: NIH Grant P51-OD011132
Georgia Clinical and Translational Science Association
COVID CURE award

Title: Covid-19 in rhesus macaques produces behavioral, cognitive, and neurological characteristics similar to clinical symptoms of post-acute sequelae of sars-cov-2 infection (pasc)

Authors: *J. RAPER[1,2], S. FREEMAN[3], R. RICHARDSON[3], M. ALI[4], W. WENG[4], A. VAN SCHOOR[3], E. VIOX[3], T. R. WICHE SALINAS[3], M. PAIARDINI[3];
[1]Pediatric Neurol., Emory Univ. SOM, Atlanta, GA; [2]Pediatrics, Div. of Neurol., Emory Univ. Sch. of Med., Atlanta, GA; [3]Emory Natl. Primate Res. Ctr., Atlanta, GA; [4]Emory Univ., Atlanta, GA

Abstract: A significant proportion of COVID-19 patients have lingering symptoms for weeks or even months following initial recovery. These “long COVID-19” symptoms or Post-Acute Sequelae of SARS-CoV-2 infection (PASC) include fatigue, sleep disorders, brain fog, loss of taste or smell, anxiety, and depression. Given the persistence of this viral pandemic, there is a great need to broaden our understanding of the viral pathophysiology to help identify novel targets for further therapeutic development. Animal models are a powerful biomedical research tool for investigating neuropathology, narrowing down novel targets, and testing the efficacy of therapeutics to treat PASC symptoms. We propose that nonhuman primates are an ideal animal model to investigate the neuropathological consequences of SARS-CoV-2 infection. Seven adult rhesus macaques between 6-18 years of age were assessed before and after intranasal infection with SARS-CoV-2 ($1.1x10^6$ PFU of 2019-nCoV/USA-WA1/2020). Despite what seemed to be a rather mild infection with viral clearance similar to human patients (2 weeks), we detected behavioral, cognitive, and neurological changes up to 4 months post-infection. Compared to baseline/pre-infection, SARS-CoV-2 infection led to sleep disturbances as shown by increased activity overnight. Cognitive impairments were detected at 85 days post-infection. SARS-CoV-2 infection altered taste and smell, such that monkeys exhibited decreased preference for their top 5 most highly preferred foods from baseline. Together these data indicate that rhesus macaques can exhibit behavioral, cognitive, and neurological characteristics similar to those reported by PASC patients. As COVID-19 infections and the associated mortality rates continue to recede, the world will be left with a large population of people who will continue to suffer from this debilitating, long-term, post-covid syndrome. We propose that the nonhuman primates provide an ideal translational model to expand our knowledge of SARS-CoV-2 pathophysiology and identify potential targets for much needed therapeutic interventions for PASC COVID-19 patients.

Disclosures: J. Raper: None. **S. Freeman:** None. **R. Richardson:** None. **M. Ali:** None. **W. Weng:** None. **A. van Schoor:** None. **E. Viox:** None. **T.R. Wiche Salinas:** None. **M. Paiardini:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.15/PP2

Topic: F.04. Neuroimmunology

Support: Rita Allen Foundation
NIH R01NS121259
NIH R01NS118563

Title: Peripheral Interleukin-10-producing monocytes contribute to sex difference in pain resolution in mice and humans

Authors: J. SIM[1], K. MONAHAN[1], C. SUGIMOTO[1], E. O'GUIN[1], S. MCLEAN[2], L. ALBERTORIO-SAEZ[3], Y. ZHAO[3], S. LAUMET[1], A. DAGENAIS[1], K. INYANG[1], M. BERNARD[1], J. K. FOLGER[1], A. J. ROBISON[1], S. LINNSTAEDT[3], ***G. LAUMET**[1]; [1]Physiol., Michigan State Univ., East Lansing, MI; [2]Univ. of North Carolina Chapel Hill, Durham, NC; [3]Univ. of North Carolina at Chapel Hill, Durham, NC

Abstract: Chronic pain is more prevalent in women. It is important to understand the mechanisms of sexual dimorphism to reduce the sexual inequality in pain. The immune system plays a critical role in pain and is sexually dimorphic. This raises the possibility that neuroimmune interactions may contribute to sex differences in the resolution of pain. Here, we used an inflammatory pain model and discovered that monocytes were the largest population of infiltrated immune cells and the major cellular source of interleukin (IL)-10 in the inflamed skin . Skin monocytes communicate with IL-10 receptors expressed on somatosensory neurons, and blocking this communication impeded pain resolution. We found greater levels of IL-10 and monocytes in the skin of males compared to females during resolution. Males also present a higher percentage of IL-10-producing monocytes, which was associated with a faster resolution of pain, than females. Manipulation of sex hormones by gonadectomy reversed the sex difference in number of IL-10-producing monocytes and pain resolution. Strikingly, we replicated this finding in the AURORA study, a longitudinal study of pain development after motor vehicle collision. In this study, men had higher RNA-seq approximated circulating monocyte levels ($t=3.14$, $p=0.002$), higher IL-10 ($t=2.70$, $p=0.008$) levels, and faster pain resolution than women ($t=-4.86$, $p<0.001$). Similarly, to mice, monocyte and IL-10 levels were positively associated with pain recovery, suggesting a mechanism for earlier recovery in men. Our data, from both mice and humans, demonstrate acritical neuro-immune mechanisms underlying the resolution of pain and a role for monocytes and IL-10 in the sexual dimorphism of pain resolution.

Disclosures: J. Sim: None. **K. Monahan:** None. **C. Sugimoto:** None. **E. O'guin:** None. **S. McLean:** None. **L. Albertorio-Saez:** None. **Y. Zhao:** None. **S. Laumet:** None. **A. Dagenais:** None. **K. Inyang:** None. **M. Bernard:** None. **J.K. Folger:** None. **A.J. Robison:** None. **S. Linnstaedt:** None. **G. Laumet:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.16/PP3

Topic: F.04. Neuroimmunology

Support: RO1 MH52716-031
R01DA039062-11

Title: Is microglial phagocytosis of SDN neurons a function of estradiol induced mast cell degranulation?

Authors: *C. V. DIONISOS, M. M. MCCARTHY;
Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neuro-immune cell crosstalk in the brain is of increasing relevance to sexual differentiation of the developing brain. Sex differences in neuroimmune interactions established during a critical period of development shape neuronal organization in the sexually dimorphic nucleus (SDN) of the preoptic area (POA). Contrary to the longstanding belief that the sex difference in SDN volume is a function of increased cell-autonomous neuronal death in females, Pickett et al (PNAS, 2023) found that differing rates of microglial phagocytosis drive the sex difference of SDN size. In females, microglia phagocytose more neurons than in males, specifically neurons that are otherwise viable if not consumed, temporarily leading to a smaller SDN. The source of the sex difference in phagocytosis, however, remains unknown. We hypothesize that high levels of estradiol in males during development spares SDN neurons from being phagocytosed by microglia. Lenz et al (J. Neurosci., 2018) identified another immune cell that could fill the knowledge gap: the mast cell, a component of the innate immune system. Estradiol induces mast cell degranulation, leading to masculinization of the POA and male typical sociosexual behaviors. Synthesizing the results from these studies, we further hypothesize that estradiol mediated mast cell degranulation acts on microglia to decrease phagocytosis of SDN neurons in males. Microglia in females are more morphologically phagocytic around the SDN as a result of less circulating estradiol. When mast cell degranulation is pharmacologically blocked with a mast cell stabilizer, we expect estradiol to have no effect on SDN size, as we have removed the direct regulator of microglial phagocytosis. The SDN is a regulator of same versus opposite sex odor preference and we will explore the impact of sparing female SDN neurons from phagocytosis on this parameter as well. This investigation will further highlight mast cells as mediators of brain sexual differentiation under both homeostatic and inflammatory conditions during development.

Disclosures: C.V. Dionisos: None. **M.M. McCarthy:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.17/PP4

Topic: F.04. Neuroimmunology

Support: NIA Grant R15AG052935

Title: Sex-differences in LPS-induced associative learning deficits: influence of dose and age

Authors: *L. SINGLETON, T. G. HOEFLER, R. K. PATEL, N. T. PIROZZI, J. CARROLL, K. F. THOMPSON, O. L. ANTON, R. A. KOHMAN;
Psychology, Univ. of North Carolina Wilmington, Wilmington, NC

Abstract: Sex differences in immune function are robust, with females typically mounting stronger, more rapid immune responses compared to males. Despite this, most preclinical research on the functional consequences of inflammation is conducted with males. Recent data from our laboratory demonstrate that acute activation of the immune system by the bacterial endotoxin, lipopolysaccharide (LPS; 0.25 mg/kg), impairs associative learning in male, but not female, mice (Patel et al., 2023). Given these sex differences in susceptibility to LPS-induced learning deficits, we sought to determine whether higher doses of LPS are required to impair cognitive function in female mice. Female C57BL6/J mice (4-5 months) were intraperitoneally administered LPS at a dose of 0.25, 0.325, 0.5, or 1.0 mg/kg or saline. Four hours following treatment, associative learning was assessed using a two-way active avoidance conditioning task. Females showed no disruptions in associative learning, regardless of the LPS dose administered. Assessment of central and peripheral inflammation is currently in progress. Further, investigation of whether females become susceptible to LPS-induced learning deficits with age is currently being evaluated in young and middle-aged males and females. Presently, the data indicate that females are resistant to LPS-induced learning deficits at doses of up to 1 mg/kg. Ongoing research will determine whether this resilience to learning disruption persists in aging females.

Disclosures: L. Singleton: None. **T.G. Hoefler:** None. **R.K. Patel:** None. **N.T. Pirozzi:** None. **J. Carroll:** None. **K.F. Thompson:** None. **O.L. Anton:** None. **R.A. Kohman:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.18/PP5

Topic: F.04. Neuroimmunology

Support: NSTC111-2320-B-039 -034 -MY2

Title: Electroacupuncture as an eosinophil-targeting treatment in OVA-induced allergic rhinitis in mice model

Authors: *Q. V. B. TRAN, Y.-H. CHEN;
China Med. Univ., North District, Taiwan

Abstract: Allergic rhinitis (AR) is an upper airway allergy triggered by allergen exposure, leading to IgE-mediated inflammation. Acupuncture is used to treat various diseases, including AR. It has been shown to alleviate AR symptoms and regulate T cell activity, reducing inflammation markers such as IgE, cytokines, and mediators. However, the underlying mechanism of acupuncture in AR treatment remains unclear. This study aims to investigate the effects of electroacupuncture (EA) on inflammation cells and neuroimmune regulation in AR. The trial examined EA at acupoints LI4 (Hegu) and LI11 (Quchi) and induced different levels of AR intensity through 7-day and 28-day ovalbumin (OVA) challenges. In mice subjected to the 7-day OVA challenge, nasal rubbing behavior was assessed, with the EA group showing a significant decrease in rubbing frequency after 7 days of treatment. Olfactory impairment was evaluated using a buried food pellet test, with the EA group exhibiting a significantly shorter detection time compared to the OVA group. Inflammatory indicators were also analyzed, revealing elevated OVA-IgE and mRNA expression of interleukins IL-5, IL-13, and IL-4 in the OVA group, which were significantly reduced in the EA group. Moreover, EA treatment decreased the expression of the eosinophil marker RNASE2A but did not affect the mast cell marker MCPT1. In the 28-day OVA group, histological examination showed signs of nasal inflammation, including epithelial cell hypertrophy and eosinophil migration, which were alleviated by EA treatment. However, EA only significantly reduced the number of eosinophils in the tissue. In summary, EA improved nasal allergic symptoms, nasal inflammation, and reduced inflammatory markers in AR. Specifically, it affected eosinophils but not mast cells. These findings suggest that EA at acupoints LI4 and LI11 may alleviate AR inflammation mediated by the IgE pathway and improve olfactory impairment. EA shows promise as a treatment for AR.

Disclosures: Q.V.B. Tran: None. **Y. Chen:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.19/PP6

Topic: F.04. Neuroimmunology

Support: NIH F31 DK127728
NIH T32 DK07563

Title: Solitary tract neuron cytokine receptor signaling mediates bodyweight recovery following inflammatory challenge

Authors: *P. FATHI[1,2], J. E. AYALA[2,3];
[2]Mol. Physiol. and Biophysics, [3]Vanderbilt Brain Inst., [1]Vanderbilt Univ., Nashville, TN

Abstract: The nucleus of the solitary tract (NTS) comprises a diverse neuronal population that responds to sensory information conveyed through vagal afferents. Preproglucagon NTS (NTS^{PPG}) neurons are the main source of the food-intake lowering hormone glucagon-like peptide-1 (GLP-1) in the brain and promote satiation when stimulated. A subset of NTS^{PPG} neurons express the IL-6 receptor (IL-6R) and functionally respond to the inflammatory cytokine IL-6. We hypothesized that NTS^{PPG} IL-6R activation would stimulate these neurons, thereby potentiating satiation signals. Therefore, loss of IL-6R expression in NTS^{PPG} was predicted to impair satiation and promote weight gain under conditions that promote large meal intake, such as exposure to 60% high fat diet (HFD). To test this hypothesis, we generated NTS^{PPG} neuron IL-6R knockout ($PPG^{IL6R\text{-}KO}$) mice and found that chronic HFD exposure did not affect body weight. Feeding microstructure analysis showed that when switched to a HFD, meal size by calorie increased in control but not $PPG^{IL6R\text{-}KO}$ mice. Using a concentrated Ensure caloric preload test we showed that $PPG^{IL6R\text{-}KO}$ mice retained sensitivity to the satiating effect of an Ensure preload across an 8-week HFD feeding regimen whereas control mice did not. These findings suggest that NTS^{PPG} IL-6R signaling actually impairs satiation under metabolic challenges and promotes larger meals. We next tested the effects of inflammatory challenges on food-intake and bodyweight in $PPG^{IL6R\text{-}KO}$ using acute endotoxemia (high-dose LPS, 2.5 mg/kg) and colitis (7 days of 3% dextran-sodium sulfate, DSS) models. Following LPS administration, $PPG^{IL6R\text{-}KO}$ mice had significantly lower cumulative caloric intake and a tendency to lose more weight. Following 3% DSS colitis induction we found that $PPG^{IL6R\text{-}KO}$ mice had larger weight loss, and only 40% of PPG^{IL6RKO} mice showed complete bodyweight recovery compared to 100% of controls. These results suggest that NTS^{PPG} IL-6R signaling provides a fitness advantage in response to an infection-like inflammatory challenge by inhibiting NTS^{PPG} neurons and, thus, preserving food intake. This uncovers a novel role for cytokine regulation of a subset of brainstem neurons that could be leveraged towards strategies for improving survival and maintaining bodyweight under pro-inflammatory conditions.

Disclosures: P. fathi: None. **J.E. Ayala:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.20/PP7

Topic: F.04. Neuroimmunology

Support: NHMRC Ideas Grant
NHMRC Principal Research Fellowship

Title: Paternal immune activation by Poly I:C modulates sperm noncoding RNA profiles and causes transgenerational changes in offspring behavior and physiology

Authors: E. A. KLEEMAN[1], S. REISINGER[1], P. ADITHYA[1], B. HOUSTON[2], G. STATHATOS[2], A. L. GARNHAM[3], S. MCLAUGHLIN[1], M. O'BRYAN[2], C. GUBERT[1], *A. J.

HANNAN[1];
[1]Florey Inst. of Neurosci. and Mental Health, Univ. of Melbourne, Melbourne, Australia; [2]Bio21 Inst., Univ. of Melbourne, Melbourne, Australia; [3]Walter and Eliza Hall Inst. of Med. Res., Melbourne, Australia

Abstract: Paternal pre-conceptual environmental experiences, such as stress and diet, can affect offspring brain and behavioral phenotypes via epigenetic modifications in sperm. Furthermore, maternal immune activation due to infection during gestation can reprogram offspring behavior and brain functioning in adulthood. However, the effects of paternal pre-conceptual exposure to immune activation on the behavior and physiology of offspring (F1) and grand-offspring (F2) are not currently known. We explored effects of paternal pre-conceptual exposure to viral-like immune activation on F1 and F2 behavioral and physiological phenotypes using a C57BL/6 male mouse model. Males were treated with a single injection (intraperitoneal) of the viral mimetic Polyinosinic:polycytidylic acid (Poly I:C: 12 mg/kg) then bred with naïve female mice four weeks after the Poly I:C (or 0.9% saline control) injection. Both the F1 and F2 male and female offspring of Poly I:C treated fathers (n=10-17 per group) showed a reduced bodyweight trajectory in the first four weeks of life, with the F2 offspring also having significantly reduced bodyweight in adulthood. The F1 male and female offspring of Poly I:C treated fathers (n=10-17 per group) displayed increased depression-like behavior in the Porsolt swim test and an altered stress response in the novelty-suppressed feeding test. Additionally, the F1 male offspring only showed significantly increased immune responsivity after a Poly I:C immune challenge (12 mg/kg). Furthermore, the F2 male grand-offspring (n=11) took longer to enter and travelled significantly shorter distances in the light zone of the light/dark box. An analysis of the small noncoding RNA profiles in sperm from Poly I:C treated males revealed significant effects of Poly I:C on the sperm microRNA content at the time of conception. Notably, eight miRNAs with an FDR < 0.05 (miR-141-3p, miR-126b-5p, miR-669o-5p, miR-10b-3p, miR-471-5p, miR-463-5p, miR-148b-3p, and miR-181c-5p) were found to be significantly downregulated in the sperm of Poly I:C treated males. Collectively, we demonstrate that paternal pre-conceptual exposure to a viral immune challenge results in both intergenerational and transgenerational effects on brain, behavior and physiology that may be mediated by alterations in the sperm small noncoding RNA content.

Disclosures: E.A. Kleeman: None. **S. Reisinger:** None. **P. Adithya:** None. **B. Houston:** None. **G. Stathatos:** None. **A.L. Garnham:** None. **S. McLaughlin:** None. **M. O'Bryan:** None. **C. Gubert:** None. **A.J. Hannan:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.21/PP8

Topic: F.04. Neuroimmunology

Support: PSU Bridge Grant
PSU Microgrant

Title: Neuroimmune dysregulation in alcohol+nicotine co-use: A critical role for nicotinic α7 receptors.

Authors: C. A. RANDALL[1], *P. RANDALL[2,3];
[1]Anesthesiol. and Perioperative Med., [2]Anesthesiol. / Pharmacol., [3]Pharmacol., Penn State Col. of Med., Hershey, PA

Abstract: More than 80% of adults with an alcohol use disorder (AUD) also use nicotine in some form. When these drugs are regularly used in combination, rates of negative health outcomes including orofacial cancers, along with heart, liver, and lung diseases increase substantially. Compounding this issue is the growing popularity of electronic nicotine delivery devices (ENDS) which until recently, were not nearly as regulated as traditional tobacco products. Moreover, these products are easily accessed by adolescents where they see their fastest growth in use. Despite such high rates of co-use, relatively few studies focus on nicotine-alcohol co-use and even fewer on their underlying neuro-biological interactions. Of particular note, both drugs affect neuroimmune function but in an opposing manner. Studies in the Randall lab focus on these differential interactions and how modulating them may ultimately provide a novel avenue for the treatment of both alcohol and nicotine dependence. The current studies are focused on the interplay of pro-inflammatory TLR3 receptors and anti-inflammatory nicotinic α7 receptors in the nucleus accumbens (Acb) and medial prefrontal cortex - prelimbic (mPFC-PL) of rats trained to self-administer both alcohol and nicotine using a co-access model. In this model, adult male and female Long-Evans rats were trained to respond on a lever (FR2) to receive 0.1ml of 2% sucrose/15% alcohol. At the same time, rats could respond on a second lever in the chamber (FR2) to activate the nicotine vaporizer (10 mg/mL nicotine solution) for 6 seconds. Following acquisition, rats continued daily self-administration sessions for a minimum of 2 months to establish stable baselines. Following this, all rats were implanted with bilateral microinjection cannulae targeting either the Acb or mPFC-PL. Following recovery and re-establishment of baseline, testing began. On test sessions, rats received bilateral microinjections of the nicotinic α7 agonist GTS-21 (0, 125, 250 µg/side). Microinjections occurred once per week until all rats had received all conditions. Here, we demonstrate that increasing doses of GTS-21 decrease alcohol self-administration with a trend towards decreasing nicotine self-administration. These findings suggest the α7 receptor may be an important target for future pharmacotherapies with the potential of treating both alcohol and nicotine dependence.

Disclosures: C.A. Randall: None. **P. Randall:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.22/PP9

Topic: F.04. Neuroimmunology

Support: R00DA045795
P30DA033934
VCU SoM Startup Funds

Title: A zinc finger transcription factor tunes social behaviors by controlling transposable elements and immune response in prefrontal cortex

Authors: *N. L. TRUBY[1], R. K. KIM[3], G. SILVA[2], X. QU[1], J. PICONE[4], R. L. NEVE[5], X. CUI[1], J. LIU[1], P. J. HAMILTON[1];
[2]Virginia Commonwealth Univ., [1]Virginia Commonwealth Univ., Richmond, VA; [4]Virginia Commonwealth Univ. Neurosci. Grad. Program, [3]Virginia Commonwealth Univ. Neurosci. Grad. Program, Richmond, VA; [5]Gene Delivery Technol. Core, Massachusetts Gen. Hosp., Boston, MA

Abstract: Social behaviors are central to the health of society and the individual and are disrupted in a number of psychiatric illnesses. However, the neurobiological origins of complex social behaviors are incompletely understood. The *Zfp189* gene product is a KRAB zinc finger transcription factor whose expression and function in the rodent prefrontal cortex (PFC) was previously determined to be protective against stress-induced social deficits. To interrogate the function and gene targets of ZFP189, we reprogrammed the endogenous $ZFP189^{WT}$ by replacing the repressive KRAB domain with an enhanced transcriptional activation domain (VP64-p65-Rta ($ZFP189^{VPR}$)) or by removing the functional moiety entirely ($ZFP189^{DN}$). Upon packaging these ZFP189 variant constructs in viral vectors and delivering to mouse PFC, we interrogated the transcriptional and behavioral adaptations mediated by these synthetic ZFP189 transcription factors. We observed that dysregulation of ZFP189-mediated transcription in this brain area, achieved by delivery of synthetic $ZFP189^{VPR}$, precipitates social behavioral deficits in terms of social interaction, motivation, and the cognition necessary for the maintenance of social hierarchy, without other observable behavioral deficits. By performing RNA sequencing in virally manipulated prefrontal cortex tissues, we discovered that ZFP189 transcription factors of opposing regulatory function have opposite influence on the expression of genetic transposable elements as well as genes that participate in immune functions. Additionally, we observe that delivery of cytokine TNFα to the PFC rescues social deficits induced by $ZFP189^{VPR}$. Collectively, this work indicates that ZFP189 function in the prefrontal cortex coordinates transcriptional neuroadaptations necessary for social behaviors by directly binding transposable element-rich regions of DNA to regulate immune-related genes. Given the evidence for a co-evolution of social behavior and the brain immune response, we posit that ZFP189 may have evolved to augment brain transposon-associated immune function as a way of enhancing an animal's capacity for functioning in social groups.

Disclosures: N.L. Truby: None. **R.K. Kim:** None. **G. Silva:** None. **X. Qu:** None. **J. Picone:** None. **R.L. Neve:** None. **X. Cui:** None. **J. Liu:** None. **P.J. Hamilton:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.23/PP10

Topic: F.04. Neuroimmunology

Support: Institute for Translational Sciences at UTMB, supported in part by a Clinical and Translational Science Award NRSA (TL1) Training Core (TL1TR001440) from the National Center for Advancing Translational Sciences, NIH
UTMB startup funding
The Texas STAR award

Title: Activation of MRGPRX2^{+} Meningeal Mast Cells Leads to Migraine-like Pain

Authors: *S. SBEI[1,2], T. MONCRIEF[3], N. LIMJUNYAWONG[4], Y. ZENG[3], D. GREEN[3]; [2]Inst. for Translational Sci., [3]Dept. of Neurobio., [1]Univ. of Texas Med. Br., Galveston, TX; [4]Ctr. of Res. Excellence in Allergy and Immunology, Res. department, Siriraj Hospital, Mahidol Univ., Salaya, Thailand

Abstract: Background: As one of the key effector cells in the inflammatory process, mast cells have essential functions in allergies and fighting infections. More recently, mast cells have been shown as important links between the nervous and immune systems. Mast cells can be found in close proximity to peripheral nerve endings and, due to their significant spatial advantages over other immune cells, are one of the first to respond to sensory nerve activation. We recently showed that mast cell specific receptors, MrgprB2 and its human homologue MRGPRX2, are involved in neurogenic inflammation and pain.
Goals: We are interested in translating our previous findings to another pain phenotype; migraine. We generated a humanized mouse line that expresses MRGPRX2 on meningeal mast cells, thus allowing us to study the role receptor of this receptor in migraine pain *in vivo*.
Methods: Calcium imaging was used to study MRGPRX2^{+} cell activation by PACAP1-38. Calcium imaging and β-hexosaminidase release assays were used to find the dose response. Minimally invasive dural stimulation model was used to apply PACAP1-38 to the MrgprB2-Cre^{+} MRGPRX2^{+} (X2^{+}) or MrgprB2-Cre^{-} X2^{+} (X2^{-}) mouse meninges. Facial mechanical hypersensitivity was measured in male mice prior to dural injection and then 1hr, 2hrs, 4hrs, and 24hrs after dural stimulation with PACAP1-38 (n=11-10/per group) using Von Frey filaments. Flow Cytometry was utilized to quantify X2^{+} cells in the extracted meninges of dural-stimulated vs sham X2^{+} and X2^{-} mice.
Results: The mast cell receptor MrgprB2/X2 is expressed in the meninges and its activation leads to migraine-like pain behavior. The neuropeptide PACAP1-38 activates of MrgprB2, therefore stimulating mast cell release leading to migraine-like pain. Similarly, PACAP1-38 activates mouse meningeal mast cells that express the human MRGPRX2 receptor.
Conclusions: Here, we demonstrate, for the first time, a novel transgenic mouse line that functionally expresses the human MRGPRX2 in connective tissue mast cells, including meningeal mast cells, and responds to PACAP1-38 to contribute to migraine-like pain, in mice.

Disclosures: S. Sbei: None. **T. Moncrief:** None. **N. Limjunyawong:** None. **Y. Zeng:** None. **D. Green:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.24/PP11

Topic: F.04. Neuroimmunology

Support: NIH Medical Sciences Center Grant Award P20GM121312
NIMH K99MH126950
NIDA R01DA050677

Title: Opioid use indicative of increased serum soluble intercellular adhesion molecule 1 concentrations and altered brain reward processing

Authors: *V. COUSSA[1], L. K. FIGUEROA-HALL[2], K. BURROWS[1], R. KUPLICKI[3], T. VICTOR[1], K. K. THOMAS[1], D. R. WALLACE[4], R. L. AUPPERLE[1], S. KHALSA[1], J. SAVITZ[1], K. TEAGUE[5], M. PAULUS, 74136[1], J. L. STEWART[1];
[1]Laureate Inst. for Brain Res., Tulsa, OK; [2]Pharmacol. & Physiol., Oklahoma State Univ. Ctr. For Hlth. Sci., Tulsa, OK; [3]Laureate Inst. For Brain Res., Tulsa, OK; [4]Pharmacology/Physiology & Toxicology, Oklahoma State Univ., Catoosa, OK; [5]Surgery and Psychiatry; Biochem. and Microbiology; Pharmaceut. Sci., OU Col. of Med., Tulsa, OK

Abstract: Substance use affects brain regions involved in impulsivity and reward processing. Elevated levels of serum soluble intercellular adhesion molecule 1 (sICAM-1) may play a role in modulating this relationship. sICAM-1 concentrations are upregulated during altered inflammatory processes that may stem from substance use disorder (SUD). This study investigated the associations between sICAM-1 and brain reward processing in cannabis and opioid use disorder groups compared to healthy comparison (HC) subjects. A subset of Tulsa 1000 study participants was matched on age, sex, and body mass index, and analyzed using respondents' preferred drug of choice (as well as use in the past year and primary intake) as an indicator for primary substance use [cannabis (n=21; 43% male) and opioids (n=39; 49% male)], alongside HC (n=30; 40% male). Serum sICAM-1 concentration was measured using MSD multi-spot assay kits. The monetary incentive delay (MID) task was performed during functional magnetic resonance imaging (fMRI). We observed that opioid users exhibited: (1) higher serum sICAM-1 concentrations compared to HC (d = 0.901) and cannabis users (d = 0.695); (2) lower % fMRI signal change in the right dorsal caudate compared to HC during win anticipation trials on the MID task (d = 0.895); and (3) a negative correlation between sICAM-1 concentrations and the MID gain vs. no gain contrast in the bilateral dorsal caudate, nucleus accumbens, and thalamus (R^2 > 0.172). In contrast, HC exhibited a positive correlation between sICAM-1 concentrations and the MID gain vs. no gain contrast in the same brain regions (R^2 > 0.168), there was no significant relationship observed between sICAM-1 and MID gain vs. no gain for the cannabis users. The significantly higher sICAM-1 concentration in the opioid users compared to the other two groups may be due to opioid-induced activation of the toll-like receptor 4 (TLR4) inflammatory pathway. It is well established in pre-clinical models that opioids activate

TLR4 in a similar fashion to lipopolysaccharide, leading to the expression of inflammatory biomarkers, including sICAM-1. The reduced dorsal caudate reward activation in the opioid users and the elevated sICAM-1 concentration is somewhat consistent with other studies' MID findings in amphetamine, cocaine, and alcohol use groups. However, among the other regions of the striatum and mesocortical-limbic circuits, the mechanism of the neural substrates involved in producing reward responses is less clear. Here, we show for the first time how the relationship between sICAM-1 concentrations and induced inflammation from drug use may correlate to opioid use and brain reward processing.

Disclosures: **V. Coussa:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **L.K. Figueroa-Hall:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **K. Burrows:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **R. Kuplicki:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **T. Victor:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **K.K. Thomas:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **D.R. Wallace:** None. **R.L. Aupperle:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **S. Khalsa:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **J. Savitz:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **K. Teague:** None. **M. Paulus:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research. **J.L. Stewart:** A. Employment/Salary (full or part-time):; Laureate Institute for Brain Research.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.25/PP12

Topic: F.04. Neuroimmunology

Support: Medical Research Council 2021-02251_VR
The Swedish Brain Foundation FO2022-0140

Title: Identification of a novel pharmacological target for the treatment of cognitive dysfunction

Authors: *X. LI[1,2], A. FAKA[2], S. IMBEAULT[2], Y. ZHENG[2], G. ENGBERG[2], L. SCHWIELER[2], S. ERHARDT[2];
[1]Inst. of Physiol. and Pharmacol., Karolinska Inst., Stockholm, Sweden; [2]Inst. of Physiol. and Pharmacol., Karolinska Institutet, Stockholm, Sweden

Abstract: Background: Cognitive impairment is commonly observed in patients with psychiatric disorders and infectious diseases, and there is a lack of pharmacological treatment for this condition. Growing evidence suggests immune activation to be important for cognitive dysfunction. Specifically, immune activation increases the levels of brain kynurenic acid (KYNA), a neuroactive metabolite interfering with neurotransmission and impairing cognitive

functioning. Kynurenine aminotransferases (KAT I-IV) convert kynurenine to KYNA. Under normal conditions, KAT II is responsible for 70% of the production of KYNA. However, the role of KATs in KYNA production during immune activation is not clear. **Methods**: Quantitative reverse transcription was used to analyze the mRNA expression levels and western blot to analyze the protein expression levels of KAT isoforms (I-IV). To obtain single-cell transcriptomic profiles, we used publicly available human postmortem data sources. KYNA levels were quantified using an ultra-performance liquid chromatography-tandem mass spectrometry system. **Results**: Our study provides evidence that KAT II is not the primary enzyme responsible for KYNA production under conditions of immune activation. Instead, we found that KAT III is the most prominent enzyme. We observed an upregulation of KAT III expression in the brains of mice, human fibroblasts, epidermal cells, and monocytes, following immune stimulation. Furthermore, we detected increased expression of KAT III in post-mortem brain samples from COVID-19 patients. In addition, we found that pro-inflammatory cytokines induced the expression of KAT III in a human cell model, and that a selective KAT III inhibitor effectively reversed the immune-induced KYNA. **Conclusions**: Our study highlights the critical role of KAT III in the production of KYNA under conditions of immune activation. These findings have significant implications for the development of therapeutic strategies aimed at addressing the cognitive impairments commonly observed in patients with psychiatric disorders and infectious diseases.

Disclosures: **X. Li:** None. **A. Faka:** None. **S. Imbeault:** None. **Y. Zheng:** None. **G. Engberg:** None. **L. Schwieler:** None. **S. Erhardt:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.26/PP13

Topic: F.04. Neuroimmunology

Support: 2R01MH052716-26

Title: Mast cell visualization in whole cleared neonatal rat brains over the first two weeks of postnatal development

Authors: *A. A. MAXIMOVA, A. C. BLANCHARD, M. M. MCCARTHY;
Pharmacol., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Crosstalk between peripheral immune cells and the brain occurs in healthy conditions and during the progression of neurodevelopmental diseases. Identifying the role peripheral immune cells play during critical periods in brain development will lend insight into neuroimmune interactions at baseline and how this communication could go awry in neuropsychiatric diseases. Mast cells (MCs) are innate immune cells capable of releasing histamine, cytokines, and growth factors during host defense, allergy, and tissue remodeling.

During neurodevelopment, a small MC population drives synaptic patterning and sexual differentiation of the preoptic area (POA) (Lenz et al., 2018 J. Neurosci), but little is known about other populations of brain MCs during brain development. We found a robust MC population adjacent to the hippocampus, peaking in cell number around postnatal (PN) day 10 and rapidly declining by PN14, a pattern reminiscent of a critical period. To further characterize the dynamics of this MC population we undertook whole-brain clearing and 3D imaging to map out the precise localization of these peri-hippocampal MCs from embryonic through postnatal time points. We hypothesize that MCs are present in the intact brain from late embryonic time points to the 2nd postnatal week of life in the rat and exhibit a variety of activation/degranulation morphological states within the peri-hippocampal niche. Using Sprague-Dawley rat pups bred in house, whole neonatal brains were collected in males and females from embryonic (E20) to postnatal (PN0, PN4, PN7, PN11, PN14, and PN20) time points, cleared using the CUBIC technique, and stained for mast cells using fluorescent rhodamine-conjugated avidin. Cleared brains were imaged using a Zeiss LS7 LightSheet microscope to visualize MCs in a 3D environment. Analysis is ongoing using Zeiss Zen and Leica Aivia software to characterize number, location, and enrichment of MCs in the peri-hippocampal region within a whole cleared brain, including comparing these parameters across time points and sex in early postnatal development. Machine learning algorithms created in the Aivia software will differentiate between MC morphological states (i.e., degranulated/intact) and further inform our understanding of the baseline activation state of brain MCs in development. Overall, visualization of MCs in 3D whole cleared brains will improve our understanding of the dynamics of peri-hippocampal MCs and help us explore how MCs can shape postnatal neurodevelopment.

Disclosures: **A.A. Maximova:** None. **A.C. Blanchard:** None. **M.M. McCarthy:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.27/PP14

Topic: F.04. Neuroimmunology

Support: CNPq
FAPERJ
FIOCRUZ
The Laboratório de Pesquisa em Malária is an Associated Laboratory (AL) of the Instituto Nacional de Ciência e Tecnologia (INCT) in Neuroimmunomodulation supported by the CNPq, and AL of the Rio de Janeiro Neuroinflammation Network financed by FAPERJ

Title: Investigation of neuroinflammatory aspects of cognitive dysfunction in non-severe experimental malaria

Authors: *P. ROSA GONÇALVES[1], L. S. CHAGAS[2], R. F. ALMEIDA[3], L. P. S. VIEIRA[1], B. N. SIQUEIRA E SILVA[1], C. C. A. EVARISTO[1], C. C. T. L. GRESS[1], F. L. RIBEIRO GOMES[1], C. A. SERFATY[2], C. T. DANIEL-RIBEIRO[1,4];
[1]Laboratório de Pesquisa em Malária, Inst. Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [2]Laboratório de Plasticidade Neural, Dept. de Biologia, Univ. Federal Fluminense, Niteroi, Brazil; [3]Ctr. de Ciências Químicas, Farmacêuticas e de Alimentos, Univ. Federal de Pelotas, Pelotas, Brazil; [4]Ctr. de Pesquisa, Diagnóstico e Treinamento em Malária, Fiocruz and Secretaria de Vigilância em Saúde, Ministério da Saúde, Rio de Janeiro, Brazil

Abstract: Cognitive deficits and behavioral changes are associated, in both human and experimental murine malaria, with cerebral malaria (CM) and even with non-severe malaria (nSM), the predominant clinical form of the human disease worldwide. From integrative immunology, the infection induces a systemic inflammation with great potential of immune response dysregulation. The hippocampus, a brain structure intimately involved in memory processes, by the glutamatergic neurotransmitter mechanism, play a pivotal role in learning and memory processes. Glutamatergic excitotoxicity and neuroinflammation have been identified in experimental CM. To date, no effective therapy for malaria cognitive impairments is available and the understanding of neuropathogenesis, especially in nSM, is poor. This study aims to investigate hippocampal cellular and molecular neuroimmune aspects of cognitive dysfunction of experimental nSM and its relation to peripheral cytokine levels. C57BL/6 mice were infected with *Plasmodium berghei* ANKA and treated, for seven days, with chloroquine (CQ) from day 4 (D4) after infection on, before the onset of any clinical sign of CM. Behavioral tests were performed 12 days after the end of treatment (dpt) to assess learning and memory. The hippocampus was collected at D4 and 12 dpt and glial and microglial activation were analyzed by immunoreactivity of GFAP and Iba1, respectively. At D4 of infection, before the CQ treatment, qualitative analysis of $Iba1^+$ cells of the hippocampus of parasitized mice showed a reduction in the process complexity of microglia in comparison to the extended and intensively branched processes displayed in the uninfected mice, characteristic of the homeostatic microglia. Preliminary results of morphological alterations in $GFAP^+$ cells suggest that astrocytes are responding to infection in different hippocampal regions. As expected, an increase in serum cytokines was evidenced in the parasitized mice, by flow cytometry, such as interleukin (IL) 6, IL-10, TNF-α and IFN-ɣ. IL-2, IL-4, IL-17A did not change their levels and there was a decrease in TGF-β1 levels. Infected and treated mice exhibited memory deficits in the object location and recognition tasks. These preliminary results suggest that a neuroinflammatory context may be involved in the cognitive impairment of experimental malaria, including in its non-severe form.

Disclosures: P. Rosa Gonçalves: None. **L.S. Chagas:** None. **R.F. Almeida:** None. **L.P.S. Vieira:** None. **B.N. Siqueira e Silva:** None. **C.C.A. Evaristo:** None. **C.C.T.L. Gress:** None. **F.L. Ribeiro Gomes:** None. **C.A. Serfaty:** None. **C.T. Daniel-Ribeiro:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.28/PP15

Topic: F.04. Neuroimmunology

Support: 2R01MH052716-26

Title: Exploring whether peri-hippocampal mast cells delay perineuronal net formation in the developing rat brain.

Authors: *K. ENGEL, A. BLANCHARD, M. MCCARTHY;
Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Perineuronal nets (PNNs) are lattice like structures that surround interneurons in the mature hippocampus. Developmentally, PNNs are constructed by post-natal day 19 (PN19) in the rat, where they terminate the post-natal critical period by surrounding the cell body, axon, and dendritic projections in their extracellular matrix (ECM) envelopes, terminating synaptogenesis. The variables controlling the developmental timing of PNNS are unknown. We recently discovered a large and transient population of an innate immune cell, the mast cell (MC), lining the lateral ventricles, a region we refer to as the peri-hippocampus. We have visualized these MCs as early as embryonic-day 15 (E15) with peak numbers ~PN10 and diminishing by post-natal day 20 (PN20), anteceding the formation of PNNs. These MCs constitutively undergo “piecemeal” degranulation of granules containing a wide array of inflammatory mediators, such as proteases known to disrupt the ECM. These combined observations lead us to hypothesize that peri-hippocampal MCs delay PNN formation in the developing hippocampus. To test this hypothesis, we will use data from previous bulk RNA sequencing on the peri-hippocampal MCs to evaluate if peri-hippocampal MCs express proteases (eg. MCPT9) and whether there are transcriptomic differences compared to peripheral and hypothalamic MCs. To identify whether the MCs are releasing MCPT9, we will use pharmacological agents to either increase (compound 48/80), decrease (Ketotifen), or have no effect (saline) on MC degranulation via i.c.v. injection of Sprague Dawley rat pups from PN6-8. On PN10, CSF will be collected through the cisterna magna and protease levels quantified by ELISA. To determine whether there is an effect on PNN formation, hippocampal tissue will be collected on PN10 for Western Blot analysis and immunohistochemistry to quantify both components of PNNs and morphology. Overall, we predict the constitutively degranulating peri-hippocampal MC population will prevent or diminish PNN formation in early hippocampal development by releasing inflammatory mediators, potentially inhibiting CSPG (chondroitin sulfate proteoglycan) binding around hippocampal neurons. This mechanism could illustrate a novel function of MCs in brain development.

Disclosures: K. Engel: None. **A. Blanchard:** None. **M. McCarthy:** None.

Poster

PSTR494. Consequences of Immune Activation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR494.29/PP16

Topic: B.04. Synaptic Transmission

Title: Focused ultrasound stimulation or surgical removal of the celiac-superior mesenteric ganglion complex alters pro-inflammatory cytokine levels in murine endotoxemia

Authors: *S. PALANDIRA[1,2], A. FALVEY[1], K. J. TRACEY[1,2,3], V. A. PAVLOV[1,2,3]; [1]The Feinstein Inst. For Med. Res., Manhasset, NY; [2]Elmezzi Grad. Sch. of Mol. Med., Manhasset, NY; [3]Donald and Barbara Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY

Abstract: Recent evidence has identified the celiac-superior mesenteric ganglion complex (CSMGC) as an important relay center in the efferent arm of the vagus nerve-based immunoregulatory mechanisms termed *the inflammatory reflex.* In the CSMGC, efferent vagus nerve cholinergic fibers interact with catecholaminergic neurons, which innervate the spleen and this interaction is essential for controlling pro-inflammatory cytokines and inflammation. Despite being an important component of the inflammatory reflex, the specific role of the CSMGC in the regulation of inflammation remained enigmatic and the possibilities for its direct targeting to control inflammation remained underexplored. To provide insight, we examined the effect of focused ultrasound (FUS) stimulation applied to the CSMGC or the surgical removal of the CSMGC on the levels of the pro-inflammatory cytokine TNF in mice with endotoxemia. FUS stimulation or sham stimulation was applied in anesthetized male C57BL/6 mice through a probe placed on the abdomen over the area of CSMGC, which was identified using color doppler ultrasound. Pulsed waves at 1.1MHz and 200mV per pulse were delivered for 5mins. In ganglionectomy experiments, in female ChAT-TdTomato mice, the CSMGC was precisely localized based on endogenous fluorescence associated with cholinergic preganglionic fibers innervating this ganglionic complex. Mice underwent surgical ganglionectomy or sham surgery. Five mins post FUS stimulation, or seven days post ganglionectomy, mice were injected with lipopolysaccharide (endotoxin, 0.25mg/kg, i.p.) and euthanized 90 minutes later, and blood and spleen were collected and processed for cytokine analysis. Compared with sham stimulation, FUS stimulation of the CSMGC (n=8 per group) significantly decreased serum TNF levels (1341 ± 277.7 pg/ml vs 705.6 ± 254 pg/ml, P = 0.0006) in endotoxemic mice. There was a significant increase in serum TNF (1360 ± 942.9 pg/ml vs 456.1 ± 274.4 pg/ml, P = 0.0281) and in splenic TNF levels (52.41 ± 25.04 pg/mg protein vs 22.72 ± 8.215 pg/mg protein, P = 0.0006) in ChAT-TdTomato mice with a surgically removed CMSGC compared with the sham group (n=8 per group) during endotoxemia. In situ microscopic examination of the CSMGC area in ganglionectomized mice vs controls confirmed the success of the procedure. For the first time these results indicate the anti-inflammatory efficacy of non-invasive FUS stimulation of the CSMGC and reveal the tonic anti-inflammatory function of this ganglionic complex. These findings characterize the CSMGC as a new therapeutic target to control pro-inflammatory cytokine levels and inflammation with potential for clinical translation.

Disclosures: S. Palandira: None. **A. Falvey:** None. **K.J. Tracey:** None. **V.A. Pavlov:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.01/PP17

Topic: F.06. Autonomic Regulation

Support: JSPS KAKENHI Grant Number JP22J20706
CREST Grant JPMJCR21P1
A-STEP Grant JPMJTR20UT

Title: Intestinal GLP-1 enhances insulin action via activation of vagal afferents by interacting with pancreatic insulin

Authors: *K. OHBAYASHI[1], T. YADA[2], Y. IWASAKI[1];
[1]Kyoto Prefectural Univ., Kyoto, Japan; [2]Kansai Electric Power Med. Res. Inst., Gifu, Japan

Abstract: [Background] Glucagon-like peptide-1 receptor agonists (GLP-1RAs), which are clinical drugs used for diabetes, stimulate insulin secretion from pancreatic beta cells and ameliorate hyperglycemia. However, endogenous intestinal GLP-1 is highly unstable, and its physiological function has not been completely elucidated. Recently, we found that the rare sugar D-allulose (Allu) stimulates release of intestinal GLP-1, thereby improving glucose tolerance by activating vagal afferents (Y. Iwasaki, Nat Commun, 2018). However, the detail mechanisms remain unclear. [Aim] We investigated the effects of intestinal GLP-1 on hyperglycemia and the underlying mechanisms including vagal afferents. [Methods] Blood glucose (BG) and plasma insulin levels were measured in both healthy and diabetic mice after oral administration of Allu or intraperitoneal injection of the GLP-1 receptor agonist exendin-4 (Ex4). [Results] A single oral administration of Allu increased intestinal GLP-1 secretion and significantly improved hyperglycemia in type 2 diabetic mice, including diet-induced obese mice and *db/db* mice, without stimulating insulin secretion. These beneficial effects were completely blunted in GLP-1R knockout mice and by pretreatment with a GLP-1R antagonist. These results suggest that the intestinal GLP-1/GLP-1R signaling enhances insulin action, resulting in the amelioration of hyperglycemia. On the other hand, in healthy and type 1 diabetic Akita mice, Allu also promoted GLP-1 secretion but failed to alter BG. Comparing the results in healthy and diabetic mice indicates that intestinal GLP-1 enhances insulin action in a manner dependent on plasma insulin levels. Co-administration of Allu and insulin secretagogue in healthy mice additively activated vagal afferent neurons and enhanced the BG-lowering effect of the insulin secretagogue. Denervation of the common hepatic branch of vagal afferents attenuated the cooperative action of intestinal GLP-1 and pancreatic insulin on BG. The therapeutic effects on insulin resistance and hyperglycemia were more rapid and potent with Allu compared to Ex4. [Conclusions] The interaction between intestinal GLP-1 and pancreatic insulin activates the hepatic branch of vagal afferents, thereby ameliorating insulin action and hyperglycemia.

Disclosures: K. Ohbayashi: None. **T. Yada:** None. **Y. Iwasaki:** 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.; Matsutani Chemical Industry Co., Ltd..

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.02/PP18

Topic: F.06. Autonomic Regulation

Support: R15DK121246

Title: Skeletal Muscle Thermogenesis of Mice in Response to Different Predator Odors

Authors: R. GIACOMINO[1], G. JANG[1], D. WALTER[1], C. A. WATTS[2], *C. M. NOVAK[1]; [1]Dept. of Biol. Sci., Kent State Univ., Kent, OH; [2]Diabetes Inst., Univ. of Washington Sch. of Med., Seattle, WA

Abstract: Skeletal muscle thermogenesis, or the heat produced by muscles, can amplify energy expenditure. In previous studies, our research program has used predator odor (PO) as a thermogenic stimulus in mice and rats. By quantitatively measuring the change in skeletal muscle thermogenesis in response to different odors, we can answer questions involving sex-based differences, effects of repeated exposure to stimuli, and the qualitative advantages of some odors over others. To address these questions, remote temperature transponders were used to monitor the skeletal muscle temperature in mice while in the presence of 3 different predator odors—ferret bedding, cat fur, rat bedding—and a control odor. Prior to thermogenesis assessment, the mice were habituated to the testing conditions to decrease the stress-related thermogenic effect of the procedure itself. Then, the mice were assessed in three separate trials per odor, in randomized order. During the four rounds of habituation, females had elevated muscle temperature for a longer period than males. Female mice, unlike males, decreased their thermogenic response upon repeated exposure to the control odor. Little difference between sexes was found for the rat and ferret odors, which elicited robust muscle thermogenesis in both males and females. Upon repeated exposure, cat odor provoked less of a thermogenic effect in both males and females. Overall, all 3 odors caused an increase in skeletal muscle thermogenesis, but ferret odor caused the greatest increase, while control odor caused only a minimal increase. Based on this, we conclude that female mice require additional habituation to prevent excessive muscle thermogenesis in response to testing conditions. Without additional habituation, we might mistakenly conclude that there is a sex difference in the muscle thermogenic response to predator threat. Also, ferret odor causes the most robust and consistent increase in skeletal muscle thermogenesis in comparison to the other POs tested. Future investigation of energy expenditure after exposure to predator odor should consider using ferret odor, as it remains effective after multiple exposures.

Disclosures: R. Giacomino: None. **G. Jang:** None. **D. Walter:** None. **C.A. Watts:** None. **C.M. Novak:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.03/PP19

Topic: F.06. Autonomic Regulation

Support: NIH-R01-MH128688
NIH R00-MH106744
Whitehall Foundation
Rutgers BHI

Title: Neural circuits for parental behavior adaptations to environmental temperature.

Authors: *Z. ADAHMAN[1], R. OYAMA[1], B. SAHOO[1], T. ABE[2], J. RICEBERG[3], I. CARCEA[1];
[1]Rutgers, The State Univ. of NJ - Newark, Newark, NJ; [2]Neurosci. and the Mortimer B. Zuckerman Mind Brain Behavior Institute,, Columbia Univ., New York, NY; [3]Icahn Inst. of Med. at Mount Sinai & Albany Med. Col., New York, NY

Abstract: Thermoregulation is essential for survival in mammals. However, autonomic and behavioral thermoregulatory mechanisms often mature postnatally. Parental care is therefore essential for neonate survival in most mammalian species. Using a mouse model, we characterized adaptations in parental behavior during either drops or increases in environmental temperature. Dams shepherds naïve virgins (female alloparents) towards pups in the nest area most accurately in cold temperature (CT) compared to room temperature (RT) and warm temperature (WT) (ratio of shepherding to total pursuits at CT is 0.95± 0.05, RT is 0.77±0.07, and WT is 0.43±0.05 Both dams and virgins build the largest nests in CT and smaller nests in WT (average nest area, RT is 81.53±12.40cm^2, CT is 103.90±11.59cm^2, and WT is 38.38±2.49 cm^2, p=0.0001, N=6). Environmental temperature also modulates pup retrieval. Mice retrieve slower in both CT and WT, unless a thermoneutral (TN) nest is also provided (latency to retrieve (sec) at CT 226.0±68.49, and at CT with TN nest 107.7±47.06). To understand the mechanisms controlling temperature-modulated parental behavior, we tested maternal care in TRPM8 knockout mice (TRPM8 KO), lacking both cold and warm perception. We observed an increase in nest area built at RT compared to wildtypes (by wildtype RT is 81.53±12.40cm^2 & TRPM8KO RT is 99.04 ±1.512cm^2). In addition, we performed whole-brain neural activity mapping after exposure to different environmental temperatures. The immediate early gene Npas4 expression increased in 26.18% and 54.17% of brain structures due to CT and WT exposure, respectively. However, 25.28% of brain structures showed an increase in Npas4 after either CT and WT exposures, whereas 8.57% of brain structures had decrease in Npas4 in both CT and WT. Among these, the paraventricular hypothalamic nucleus (PVN), which showed increased Npas4 during both CT and WT because of its dual role in regulating social behavior and autonomic functions. Within the PVN, CT activates vasopressin neurons. Vasopressin (AVP) plays important roles in maternal care and other aspects of social behaviors. We performed synaptic anterograde tracing from AVP neurons and found connections with structures implicated in thermoregulatory responses (lateral parabrachial nuclei), maternal

behavior (periductal gray area), and other social behaviors (anterior cingulate cortex, ventral tegmental area, dorsal raphe nuclei). Future directions include using a combination of in-vivo neural activity recordings and chemogenetics manipulation to tease apart the neural circuit involving AVP cells in parental behavior adaptations to environmental temperature.

Disclosures: Z. Adahman: None. **R. Oyama:** None. **B. Sahoo:** None. **T. Abe:** None. **J. Riceberg:** None. **I. Carcea:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.04/PP20

Topic: F.06. Autonomic Regulation

Support: R01NS1315

Title: Two-photon imaging of the area postrema in mice models

Authors: *E. STACY[1], A. MATUNIS[1], Z. HUBBARD[1], S. TAMURA[1], K. ABE[1], R. IWAMOTO[1], Y. KAMBE[2], T. K. SATO[2], T. R. SATO[1];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]Kagoshima Univ., Kagoshima, Japan

Abstract: The area postrema (AP) is a structure located at the dorsal surface of the medulla oblongata at the caudal end of the fourth ventricle. Past studies have suggested that the AP structure detects emetic toxins in the blood and plays a role in integrating interoceptive information. However, due to its small size, it has been a challenge to study the functional properties of the neurons in this area. In the current proposal, we developed a novel approach to expose and visualize the AP in-vivo. This was partially accomplished by applying current to the cerebellum, causing it to shirk and expose the underlying structures. First, by directly injecting AAV-CAG-tdTomato into AP under visual guidance, we confirmed that neurons in this area project to the solitary nucleus, the dorsal motor nucleus of the vagus and parabrachial nucleus. Second, by combining this approach with in-vivo two-photon imaging, we were able to visualize the activity of neurons in the AP. We are currently studying the relationship between the activity patterns of neurons in the AP and their projection patterns.

Disclosures: E. Stacy: None. **A. Matunis:** None. **Z. Hubbard:** None. **S. Tamura:** None. **K. Abe:** None. **R. Iwamoto:** None. **Y. Kambe:** None. **T.K. Sato:** None. **T.R. Sato:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.05/PP21

Topic: F.06. Autonomic Regulation

Support: American Diabetes Association

Title: Molecular taxonomy of vagal efferent neurons and their response to inflammation

Authors: *M. JALIL, J. N. CAMPBELL;
Biol., Univ. of Virgina, Charlottesville, VA

Abstract: The vagus nerve can sense and suppress systemic inflammation but also regulates a plethora of other functions including heart rate, digestion, and metabolism etc. Identifying the specific vagal neurons governing the inflammation suppression will reveal therapeutic targets for treating inflammatory disease and injury. To identify if vagal neurons are activated by inflammation, we performed immunofluorescence for the immediate-early gene product and marker of activated neurons, cFos, and the transcription factor Phox2b in the dorsal motor nucleus of the vagus (DMV) of adult mice 90 minutes after administering intraperitoneal vehicle or bacterial lipopolysaccharide (LPS) 5mg/kg to trigger systemic inflammation. Our results show that the vast majority of DMV neurons in the LPS-treatment show cFos expression and were also immunoreactive for Phox2b. Then, to molecularly identify the affected neuronal subtypes and their response to inflammation, we profiled ~1500 Phox2b+ DMV neurons using single nuclei RNA-sequencing 90 mins after administering LPS or vehicle. We then compared the gene expression profiles of each neuron subtype between treatments and identified general and subtype-specific transcriptional changes due to LPS treatment in DMV. Clustering DMV neurons based on their transcriptomic similarity revealed nine candidate subtypes, which we annotated according to candidate subtype-specific marker genes. Our results indicate that DMV subtype marked by expression of Col6a3 gene is the most sensitive to LPS treatment based on the number of genes affected relative to vehicle. This subtype shows upregulation of neuronal activation markers like Fos and immune-related genes such as Mcl1. Additionally, our single-nuclei RNA-seq dataset also identifies three previously uncharacterized neuronal subtypes, including heterogeneity within previously identified subtypes. For instance, our analysis differentiates Sox5+/Calb2+ DMV neurons from Trpv1+ DMV neurons, previously reported as one subtype, and resolves two subtypes of Nppb+ DMV neurons. Overall, our results provide a comprehensive molecular atlas of DMV neurons subtypes and their transcriptional responses to inflammation and so shed light on potential molecular and cellular mechanisms involved in the vagal anti-inflammatory pathway.

Disclosures: **M. Jalil:** None. **J.N. Campbell:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.06/PP22

Topic: F.06. Autonomic Regulation

Support: Alberta Children's Hospital Research Institute
Canadian Institutes of Health Research
Hotchkiss Brain Institute

Title: Bradykinin and IL-1beta increase the heat sensitivity of vagal TRPV1; relevance for Febrile Seizures

Authors: *O. EROME-UTUNEDI[1], K. T. BARRETT[1], A. ROY[2], C. GAVRILOVICI[1], M. SCANTLEBURY[1];
[1]Neurosci., Univ. of Calgary, Calgary, AB, Canada; [2]Anesthesiol. & Critical Care Med., The George Washington Univ. Sch. of Med. & Hlth. Sci., Washington DC, WA

Abstract: Febrile Seizures (FS) are generalized seizures that occur with a fever. They affect children primarily between six months and five years old and are the most common childhood seizure disorder. Due to its unknown mechanism, current treatments for FS target its symptomology and these treatments' associated risks struggle to outweigh their benefits. Previous studies indicate that FS are triggered by respiratory alkalosis resulting from hyperthermia-induced hyperventilation. Ongoing work in our lab shows that lipopolysaccharide (LPS)-induced inflammatory sensitization and heat activation of transient receptor potential vanilloid-1 (TRPV1) receptors in vagal nodose ganglia (VNG) possibly underlies this hyperpnea-induced respiratory alkalosis that triggers FS. TRPV1 is a heat-sensitive nociceptive channel that is activated at temperatures above 43°C, and inflammatory mediators bradykinin (BK), IL-1β and TNF-α have been found to sensitize heat-sensitive nociceptive channels. Therefore, in this study, we investigated which inflammatory mediators, individually or in conjunction, are responsible for sensitizing vagal TRPV1 in the breathing mechanism underlying FS.We hypothesized that BK and IL-1β sensitize vagal TRPV1 to heat, and when combined, their effects are potentiated. To test this hypothesis, we exposed vagus nerves attached to VNG from naïve postnatal day (P) 9-11 Sprague-Dawley rats to four 80-second heat (45°C) challenges in the presence or absence of BK and IL-1β in vitro. We assessed the maximum vagus nerve activity (VNA) in response to each heat challenge, the temperature at which 20% of this maximum activity (TI20) was observed, and the latency to both the maximum VNA and TI20.We found that compared to vehicle-treated controls, IL-1β increased the maximum VNA in response to heat, while BK caused a 0.7°C (0.70±0.22°C, p=0.017, df=14, Kenward-Roger) reduction in the TI20 and significantly reduced the latency to both the maximum VNA and TI20 by 13 sec (12.88±4.59 sec, p=0.035, df=14, Kenward-Roger) and 15 sec (15.12±5.34 sec, p=0.033, df=14, Kenward-Roger) respectively. This effect of BK, but not IL-1β, was exacerbated in subsequent heat challenges.These findings indicate that during inflammation, BK and IL-1β differentially enhance the heat sensitivity of neonatal rat vagus nerves, and in conjunction may be responsible for the vagal TRPV1-mediated inflammation-induced thermal hyperpnea that leads to respiratory alkalosis and consequent FS. These novel findings provide a better understanding of the mechanism underlying FS genesis and identify possible molecular targets for the development of FS treatments.

Disclosures: O. Erome-Utunedi: None. **K.T. Barrett:** None. **A. Roy:** None. **C. Gavrilovici:** None. **M. Scantlebury:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.07/PP23

Topic: F.06. Autonomic Regulation

Title: Sex differences in morphine effects on expression of thermogenic and immune genes in brown adipose tissue.

Authors: S. BOWLES[1], S. LUZ[1], K. EIGO[1], P. SEALE[2], ***S. BHATNAGAR**[3];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania, Philadelphia, PA;
[3]Children's Hosp. of Philadelphia and Univ. of Pennsylvania, Philadelphia, PA

Abstract: Brown adipose tissue (BAT) is a class of adipose tissue found in mammals that has the unique capacity for adaptive thermogenesis. BAT activity is regulated by sympathetic activation of β3-adrenergic receptors and driven by the inner mitochondrial protein uncoupling protein 1 (UCP1) and through a key transcriptional regulator of thermogenic genes in adipose tissue, PPARG coactivator 1-alpha (Pgc1α). Pharmacological manipulation of opioid receptors induces changes in body temperature. However, little is known about the impact of opioids on BAT activity and thermogenic genes. In addition, sex differences in BAT activity and thermogenic gene expression are not well understood. In the studies described here, adult male and female rats were either administered a single i.p injection of morphine (10mg/kg) for one day (acute exposure) or for five consecutive days (repeated exposure). Adipose tissue samples were collected three hours after the administration. Acute morphine significantly increased UCP1 expression in BAT of males compared to saline but had no impact in females. Additionally, males showed no changes in cytokine expression (TNFα, IL1β, and IL-6). However, acute morphine significantly increased TNFα in females. With repeated administration of morphine, UCP1 expression was elevated in the BAT of both males and females compared to saline. However, Pgc1α was only increased in males given morphine compared to saline. Additionally, there was no change in proinflammatory cytokines in males, though females showed a significant decrease in TNFα and IL1β expression. White adipose tissue (WAT) can be recruited to become thermogenic. Repeated morphine did not induce a significant effect on expression of thermogenic genes in the subcutaneous inguinal WAT in either males or females but differences between males and females were observed in expression of thermogenic and immune genes in epididymal WAT. Taken together, these data suggest that both acute and repeated administration of morphine initiate expression of thermogenic genes and immune genes in BAT differentially between males and females. Future studies aim to explore the neural substrates that mediate these sex differences in BAT activity and thermoregulation through opioid receptors.

Disclosures: S. Bowles: None. **S. Luz:** None. **K. Eigo:** None. **P. Seale:** None. **S. Bhatnagar:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.08/PP24

Topic: F.06. Autonomic Regulation
IRP NIH ZIA DK075062
IRP NIH ZIA DK075063

Title: Thermoregulatory roles of preoptic area histamine receptor H1 neurons

Authors: *R. PIÑOL[1], A. VALERI[2], O. GAVRILOVA[2], C. XIAO[2], M. REITMAN[2];
[1]NIH-NIDDK-DEOB, Bethesda, MD; [2]NIDDK, NIH, Bethesda, MD

Abstract: The preoptic area plays an important role in the regulation of body temperature, contributing to behavioral and physiological thermoregulatory functions, including torpor, fever and defense from cold and hot. Activation of multiple, possibly overlapping preoptic neuronal populations can decrease body temperature. Three populations, when activated, increase body temperature: two contributing to fever and one, BRS3-expressing, to cold defense. The histamine receptor H1 is widely expressed in the brain, including in preoptic nuclei. Nanoinjection of histamine in the preoptic area can increase body temperature and preoptic histamine levels are correlated with wakefulness. We developed a Hrh1-Cre mouse to test the role of preoptic histamine receptor H1 neurons in thermoregulation. Optogenetic activation of preoptic histamine receptor H1 neurons increased body temperature and physical activity in both sexes and, in females, nest building. Chemogenetic inhibition did not change body temperature or physical activity during the light phase but attenuated the dark phase body temperature increase, without changing physical activity. In addition, inhibition of preoptic area histamine receptor H1 neurons reduced cold-associated nest building in both sexes. Preoptic area histamine receptor H1 neurons project widely, including to histamine-producing nuclei. In conclusion, preoptic histamine receptor H1 neurons participate in several thermoregulatory actions, including body temperature regulation, physical activity, and nesting. These neurons potentially mediate circadian histamine-associated body temperature changes and nesting behavior.

Disclosures: R. Piñol: None. **A. Valeri:** None. **O. Gavrilova:** None. **C. Xiao:** None. **M. Reitman:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.09/PP25

Topic: F.06. Autonomic Regulation

Support: NS085477 (Saper), NS072733 (Saper), HL095491 (Saper)
NS122589 (Arrigoni), NS112175 (Kaur) and OD028635 (Banks)
University of Tsukuba/Japan Agency for Medical Research (Saper)
BIDMC Career Development Award (Machado)
Boston Nutrition Obesity Research Center Pilot & Feasibility award # 3P30DK046200-29 (Machado)
Mathers Foundation # MF-2204-02555 (Machado)
NIH T32HL00790

Title: Ep3r-expressing preoptic neurons act as a two-way master switch for thermoregulation

Authors: *N. MACHADO[1], F. RAFFIN[2], S. KAUR[3], A. BANKS[1], N. LYNCH[1], O. FANARI[1], O. RAMÍREZ PLASCENCIA[4], S. ATEN[1], J. D. LIMA[5], S. BANDARU[1], R. PALMITER[6], E. ARRIGONI[7], C. B. SAPER[8];
[1]Harvard Med. School, BIDMC, Boston, MA; [2]Beth Israel Deaconess Med. Ctr., Boston, MA; [3]Neurol., Beth Israel Deaconess Med. Ctr. and Harvard M, Boston, MA; [4]Neurol., BIDMC/ Harvard Med. Sch., Boston, MA; [5]Univ. of Sao Paulo, Sao Paulo, Brazil; [6]Univ. Washington, Seattle, WA; [7]BIDMC and Harvard Med. Sch., Boston, MA; [8]James Jackson Putnam Prof, Harvard Med. Sch. Dept. of Neurol., Boston, MA

Abstract: Many species use a temporary drop in body temperature and metabolic rate (torpor) to survive food scarcity. A profound reduction in body temperature (Tb) similar to that seen in torpor is observed with activation of preoptic neurons that express the neuropeptides PACAP, BDNF, or QRFP, the vesicular glutamate transporter (Vglut2) or the leptin receptor, estrogen receptor 1, or prostaglandin E receptor 3 (EP3R, encoded by the gene *Ptger3*) in mice. However, most of these genetic markers are found on multiple populations of preoptic neurons and only partially overlap with one another. We hypothesized that expression of the EP3R marks a unique population of preoptic neurons required both for lipopolysaccharide (LPS)-induced fever and for torpor. We first quantified the co-expression of EP3R and PACAP using in situ hybridization, finding that 60.3% ± 8.8 SEM of $MnPO^{EP3R}$ neurons expressed the gene encoding PACAP, *Adcyap1,* and 45.6% ± 2.8 SEM of $MnPO^{PACAP}$ neurons expressed *Ptger3*. Then, we crossed $Ptger3^{flox/flox}$ mice with $Adcyap1^{Cre}$ mice to generate mice with EP3R inactivated in PACAP neurons. We found that control mice had a typical fever response after LPS treatment (~1.5 ºC peak in Tb). However, the PACAP+EP3R-null mice had a ~0.5 ºC fall in Tb in response to LPS. As EP3R is mainly inhibitory, we used fiber photometry to investigate whether an EP3R-mediated inhibition of the $MnPO^{PACAP}$ neurons underlies the generation of febrile responses and found a substantial reduction in GCaMP signal from $MnPO^{PACAP}$ neurons during fever. Using an optogenetic method, we also inhibited $MnPO^{PACAP}$ cell bodies or their synaptic terminals in the DMH or RPa. This protocol induced an elevation of Tb by 0.5-0.6 °C that lasted >2 h after a 15 min inhibition of the cell bodies or their terminals in the DMH. To test whether the expression of EP3R identifies a critical population of preoptic neurons that are necessary to cause torpor responses, we used both chemogenetic and optogenetic methods associated with $Ptger3^{Cre}$ mice to permit selective activation of the $MnPO^{EP3R}$ neurons. Using these approaches, we demonstrated that stimulation of $MnPO^{EP3R}$ cells causes a prolonged drop of Tb from ~36°C to 22-27 °C and reduces energy expenditure by ~80%. In vitro GCaMP-based studies revealed that $MnPO^{EP3R}$ cells sustain a stably active state after a brief stimulation. Finally, we tested the

necessity of $MnPO^{EP3R}$ neurons in modulating Tb and EE in a natural condition or physiological challenges. We found that deletion of these neurons causes an elevation of Tb during the sleep phase and prevents fever or torpor. These properties endow $MnPO^{EP3R}$ neurons with the ability to act as a two-way master switch for thermoregulation.

Disclosures: **N. Machado:** None. **F. Raffin:** None. **S. Kaur:** None. **A. Banks:** None. **N. Lynch:** None. **O. Fanari:** None. **O. Ramírez Plascencia:** None. **S. Aten:** None. **J.D. Lima:** None. **S. Bandaru:** None. **R. Palmiter:** None. **E. Arrigoni:** None. **C.B. Saper:** None.

Poster

PSTR495. Thermoregulation and Vagus Innervation

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR495.10/PP26

Topic: F.06. Autonomic Regulation

Support: National Natural Science Foundation of China grants (32071010)
Shanghai Pujiang Program (20PJ1415000)
the Shanghai Municipal Science and Technology Major Project (2018SHZDZX05)
National Science and Technology Innovation 2030 Grants (2022ZD0206100)
the Lingang Laboratory (grant no. LG-QS-202203-09)

Title: Cold-sensitive ventromedial hypothalamic neurons control homeostatic thermogenesis.

Authors: *C. FENG, Y. WANG, X. ZHA, H. CAO, S. HUANG, X. XU, Z. LIANG, Z. ZHANG;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: Homeostatic thermogenesis is a critical protective feature of endotherms. However, the specific neuronal types involved in cold-induced thermogenesis remain largely unknown. Using functional magnetic resonance imaging and *in-situ* hybridization, we screened for and found preprodynorphin(PDYN)-expressing neurons in the ventromedial hypothalamus (VMH) as cold-sensitive cells. In addition, a reduction and elevation of temperature triggered an increase and decrease of calcium activity in these $dmVMH^{Pdyn}$ neurons, respectively. Optogenetic activation of $dmVMH^{Pdyn}$ neurons led to a concurrent increase in the brown adipose tissue and internal body temperature as well as the heart rate and blood pressure, whereas optogenetic inhibition showed opposite results, indicating an active role of $dmVMH^{Pdyn}$ neurons in homeostatic thermogenesis. Furthermore, we found that $dmVMH^{Pdyn}$ neurons are linked to known thermoregulation circuits. Importantly, $dmVMH^{Pdyn}$ neurons also showed robust activation during mouse social interaction, and optogenetic inhibition suppressed social interaction and associated hyperthermia. What is more, we also found a robust role of $dmVMH^{Pdyn}$ neurons prevent metabolic-side effect of olanzapine. Such functions of $dmVMH^{Pdyn}$

neurons allow coordinated regulation of energy metabolism , social behaviors and cognitive diseases.

Disclosures: **C. Feng:** None. **Y. Wang:** None. **X. Zha:** None. **H. Cao:** None. **S. Huang:** None. **X. Xu:** None. **Z. Liang:** None. **Z. Zhang:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.01/PP27

Topic: G.04. Emotion

Support: R15MH110951

Title: Salience and fronto-parietal network resting-state functional connectivity linked to adaptive and maladaptive emotion regulation in individuals with high-trait anxiety

Authors: M. MATTSON[1], C. PENDELL[2], M. OJA[1], G. WESTRICK[1], J. CARLSON[1], *L. FANG[1];
[1]Northern Michigan Univ., Marquette, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Emotion regulation strategies are used to monitor and process an individual's emotional response to the environment. Adaptive emotion regulation aids in mitigating the negative impact of the response. In contrast, maladaptive emotion regulation potentiates the negative affective state, such as maintaining and amplifying anxiety symptoms. Previous research has shown that the prefrontal cortex (PFC), anterior insula, amygdala, and supplementary motor area play an important role in emotion regulation. Anxiety disorders have been linked with impaired emotion regulation - individuals suffering with anxiety have been observed to have atypically low engagement of the PFC and abnormal connectivity between the PFC and amygdala. However, research on how various intrinsic functional networks are implicated in different emotion regulation strategies is still scarce. The present study aimed to explore how the intrinsic functional connectivity involved in emotion processing and cognitive control is associated with adaptive and maladaptive emotion regulation in high trait anxious individuals. We collected resting-state fMRI data from 69 participants with high-trait anxiety. In addition, the usage of adaptive and maladaptive emotion regulation strategies were measured using the Cognitive Emotion Regulation Questionnaire. Adaptive and maladaptive emotion regulation scores were entered into the linear regression model along with mean motion as covariates for ROI-to-ROI (Region of Interest) connectivity analyses in the CONN toolbox. All seed regions used in analysis were from bilateral amygdala, the frontoparietal network, salience network, and default-mode network. The results showed that, at an uncorrected $p < .05$ level, relative to maladaptive emotion regulation strategies, adaptive strategies were positively associated with the connectivity between left rostral PFC and the lateral PFC, left rostral PFC and right supramarginal gyrus (SMG), as well as right rostral PFC and the right SMG. Our

findings suggest that the intrinsic functional connectivity of the salience and frontoparietal networks was associated with the difference between adaptive and maladaptive emotion regulation in high-trait anxious individuals. The greater connection between the frontoparietal and salience network may reflect greater cognitive control over emotional reactivity.

Disclosures: **M. Mattson:** None. **C. Pendell:** None. **M. Oja:** None. **G. Westrick:** None. **J. Carlson:** None. **L. Fang:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.02/PP28

Topic: G.04. Emotion

Support: NIH R01DA048096
NIH R01MH121099
NIH R01MH124115
NIH 5KL2TR00142
NIH F30DA053176

Title: Subjective feelings in non-Parkinson's controls versus individuals with Parkinson's Disease with and without impulse control disorder during a risky decision-making task

Authors: *A. JIANG[1], B. LIEBENOW[5], E. DIMARCO[2], L. P. SANDS, III[6], M. S. SIDDIQUI[3], I. U. HAQ[7], K. T. KISHIDA[4];
[1]Physiol. & Pharmacol., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [2]Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [3]Neurol., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [4]Wake Forest Sch. of Med., Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [5]Wake Forest Sch. of Med., Wake Forest Sch. of Med., Winston-Salem, NC; [6]Human Neuroimaging Lab. and Computat. Psychiatry Unit, VTC Fralin Biomed. Res. Inst., Roanoke, VA; [7]Neurol., Univ. of Miami, Miami, FL

Abstract: Subjective feelings play a crucial role in influencing impulsive actions, thereby impacting the decision-making process. A selective attentional bias can lead to impulsive actions, where individuals over attend to information that aligns with their impulsive behavior while downplaying other relevant information. Impulse control disorder (ICD) is a condition that can arise in Parkinson's disease (PD) whereby patients develop repetitive, impulsive behaviors onset after prescription of certain dopaminergic therapies. The mechanisms behind ICD are not fully known. It has been estimated that approximately 15% of PD patients taking medications develop ICD, and the condition appears more often in patients who have been prescribed dopaminergic agonists.
In previous work, we investigated how individuals with PD with and without ICD, as defined by scores in Questionnaire for Impulsive-Compulsive Disorders in Parkinson's Disease-Rating

Scale (QUIP-RS), valued options and experienced subjective feelings in a risky decision-making task when on and off their dopaminergic medication. In the task, participants completed multiple trials during which they chose between a certain reward and a gamble option consisting of 2 gain values, then rated their feelings after a third of trials. We modeled participants' rating behavior by fitting a Bayesian hierarchical version of a happiness model developed by Rutledge et al. with group-level and individual-level parameters for how much the value of the certain reward option, expected value of the gamble option, and resulting reward prediction error influence reported subjective feelings, as well as a forgetting factor to take into account how events in previous trials affect the current rating.
In PD, we found those with ICD (n=18), when off their medication, weigh the value of both the certain reward and gamble options less than patients without ICD (n=12) when reporting their subjective feelings. We now examine these parameters in comparison to healthy controls (n=40) without PD. We found weights for healthy controls to be similar to PD with ICD (on and off), and that posterior distributions for the healthy controls were credibly different than patients without ICD when off their dopaminergic medication. This suggests that individuals with PD who do not experience ICD may exhibit distinct characteristics compared to those with PD and ICD, as well as individuals without PD. To investigate further, we examined the neural correlates of the task in a subset of healthy controls (n=16) during functional magnetic resonance imaging which showed BOLD activity associated to reward and choice in areas including the caudate and accumbens.

Disclosures: A. Jiang: None. **B. Liebenow:** None. **E. DiMarco:** None. **L.P. Sands:** None. **M.S. Siddiqui:** None. **I.U. Haq:** None. **K.T. Kishida:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.03/QQ1

Topic: G.04. Emotion

Title: Relationship between psychosocial stress-induced prefrontal cortex activity and gut microbiota in healthy participants - A functional near-infrared spectroscopy study

Authors: *K. YAMAOKA[1], N. UOTSU[1], E. HOSHINO[2];
[1]FANCL Corp. Res. Inst., Yokohama, Japan; [2]Keio Univ. Global Res. Inst., Tokyo, Japan

Abstract: Brain and gut microbiota communicate in a bidirectional manner with each affecting a person's response to psychosocial stress. However, the association between stress-related brain functions and the gut microbiota is mostly investigated in animal models and patients with depression. Recently, we investigated the relationship between psychosocial stress response in the prefrontal cortex and the abundance of gut microbiota in healthy male participants, as reported in our previous publication (Yamaoka, et al., 2022). The study demonstrated that the psychosocial stress response in specific regions of the prefrontal cortex were associated with

certain gut microbiota abundance, and the participants who had high psychosocial stress resembled that noted in patients with depression. In the present study, we revisited the same dataset and applied a new analysis to further explorer the relationships between neural response to psychosocial stress and gut microbiota. Specifically, we investigate the association between psychosocial stress response in the previously observed five areas of the prefrontal cortex and the gut microbiota community structures. In the experiment, 58 healthy males participated, submitted fecal samples, and were exposed to a psychosocial stress task while brain activation data were recorded using functional near-infrared spectroscopy. The task successfully induced subjective stress and increased heart rate, as well as activation of specific regions of the prefrontal cortex (the right pre-motor cortex/supplementary motor area, right dorsolateral prefrontal cortex, right frontal pole, and right inferior prefrontal gyrus). For our current investigation, we performed cluster analysis based on their gut microbiota characteristic, classifying the participants into two clusters, which revealed a difference in gut microbiota diversity. The activation in the right frontal pole, and right inferior prefrontal gyrus were higher in the low diversity group compared to the high diversity group. Furthermore, the abundance of *Alistipes*, *Anaerofilum*, *Asaccharobacter*, *Clostridium IV*, *Odoribacter*, and *Oxalobacter*, genera that are more prevalent in patients with depression, were higher in the low diversity/ high stress response group. These results suggest a further association involving brain function and gut microbiota community structure in healthy participants, adding to the direct relationship between brain function and the individual gut microbiota types. The combination of both studies provides a deeper insight of gut-brain interactions regarding psychosocial stress.

Disclosures: K. Yamaoka: None. **N. Uotsu:** None. **E. Hoshino:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.04/QQ2

Topic: G.04. Emotion

Support: The Jordan Elizabeth Harris Foundation

Title: Characterizing affective and non-affective cognitive control performance in adolescents with epilepsy and the impact of anxiety and anhedonia

Authors: *K. KILLIAN[1], F. KING[2,5], C. PAPADELIS[5,2,6], T. L. GREER[3,7], C. M. COOPER[5,3,4,7];
[1]Univ. of Texas, Arlington, Arlington, TX; [2]Dept. of Bioengineering, [3]Dept. of Psychology, Univ. of Texas at Arlington, Arlington, TX; [4]Dept. of Bioengineering, Univ. of Texas at Arlington, Fort Worth, TX; [5]Jane and John Justin Inst. for Mind Hlth., Cook Children's Med. Ctr., Fort Worth, TX; [6]Dept. of Pediatrics, Texas Christian Univ. Sch. of Med., Fort Worth, TX; [7]Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Mood and anxiety disorders are highly comorbid in epilepsy. Anxiety occurs in approximately 40% of individuals with epilepsy, compared to the prevalence of 20-30% in the general population. Anhedonia, a cardinal symptom of depression, occurs in about 35% of individuals with epilepsy. The posited bidirectional relationship between epilepsy and these psychiatric conditions is not well understood. However, a similar relationship between affective and non-affective cognitive processing and psychiatric symptoms has been more extensively investigated (e.g., emotion processing and anxiety), and may inform our understanding of these relationships in epilepsy. The current study included individuals with epilepsy (n=23) and healthy controls (n=23), ages 10-19, who completed a range of questionnaires capturing demographic information, medical history, anxiety, anhedonia, and quality of life. Participants also completed cognitive and behavioral tasks, including the Flanker Interference task, which measures non-affective voluntary and involuntary control, and the Emotional Stroop task, which measures affective cognitive control. Differences in task performance, i.e., accuracy and response times, were assessed between the groups. Relationships between task performance and anxiety symptom level, as well as level of anhedonia were also assessed. Youth with epilepsy had slower response times than healthy controls in the Flanker Interference Task. Healthy controls showed the typical response to the Emotional Stroop task, characterized by slower response times for incongruent compared to congruent stimuli. The same was not seen for those with epilepsy. Significant associations were observed between Flanker Interference performance and levels of anxiety and anhedonia. Higher levels of anxiety were related to faster response times on the task. Greater levels of anhedonia were related to slower response time for the Flanker Interference effect. No significant associations were found between Emotional Stroop performance and levels of anxiety and anhedonia. Characterizing the relationship between epilepsy, anxiety, and anhedonia using both affective and non-affective cognitive tasks may guide development of diagnostic markers for these disorders and may improve cognitive interventions and related outcomes in adolescents with these disorders.

Disclosures: K. Killian: None. **F. King:** None. **C. Papadelis:** None. **T.L. Greer:** None. **C.M. Cooper:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.05/Web Only

Topic: G.04. Emotion

Support: DST Grant SRG 2019 000382

Title: A comprehensive computational analysis of RNA binding protein (RBP) networks potentially underlying psychiatric comorbidities (RBP) networks potentially underlying psychiatric co-morbidities

Authors: *S. JHA[1], N. M J[2];
[1]SASTRA Deemed Univ., Thanjavur, India; [2]Biotech., St Joseph's Univ., Bangalore, India

Abstract: Psychiatric disorders are a major burden on global health. They manifest as co-morbid conditions, complicating the treatment (Samsom and Wong, 2015, Front. Psychiatry 6:13). The current understanding of the regulatory basis of psychiatric co-morbidities is largely focused on epigenetic modulators, noncoding RNAs, and transcription factors (Martins and Schratt, 2021, Transl Psychiatry 11:263; Egervari et al. 2019, Mol Psychiatry 24:653-673). The clusters of RNA-binding proteins (RBPs) are now known to be predominant controllers of multiple gene regulatory processes (Harvey et al. 2018, Wiley Interdiscip Rev RNA 9:e1465). However, their involvement in gene expression dysregulation in psychiatric co-morbidities is yet to be understood. In the present work, we identified ten RBP-coding genes to be associated with psychiatric disorders, mainly major depression, schizophrenia, and bipolar disorders (QKI, ELAVL2, EIF2S1, SRSF3, IGF2BP2, EIF4B, SNRNP70, FMR1, DAZAP1, and MBNL1). Further, the analysis of transcriptomic changes in response to individual manipulation of these RBPs showed the potential influence of a large number of RBPs driving differential gene expression, suggesting functional cross-talk giving rise to multi-protein networks. Subsequently, we analyzed the transcriptome data of post-mortem human brain samples from diseased and control individuals which suggested the involvement of ~ 100 RBPs influencing gene expression changes. Further, hnRNP, SRSF, and PCBP family RBPs, Matrin3, U2AF2, KHDRBS1, PTBP1, and also PABPN1 were found to be the hub proteins of the network formed b these RBPs. These RBPs are known to regulate transcript splicing, mRNA transport, localization, stability, and translation. Thus, extensive RBP networks involving a few hub proteins could result in transcriptome-wide dysregulation of post-transcriptional modifications, potentially driving multiple psychiatric disorders. Future studies towards detailed understanding of the functional involvement of these RBPs in modulating neural homeostasis could provide novel therapeutic targets towards the treatment of psychiatric disorders (Nishanth and Jha, 2023, EJMHG, 24:2).

Disclosures: S. Jha: None. **N. M j:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.06/QQ3

Topic: G.04. Emotion

Support: Univ. of Minnesota UROP award

Title: The Relationship between cerebral asymmetry and measures of psychopathy in a non-clinical sample is moderated by both empathic challenge and biological sex

Authors: *R. LLOYD[1], R. HJELLE[2], G. GUNTHER[1];
[1]Psychology, Univ. of Minnesota, Duluth, Duluth, MN; [2]Dept. of Psychology, Univ. of Minnesota, Duluth, MN

Abstract: This study investigated the relationships among psychopathy, assessed by the Levenson Self-Report Psychopathy (LSRP) scale, cerebral laterality, and biological sex. EEG recordings from frontal cortices (L3 and L4) were taken during resting conditions and while viewing a video of an emergency field amputation, used as an empathic challenge. The ratio of alpha power from the two recording sites was taken as an index of relative activity in the two hemispheres. Eighty students from the University subject pool (47 female; 33 male) were recruited as participants. Males had significantly higher LSRP scores than did women, consistent with the initial report by Levenson et al (1995).
While LSRP scores were unrelated to cerebral laterality under resting conditions, biological females demonstrated a significant quadratic relationship between lateralization and psychopathy scores, with females on the low and high ends of the psychopathy distribution demonstrating greater lateralization to the right hemisphere than those with moderate scores. Conversely, the male sample showed no relationship between lateralization and psychopathy scores. Our data suggest that the relationship between psychopathy and cerebral laterality may be sexually dimorphic.
Batky et al (2020) failed to find a relationship between psychopathy and hemispheric asymmetry, at rest or during an emotional processing task, in incarcerated male youth, which is consistent with our findings.
Many studies suggest that the left hemisphere mediates positive affect and approach, while the right hemisphere mediates negative affect and withdrawal (Davidson, et al, 1990). However, Harmon-Jones et al (2010) present data that left hemisphere activation is also associated with approach to negative stimuli (e.g., anger), distinguishing motivational direction from affective valence.
Light et al (2009) distinguished between “empathic concern”, sharing pain or sorrow of another, which is associated with right cortical activation and “empathic happiness”, pleasure in response to someone else’s positive state which is associated with left cortical activity in young children.As in the present study, baseline assessments of laterality were unrelated to empathy, consistent with our findings, suggesting that higher scores on the LSRP are associated with less empathic concern and, thus, less right hemisphere lateralization. However, the quadratic relationship may indicate that there is more than one psychological response to the stimulus, in addition to empathy.

Disclosures: R. Lloyd: None. **R. Hjelle:** None. **G. Gunther:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.07/QQ4

Topic: G.04. Emotion

Support: NIH Grant R21MH133055-01
UAB Dept of Neurology

Title: Distinct gamma frequency responses during components of acute psychosocial stress in humans

Authors: *A. M. GOODMAN[1], A. R. NOLAN[1], K. E. DAVIS[1], D. JANKO[1], R. J. CHATFIELD[1], J. F. MAGNOTTI[2], J. P. SZAFLARSKI[1];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Prior functional magnetic resonance imaging (fMRI) work has implicated specific neurocircuitry enabling acute psychosocial stress responses in humans (Pruessner et al., 2008; Goodman et al., 2020, 2022). Decreased activity within salience (dorsomedial prefrontal cortex [PFC] and anterior insular regions) and limbic (amygdala, hippocampus) networks during stressful performance demands and increased activity with the self-reference network (rostral anterior cingulate cortex [ACC] and posteromedial cortex [PMC]) and limbic networks during negative evaluative feedback has been well-documented. As a critical extension, stereoencephalography (sEEG) allows for capturing finer spatiotemporal specificity and transitions within whole-brain networks compared to fMRI. The current study assessed 13 patients with epilepsy undergoing sEEG monitoring (2048Hz) for potential resective surgery who performed a variant of the Montreal imaging stress task. Data were preprocessed and analyzed using R Analysis and Visualization of iEEG (RAVE). Data were notch filtered for power-line noise and harmonics, bipolar referenced, and sectioned into 2s epochs time-locked to stimulus presentation. Electrodes containing non-physiological artifact were excluded, and a complex Morlet wavelet transform was applied to extract data in the gamma frequency range (70-150Hz). Electrodes were localized by coregistering the MRI and CT to MNI template and labeled with the Harvard-Oxford MNI atlas. Paired samples t-tests and Cohen's d effect size assessed each region's responses to stressful math performance (control>stress) and negative evaluative feedback (positive>negative) trial events. Gamma responses (mean dB) decreased during stressful math within the left anterior insula ($t[44]=3.05$, $p<0.01$, $d=0.26$), but increased during negative feedback within the rostral ACC ($t[16]=-3.11$, $p<0.01$, $d=0.57$) and hippocampal ($t[80]=-4.24$, $p<0.001$, $d=0.64$) regions. All remaining comparisons failed to reach the criteria for significance (all $ps>0.1$). Decreased responses within the salience network during stressful performance and increased responses within self-reference and limbic networks during negative feedback were consistent with prior fMRI findings. Rather than relying on broad neuroanatomy, refined electrode localization within functional networks may address the discrepancy for remaining regions. Regardless, our results are the first to assess gamma band specific stress activity using sEEG. Extending fMRI-based models will provide new knowledge for how and when distinct components of stress experience are processed within specific neural networks.

Disclosures: **A.M. Goodman:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **A.R. Nolan:** None. **K.E. Davis:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **D. Janko:** None. **R.J. Chatfield:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **J.F. Magnotti:** None. **J.P. Szaflarski:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.08/QQ5

Topic: G.04. Emotion

Title: Symptom improvement precedes change in cognitive processing in patients undergoing TMS for depression

Authors: *A. N. MCINNES, C. R. P. SULLIVAN, S. T. OLSEN, A. S. WIDGE;
Univ. of Minnesota, Minneapolis, MN

Abstract: Current neuromodulation therapies for major depressive disorder (MDD) most often target the dorsolateral prefrontal cortex (DLPFC), forming part of the key hub underlying decision-making circuitry. Transcranial magnetic stimulation (TMS) of DLPFC may reduce the severity of MDD by augmenting decision-making related cognitive control and redirecting maladaptive cognitive patterns. Here, we examined the temporal relationship of decision-making processes and MDD severity over the course of TMS treatment. Patients (n = 30) with MDD underwent a treatment course of daily TMS for eight weeks. Over the course of their treatment, these patients completed weekly sessions of the dot pattern expectancy (DPX) task, a behavioral tool to assess cognitive control.

We performed drift diffusion modelling of reaction time data in the DPX task. The drift rate parameter was most strongly predictive of depression symptom severity over the course of treatment, on a randomly held-out 20% of patients. This supports a relationship between cognitive control and depression severity. If changes in cognitive control underly the improvement in depression severity during TMS treatment, then changes in cognition should precede and predict changes in depression severity. To test this, we conducted a Granger causality analysis with increasing lags, to determine whether past measures of cognitive control can predict future measures of depression severity. Our test failed to support a causal relationship of cognitive control on depression severity. However, the reverse Granger causal relationship was significant. Akaike Information Criterion (AIC) values supported a causality model where changes in depression severity preceded cognitive control with a two-week time lag.

These findings suggest that while cognitive control is associated with the severity of depression, changes in cognition during treatment do not precede and predict symptom improvement during treatment that targets cognitive control circuitry. Rather, the reverse relationship is supported by these data - symptom improvement may lead to improved cognition.

Disclosures: A.N. McInnes: None. **C.R.P. Sullivan:** None. **S.T. Olsen:** None. **A.S. Widge:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.09/QQ6

Topic: G.04. Emotion

Support: 5 KL2 TR001115

Title: Insular networks in transdiagnostic emotion dysregulation and brain stimulation

Authors: *N. GERLUS[1], A. D. NEACSIU[2], J. L. GRANER[1], K. S. LABAR[1];
[1]Psychology & Neurosci., Duke Univ., Durham, NC; [2]Psychiatry & Behavioral Sci., Duke Univ. Sch. of Med., Durham, NC

Abstract: Background: The neural processes underlying emotional reactivity and emotional regulation in transdiagnostic clinical populations are not well-characterized. However, the insula is commonly implicated in aberrant affective and salience processing. This project aims to identify functional connectivity (FC) with the insula that may underlie transdiagnostic emotion dysregulation and that may be modulated by brain stimulation in dysregulated individuals.
Methods: As part of a blinded clinical trial, participants (M = 33.8 years, 82% F) with clinically significant emotion dysregulation and ≥ 1 co-morbid psychiatric disorder were cued to recall negative or neutral autobiographical memories and then asked to either experience or regulate associated emotions while they underwent a baseline MRI in a 3T scanner. Participants were then taught cognitive restructuring techniques and randomized to either active (n = 10, 20 rTMS trains at 10 Hz for 4s over the left dorsolateral prefrontal cortex [dlPFC] at 120% rMT, 26s ITI) or sham (n = 13) neurostimulation. The emotion regulation fMRI paradigm was repeated one week post-intervention. FC analyses used a gPPI approach implemented in FSL ($z > 2.3$, cluster-corrected at $p < 0.05$).
Results: Pre-intervention, compared to when participants were instructed to feel negative emotions, reframing negative emotions was associated with increased functional coupling between the right dlPFC and both the left and right insula. In addition, participants showed decreased FC between the left insula and the left superior parietal lobule, and increased FC between the right insula and right dorsomedial prefrontal cortex (dmPFC) when they reframed negative emotions compared to when they were cued to recall negative memories. In a post- minus pre-intervention contrast, participants showed significantly decreased FC between the right insula and right inferior parietal lobule during reframing negative emotions compared to when they were asked to feel negative emotions. In addition, the post- minus pre-intervention contrast showed significant changes in left insula FC with the right dmPFC in participants who received active rTMS compared to sham stimulation during reframing versus memory cue reactivity.
Conclusion: Cortico-insular connectivity is associated with emotional processing in a clinical sample and is modulated by active neurostimulation. Future directions include investigating whether memory salience differentially modulates emotional reactivity and regulation in insular networks. These networks may be promising targets for neurostimulation interventions in transdiagnostic emotion dysregulation.

Disclosures: **N. Gerlus:** None. **A.D. Neacsiu:** None. **J.L. Graner:** None. **K.S. LaBar:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.10/QQ7

Topic: H.06. Social Cognition

Title: Comparison of Social Functioning between Psychotic, Substance Use, and Mood and Anxiety Disorders

Authors: *K. FERSTER[1], A. KHAN[1], T. BEL-BAHAR[2], R. B. SHAIK[4], L. LEPOW[3], M. A. PARVAZ[5];
[1]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [2]Icahn Sch. of Med. At Mount Sinai, New york, NY; [3]Icahn Sch. of Med. At Mount Sinai, New York, NY; [4]Icahn Sch. of Medicine, Mount Sinai, New York, NY; [5]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Social cognition is a multifaceted construct and different aspects of social cognition [e.g., cognitive and affective Theory of Mind (ToM)] have shown to be impaired in different psychopathologies. However, a comprehensive assessment of social cognition requires multiple questionnaires and test, which is time consuming and often infeasible. Here, we compared social cognition between psychotic, substance use, mood and anxiety disorders, and healthy control groups using a novel task, the Edinburgh Social Cognition Test (ESCoT), that assesses cognitive ToM, affective ToM, interpersonal and intrapersonal functioning. In this single site cross-sectional observational study, 52 participants (14 individuals with Schizophrenia, 7 individuals with methamphetamine use disorder, 6 individuals with mood and anxiety disorder, and 25 healthy controls) were recruited from multiple studies at Mount Sinai Hospital. After completing a screening session and providing an informed consent, eligible participants visited Mount Sinai Hospital and completed several questionnaires, including ESCoT. Using a two-sided multiple comparison analyses, we determined significant differences between different clinical diagnoses. Notably, significant deficits were found in Cognitive ToM in individuals with Schizophrenia compared to healthy controls ($p=0.0057$) and to individuals with mood and anxiety disorder ($p=0.0281$). For Affective ToM, significant deficits were found in individuals with schizophrenia compared to healthy controls ($p=0.0018$) and to individuals with methamphetamine use disorder ($p=0.0355$). In interpersonal understanding, significant deficits were observed in individuals with schizophrenia compared to healthy controls ($p=0.0499$) and to individuals with methamphetamine use disorder ($p=0.0319$). No significant group differences were observed in intrapersonal understanding. The total ESCoT score and its subscales, cognitive and affective ToM and interpersonal functioning, showed significant deficits in individuals with schizophrenia, which is consistent with prior literature. However, the novelty of these results stem from the use of ESCoT to validate prior results. We did not see any significant group differences in the intrapersonal understanding of social norms, which is also expected given the non-social context of this domain. This work is currently underway with data being collected on more participants in each group to see whether these findings can be generalized.

Disclosures: **K. Ferster:** None. **A. Khan:** None. **T. Bel-Bahar:** None. **R.B. Shaik:** None. **L. Lepow:** None. **M.A. Parvaz:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.11/QQ8

Topic:

Support: DANA
K23MH108711
R01MH123639
MH121790

Title: Visual network is disrupted earlier than auditory network in individuals with clinical high risk for schizophrenia

Authors: *A. MURATI, J. K. LEE, D. J. VIEIRA, D. R. RUIZ-BETANCOURT, F. I. PLAZA, J. P. SANCHEZ-PEÑA, G. H. PATEL;
Columbia University/New York State Psychiatric Inst., New York, NY

Abstract: Introduction
While previous research has identified significant abnormalities in visual and auditory processing using naturalistic stimuli in schizophrenia participants (SzP), no studies have compared the integrity of these sensory modalities in individuals at clinical high-risk for developing schizophrenia (CHR). We compared the resting state functional connectivity of the language network in the superior temporal sulcus, a region implicated in both auditory and visual processing, between CHRs, SzPs, and healthy controls (HC).
Method
We collected 22 minutes of resting state fMRI data in 41 HC, 30 CHR and 42 SzP. We used a brain atlas that divides 131 cortical areas involved in social cognition into 36 network components. We then measured the functional connectivity between the language processing network and other network components. Group comparisons were made with ANOVAs and post-hoc t-tests.
Results
We found significant main effects of group in 15/36 networks in connectivity to the right language network and 11/36 networks in connectivity to the left language network ($F_{(2,110)} > 3.29$, $p < 0.05$). Post hoc analyses suggest 3 profiles of group differences. In the first, CHRs and SzPs both had less connectivity than HC between the language network and the early and late visual areas. In the second, SzP had decreased connectivity compared to HC while CHRs did not significantly differentiate from either group between the language network and the auditory and language areas. In the third, SzP had higher connectivity than either HC or CHR between the language network and the cingulo-opercular and salience network components.

Discussion
In CHRs, we observed that functional connectivity between language and visual networks were more disrupted than between language and auditory networks. The results suggest that the disruption of visual areas in CHRs may precede that of auditory areas, which may in turn aid in early identification of the risk of developing schizophrenia.

Disclosures: A. Murati: None. **J.K. Lee:** None. **D.J. Vieira:** None. **D.R. Ruiz-Betancourt:** None. **F.I. Plaza:** None. **J.P. Sanchez-Peña:** None. **G.H. Patel:** None.

Poster

PSTR496. Human Emotion: Disorders and Clinical Populations

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR496.12/QQ9

Topic: H.06. Social Cognition

Support: K23 MH108711
T32 MH018870
R01 MH121790
R01 MH123639
Dana Foundation

Title: Momentary divergences in brain state dynamics in schizophrenia are associated with a failure to perceive and understand naturalistic social situations

Authors: *D. J. VIEIRA[1,2], S. A. MIRZA[1,2], N. C. FOLEY[2,1], J. K. LEE[1,2], A. QU[1,2], G. H. PATEL[1,2];
[1]New York State Psychiatric Inst., New York, NY; [2]Columbia Univ., New York, NY

Abstract: Introduction: Perception of naturalistic social scenes requires the dynamic interplay of many cortical systems, observed as coactivation patterns (CAPs), or 'brain states', in whole-brain imaging. Here we examine how these CAP states are differentially synchronized to the sensory and narrative properties of the movie, both between CAPs and between schizophrenia participants (SzP) and healthy controls (HC).
Methods: 27 SzP and 21 healthy controls watched a visual-only 15-minute video clip while whole-brain BOLD-fMRI data was collected. *K*-means clustering was used to cluster the BOLD activity time series across 105 functionally localized ROIs into 8 CAP states. At each TR, we computed how close an individual's BOLD activity in the 105-dimensional regional activation space was to a given CAP (**Co**hesion) relative to all other CAPs (**Co**ntrast). The obtained 'CoCoFit' time series of each participant was then correlated with continuous measures of the sensory and narrative content of the movie, as well as each participant's eye-tracking data. Additionally, for every TR in which the BOLD activity of SzP significantly diverged from the HC 'representative' CAP (i.e., the CAP with the greatest CoCoFit), we correlated the fractional occupancy (FO) of the HC representative CAP with TASIT, a social cognition test. All FDR-

corrected *p* values are reported as *q*.
Results: The CoCoFit of CAP 8, which is characterized by the co-activation of TPJ-pSTS areas involved in face-emotion processing, attention, and theory-of-mind, was significantly associated with the movie's visual motion intensity (q=6x10^{-24}; HC > SzP, p=0.025), degree of social engagement among characters (q=5x10^{-7}), each individual's saccadic frequencies (q=4x10^{-6}), and the number of faces in peripheral vision (q=0.0015) but not foveal vision (q=0.41). The FO of CAP 8 during moments of SzP divergence from CAP 8 was significantly correlated with TASIT for HC (r=0.62, p=0.008) but not SzP (r=-0.02, p=0.92, $F_{1,38}$=4.72, p=0.036).
Conclusion: These results suggest the failure to occupy the TPJ-pSTS CAP in the appropriate moments is associated with the failure to perceive moving facial expressions in peripheral vision, which then leads to impaired visual exploration and poorer understanding of social situations. They also demonstrate methods for associating CAP state dynamics with the various sensory, narrative, and behavioral events during naturalistic viewing experiments.

Disclosures: D.J. Vieira: None. **S.A. Mirza:** None. **N.C. Foley:** None. **J.K. Lee:** None. **A. Qu:** None. **G.H. Patel:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.01/QQ10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045764
NIH Grant U54MD007592-28

Title: Medial prefrontal and insular cortex activity during a distress tolerance task using in vivo electrophysiology in a female rat

Authors: *M. A. PEVETO, R. SOSA JURADO, T. M. MOSCHAK;
Biol. Sci., Univ. of Texas At El Paso, El Paso, TX

Abstract: Distress tolerance (DT) is the behavioral ability to persist in challenging, goal-directed activity while experiencing psychological stress. The behavioral inability to tolerate this affective stress is known as low DT. Low DT has been associated with heightened drug-seeking behavior and a tendency to relapse in individuals suffering from addiction. The medial prefrontal (mPFC) and insular (INS) cortex are brain regions that have been implicated in drug-seeking and make up the salience network. The salience network is a connectivity network that has been proposed to influence the functions of these brain regions in ways that may underlie individual differences in patterns of action, such as those driven by the saliency of external or interoceptive cues (i.e., drug craving), during decision-making. However, no preclinical studies have examined the neural activity of these brain regions in tandem during DT. We hypothesize that INS cell firing and INS-mPFC connectivity during the DT task will correlate with subsequent cocaine seeking

and taking and that a history of cocaine self-administration will disrupt this activity and connectivity. To investigate this, a preliminary study was done in which a Neuropixel dual-probe fixture was implanted in the INS and the infralimbic (IL), prelimbic (PrL), and anterior cingulate cortex (ACC) regions of the mPFC. Neuronal activity was recorded in a freely behaving female (n=1) Long Evans rat during the task. Our data suggest a trend for IL neurons to have a more excited profile than PL neurons during the DT task, with neural activity in all regions responding to certain aspects of the task (e.g., lever presentation). Subsequently, we will have our subject go through a timeline of tasks to test our hypothesis. The rat will go through an elevated plus maze (EPM) test and 2 weeks of cocaine self-administration for 6 hr/day. They will then begin a 1-month period of experimenter-imposed abstinence after which DT, EPM, and neural activity will be reassessed. We used chi-square analyses to assess differences in neural activity across brain regions and will also do so across experimental groups. Collectively, these preliminary data support existing research demonstrating a role for the mPFC in DT and our future work will expand upon these findings by assessing drug-seeking and INS-mPFC/salience network connectivity in male and female subjects.

Disclosures: **M.A. Peveto:** None. **R. Sosa Jurado:** None. **T.M. Moschak:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.02/QQ11

Topic: G.09. Drugs of Abuse and Addiction

Support: This study was supported by the Intramural Research Program of the National Institute on Drug Abuse

Title: The Ventral Tegmental Area has glutamatergic neurons that play a role in cocaine seeking-behavior

Authors: *M. BARBANO, J. QI, E. CHEN, U. MOHAMMAD, O. ESPINOZA, M. MORALES;
Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Converging evidence indicates that both dopamine and glutamate neurotransmission within the nucleus accumbens (NAc) play a role in drug addiction. Increases of NAc dopamine release from ventral tegmental area (VTA) inputs encode the rewarding effects of drugs of abuse. In contrast, NAc glutamate release from prefrontal cortex inputs synapsing on NAc medium spiny neurons play a role in cocaine reinstatement. We have previously demonstrated that, in addition to dopamine neurons, the VTA has glutamate neurons that also target the NAc and establish excitatory synapses on parvalbumin GABAergic interneurons. Here, we determined whether this glutamatergic pathway from VTA to NAc-parvalbumin interneurons plays a role in cocaine-seeking behavior. By expressing Channelrhodopsin in mouse VTA glutamatergic

neurons, and by NAc photostimulation, we evoked local release of glutamate from VTA glutamatergic axons. By conditioned place preference (CPP) task, we evaluated the role of NAc glutamate release (evoked by local photostimulation of VTA glutamatergic inputs) during the acquisition, expression or reinstatement of cocaine-induced CPP. We found that NAc photostimulation of VTA glutamatergic inputs did not alter CPP acquisition, but inhibited both CPP expression and reinstatement behaviors. In another set of mice expressing Channelrhodopsin in parvalbumin interneurons, we found that direct photostimulation of the whole population of NAc parvalbumin neurons did not modify CPP acquisition, expression, or the reinstatement. From these findings, we concluded that (a) VTA neighboring dopamine and glutamate neurons innervating the NAc play different roles in the neurobiology of cocaine reward, (b) NAc glutamatergic inputs depending on their source (cortex or VTA) and specific neuronal targets (medium spiny neurons or parvalbumin interneurons) differentially modulate cocaine-seeking behavior, and (c) a selective population of NAc parvalbumin neurons, regulated by VTA glutamatergic neurons, play a role in cocaine-seeking behavior.

Disclosures: **M. Barbano:** None. **J. Qi:** None. **E. Chen:** None. **U. Mohammad:** None. **O. Espinoza:** None. **M. Morales:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.03/QQ12

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by the NIDA Intramural Program/NIH.

Title: The Dorsal Raphe has Dynorphin-glutamatergic and Dynorphin-serotonergic neurons that innervate the Ventral Tegmental Area

Authors: *R. GARCIA, M. MORALES;
NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: The Dorsal Raphe (DR) is among the brain area that provides major innervations to the ventral tegmental area (VTA), and although the DR is best known for containing serotonergic neurons, the DR has different classes of neurons, including glutamatergic (expressing the vesicular glutamate transporter 3 (VGluT3) and Dynorphin (Dyn) neurons. We have previously demonstrated that some of the DR neurons expressing VGluT3 alone or in combination with serotonergic markers, such as tryptophan hydroxylase (TPH), establish excitatory synapses on VTA dopamine neurons, and mediate reward. Recent studies have shown that DR-Dyn neurons innervate the VTA, but it is unclear the extent to which Dyn neurons are expressed within the different classes of DR neurons that target the VTA. In the present study, we applied anatomical techniques to characterize the class of DR-Dyn neurons that innervate the VTA. By a combination of immunohistochemistry (for the detection of TPH) and RNAscope (for the

detection of transcripts encoding VGluT3 or Dyn, we found that within the total population of DR-Dyn neurons, 34% co-expressed TPH and VGluT3 mRNA, 29% of co-expressed TPH without VGluT3 mRNA, and 13% co-expressed VGluT3 mRNA without TPH, and 23% of the neurons expressing Dyn mRNA did not co-express VGluT3 or TPH. Next, we did iontophoretic intra-VTA application of the retrograde track tracer fluorogold (FG) to identify DR-neurons innervating the VTA and by RNAscope established the phenotype of these DR-FG neurons. We found that about half of the total population of FG-Dyn labeled neurons co-expressed TPH and VGluT3 mRNA, 19% co-expressed TPH, 14% co-expressed VGluT3 mRNA, and 10% of FG-Dyn labeled neurons lacked TPH or VGluT3 mRNA. In summary, we found that most of the DR Dyn neurons projecting to the VTA, have the capability to release glutamate alone or together with serotonin.

Disclosures: R. Garcia: None. **M. Morales:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.04/QQ13

Topic: G.09. Drugs of Abuse and Addiction

Title: A subset of dorsal raphe glutamatergic neurons relays rewarding information to the ventral tegmental area

Authors: *R. I. OSNAYA[1], S. QIANWEI[2], H.-L. WANG[3], B. LIU[4], S. ZHANG[5], M. F. MORALES[4];
[1]NIDA, Baltimore, MD; [2]NIH, Baltimore, MD; [3]IRP/NIDA/NIH, Baltimore, MD; [4]IRP, NIDA, NIH, IRP, NIDA, NIH, Baltimore, MD; [5]Natl. Inst. of Health, Natl. Inst. on Drug Abuse, IRP, Natl. Inst. of Health, Natl. Inst. on Drug Abuse, IRP, Baltimore, MD

Abstract: The Ventral Tegmental Area (VTA) mediates different aspects of motivated behavior, mediated in part by dopamine neurons that integrate information from different brain structures. We had previously demonstrated that subsets of Dorsal Raphe (DR) glutamatergic neurons expressing the vesicular glutamate transport 3 (VGluT3) establish excitatory synapses on VTA dopaminergic neurons, and that activation of this DR-VGluT3 pathway to VTA-dopamine neurons is rewarding. Here, we present evidence indicating that in addition to DR-VGluT3 inputs to VTA, there is another source of DR glutamatergic neurons, expressing the vesicular glutamate transporter 2 (VGluT2) that innervates the VTA. By VTA injection of the retrograde track tracer fluorogold (FG) and phenotyping of DR-FG-neurons we detected DR-FG-neurons expressing VGluT2 mRNA. These DR-FG-VGluT2 neurons represented ~40% of the total population of FG-neurons, indicating that the subset of DR-VGluT2 neurons provides a major input to VTA. Next, to investigate a possible role of the DR-VGluT2 pathway to VTA in behavior, we injected a cre-dependent viral vector in the DR of VGluT2-cre mice to selectively expressed Channelrhodopsin-eYFP under the regulation of the VGluT2 promoter in DR-VGluT2

neurons. By immunohistochemistry, we confirmed VTA expression of eYFP-axons from DR-VGluT2 neurons, and by VTA laser-stimulation found that mice presented a preference for a chamber in which they received the laser stimulation. By intra-optical cranial self-stimulation, we found that mice preferred to turn a wheel associated with VTA-laser-stimulation of DR-VGluT2 inputs. Thus, further indicating that DR-VGluT2 input to VTA is rewarding. Next, we determined the extent to which laser stimulation of DR-VGluT2 neurons innervating the VTA changes in response to a reward (sucrose pellets). For these studies, we injected a Cre-dependent retrograde GCAMP virus within the VTA of VGluT2-Cre mice and implanted an optic fiber on the DR. We found that DR-VGluT2 neurons innervating the VTA increased their activity in response to sucrose consumption events. In another set of studies, we found that calcium transients increased in response to both an aversive (Trimethylthiazoline) and a rewarding odor (peanut oil), indicating that DR-VGluT2 neurons innervating the VTA respond to stimuli salience. In summary, while DR is best known to regulate behavior by releasing serotonin throughout the brain, we discovered an unanticipated subset of DR excitatory VGluT2-neurons that relay reward and salience information to VTA by releasing glutamate.This work was supported by NIDA/NIH.

Disclosures: R.I. Osnaya: None. **S. Qianwei:** None. **H. Wang:** None. **B. Liu:** None. **S. Zhang:** None. **M.F. Morales:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.05/QQ14

Topic: G.09. Drugs of Abuse and Addiction

Title: Ventral tegmental area glutamatergic inputs to the nucleus accumbens play a role in psychostimulant-seeking behavior

Authors: *O. ESPINOZA, U. MOHAMMAD, M. F. BARBANO, M. MORALES; Integrative Neurosci. Res. Br., Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: The ventral tegmental area (VTA) is best known for its dopamine neurons, which are activated by drugs of abuse to induce dopamine release in the nucleus accumbens (NAc). The VTA also contains non-dopaminergic neurons including glutamatergic, GABAergic, and combinatorial neurons. We have previously demonstrated that VTA neurons expressing the vesicular glutamate transporter type 2 (VGluT2) synapse onto parvalbumin GABAergic interneurons, and NAc photoactivation of these fibers drives inhibitory neurotransmission in the NAc. We investigated the role of this VTA-NAc pathway in cocaine-seeking behavior and found that photoactivation does not modify acquisition but blocks the expression and reinstatement of cocaine-induced conditioned place preference (CPP) at a concentration of 15 mg/kg. In the present study, we investigated if this effect was dependent on the dose of cocaine used. We injected a Cre-dependent viral vector into the VTA of VGluT2-Cre mice for the selective

expression of channelrhodopsin tethered to eYFP or eYFP alone in VGluT2 neurons and then implanted optical probes in the NAc to activate local release of glutamate from VTA-VGluT2 fibers. We then trained the mice in a CPP paradigm to assess cocaine-seeking behavior and found that NAc release of glutamate from VTA-VGluT2 fibers prevented the expression and reinstatement of cocaine-induced CPP at concentrations of 5 and 10 mg/kg. As a follow up, we ran the same experiment at 15 mg/kg of cocaine with an additional test day and found that NAc release of glutamate from VTA-VGluT2 fibers induces preference for the cocaine-paired side when release is paired with the saline-paired side, suggesting that the mice find the stimulation aversive. We next determined the extent to which the findings observed with cocaine were also observed with methamphetamine and found that NAc release of glutamate from VTA-VGluT2 fibers inhibited the expression and reinstatement of methamphetamine-induced CPP. Thus, we concluded that NAc release of glutamate from VTA-VGluT2 fibers plays a critical role in psychostimulant-seeking behavior by preventing cocaine and methamphetamine CPP expression and reinstatement.
This work was supported by NIDA IRP (NIH)

Disclosures: O. Espinoza: None. **U. Mohammad:** None. **M.F. Barbano:** None. **M. Morales:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.06/QQ16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA054329
Institutional Training Grant 5T32DA018926

Title: Mu opioid receptors on D2 medium spiny neurons have divergent effects on cocaine and opiate behaviors

Authors: *B. REMMERS[1], I. CHOI[1], K. MATSUMURA[1], C. BOWERING[2], A. NICOT[1], L. DOBBS[1];
[1]Neurosci., Univ. of Texas at Austin, Austin, TX; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Cocaine and opiate reward are mediated by increased dopamine transmission in the striatum. While it is not surprising that activation of *Gi*-coupled mu opioid receptors (MORs) is important for opiate reward, evidence also suggests MORs play an important role in cocaine reward. For instance, global deletion of MORs attenuates reinstatement of cocaine seeking. This effect appears to be localized to the striatum, as intra-striatal administration of MOR antagonists block the acquisition and expression of cocaine place preference. Further, this likely occurs through enhanced levels of the opioid peptide, and MOR agonist, enkephalin. Withdrawal from

long-term cocaine increases striatal enkephalin levels and augmenting striatal enkephalin tone facilitates acquisition of cocaine place preference. However, within the striatum, MORs are expressed on the two populations of GABAergic output neurons: dopamine D1 and D2 medium spiny neurons (D1-MSN, D2-MSN). Thus, it is unclear which population of MORs is important for regulating drug reward. Since D1-MSNs and D2-MSNs have opposing effects on motivated behavior, with D1-MSNs driving drug reward and D2-MSNs restraining it, we hypothesized that MORs on D2-MSNs act to inhibit these neurons and support cocaine and opiate reward. To test this, we generated a knockout mouse with a targeted deletion of MORs from D2-MSNs (D2-MORKO) and tested them in cocaine, morphine, and fentanyl locomotor sensitization and conditioned place preference. D2-MORKO mice showed a functional loss of MORs, reflected by an inability of DAMGO, a MOR agonist, to suppress GABA transmission from D2-MSNs onto neighboring D1-MSNs. Lack of MORs from D2-MSNs slowed the acquisition of cocaine place preference and attenuated the expression of cocaine preference when tested in the presence of cocaine relative to littermate controls. In contrast, D2-MORKOs showed normal acquisition and expression of morphine and fentanyl place preference compared to controls. Deletion of MORs from D2-MSNs also had no effect on acute and sensitized locomotor responses following single-dose or repeated-administration of cocaine, morphine, or fentanyl. These findings suggest a divergent role for MORs expressed in D2-MSNs in mediating cocaine and opiate reward and further suggest these MORs facilitate conditioned cocaine reward. We suspect this occurs through cocaine-enhanced enkephalin, which acts on MORs to suppress D2-MSNs and facilitate cocaine reward.

Disclosures: B. Remmers: None. **I. Choi:** None. **K. Matsumura:** None. **C. Bowering:** None. **A. Nicot:** None. **L. Dobbs:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.07/QQ17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA054329
UT Austin Rising STARs Award
Bruce Jones Predoctoral Fellowship
NIH Grant NIAAA K99/R00-025393

Title: Sex-dependent role of the endogenous opioid system in cocaine reward

Authors: *K. MATSUMURA[1], A. NICOT[2], I. CHOI[2], M. ASOKAN[3], N. N. LE[3], L. NATIVIDAD[3,4], L. K. DOBBS[1,4,5,6];
[1]Inst. for Neurosci., [2]Dept. of Neurosci., [3]Col. of Pharmacy, Div. of Pharm. & Toxicology, The Univ. of Texas At Austin, Austin, TX; [4]Waggoner Ctr. for Alcohol & Addiction Res., [5]Dept. of Neurosci., [6]Dept. of Neurol., The Univ. of Texas at Austin, Austin, TX

Abstract: Cocaine-associated contexts exert powerful control over behavior and can incite drug seeking. This kind of context-dependent cocaine seeking is encoded within the striatal circuits, and these circuits and behaviors, in part, are regulated by endogenous opioid peptides and receptors. We previously showed that increasing levels of enkephalin in the striatum facilitates acquisition of cocaine conditioned place preference (CPP), while intra-striatal infusion of mu opioid receptor antagonists attenuate expression of cocaine CPP. However, whether striatal enkephalin is necessary for acquisition of cocaine CPP and maintenance during extinction remains unknown. To address this, we generated mice with a targeted deletion of enkephalin from dopamine D2-receptor expressing striatal medium spiny neurons (D2-PenkKO) and tested them in a cocaine CPP paradigm. Low striatal enkephalin levels did not attenuate acquisition of cocaine CPP. However, expression of preference, assessed after acute administration of the opioid receptor antagonist naloxone, was blocked in females, regardless of genotype, suggesting that an opioid peptide other than striatal enkephalin is necessary for expression of cocaine preference in females, When saline was paired with the cocaine context during extinction sessions. female D2-PenkKO and wildtype littermates extinguished preference faster than males. In contrast, pairing naloxone with the cocaine context prevented extinction in female D2-PenkKOs and wildtype littermates. We conclude that while striatal enkephalin is not necessary for acquisition or maintenance of cocaine reward, females are uniquely susceptible to the effects of opioid antagonists on expression and maintenance of cocaine preference. The divergent effects of naloxone on expression and extinction of preference further indicate that different opioid peptides may be important in distinct phases of conditioning. Taken together, our data indicate endogenous opioid peptides and receptors play sex-dependent roles in mediating expression and maintenance of conditioned cocaine reward.

Disclosures: K. Matsumura: None. **A. Nicot:** None. **I. Choi:** None. **M. Asokan:** None. **N.N. Le:** None. **L. Natividad:** None. **L.K. Dobbs:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.08/QQ18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-NIDA : 5F31DA053793-02
NIH-NIGMS RISE #2R25GM082406
RCMI-Supplement Project NMHHD 3U54MD007579-37S1
INBRE- P20 GM103475-19
B.R.A.I.N. Core IM-HD MD007579

Title: Effects of chronic stress on the amygdalo-striatal circuits in rats exposed to stress and cocaine

Authors: *R. MORALES SILVA[1,2], A. AMADOR MALDONADO[1], B. DOMINGUEZ-PADOVANI[1], Y. PEREZ PEREZ[1], J. PEREZ TORRES[1], G. RODRIGUEZ TORRES[1], M. SEPULVEDA ORENGO[1,3];
[1]Ponce Hlth. Sci. Univ., Ponce, Puerto Rico; [2]Neurosci. Div., [3]Ponce Res. Inst., Ponce, Puerto Rico

Abstract: Generally, post-traumatic stress disorder (PTSD) and substance use disorder (SUD) are examined separately in preclinical studies, although they can co-occur in patients. One of the factors associated with a high relapse state is stress. Therefore, observing these two disorders closely is necessary to understand better the mechanisms underlying this comorbidity's effects on neurophysiology. Two crucial brain structures involved in the development of these disorders are the basolateral amygdala (BLA) and the nucleus accumbens (NAc). The BLA and the NAc are two essential brain structures involved in developing these disorders. The BLA concerns the development of fear memories and PTSD, while the NAc is part of the reward circuitry and plays an important role in the progression of SUD. The objective is to determine how BLA modulates the NAc-core in a combined animal model of chronic stress and SUD. The hypothesis is that chronic stress will induce neurophysiological changes to the BLA-NAc core synapses, correlating with increased cocaine-seeking behaviors. To test this hypothesis, we used unescapable footshocks for 5 days at an intensity of 0.55mA (presented randomly), followed by 6-hour sessions of extended-access cocaine self-administration for 10 days and a 1, 15, or 30-day forced abstinence period. Subsequently, we examined cue- and cocaine-induced cocaine-seeking behavior. In another cohort of animals, instead of reinstatement tests after 30 days of abstinence, rats were euthanized for whole-cell patch-clamp electrophysiology using optogenetics to stimulate the BLA synaptic terminals on the NAc core synapses to measure the synaptic changes analyzing AMPA/ NMDA currents ratio. Our data show that chronic stress before cocaine exposure increases cocaine-induced seeking behavior but only on 30 days forced abstinence period. Additionally, preliminary results from whole-cell patch-clamp recordings suggest that saline rats exposed to chronic stress increase the AMPA/NMDA currents ratio in the BLA-NAc core synapses compared to the non-stress saline and cocaine groups after abstinence. In addition, non-stress cocaine-exposed rats after abstinence have no difference in AMPA/NMDA ratio compared to non-stress saline and stress cocaine groups. Therefore, these preliminary results suggest that chronic stress-induced long-lasting changes in synaptic plasticity in BLA-NAc core synapses. In addition, cocaine exposure prevents chronic stress-induced synaptic changes after 30 days of abstinence.

Disclosures: R. Morales Silva: None. **A. Amador Maldonado:** None. **B. Dominguez-Padovani:** None. **Y. Perez Perez:** None. **J. Perez Torres:** None. **G. Rodriguez Torres:** None. **M. Sepulveda Orengo:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.09/QQ19

Topic: G.09. Drugs of Abuse and Addiction

Support: G-RISE Program (#T32GM144896)
NIH-NIGMS (#2R25GM082406)

Title: The impact of fear conditioning prior to cocaine self administration oncocaine seeking in adult rats

Authors: *Y. PEREZ-PEREZ[1], R. J. MORALES-SILVA[2], G. N. RODRIGUEZ-TORRES[2], B. A. DOMINGUEZ-PADOVANI[2], L. GODOY MUÑOZ[2], M. T. SEPULVEDA-ORENGO[2]; [1]Basic Sci., [2]Ponce Hlth. and Sci. Univ., Ponce, Puerto Rico

Abstract: The impact of fear conditioning prior to cocaine self-administration on cocaine seeking in adult rats
Yobet A. Perez-Perez, Roberto J. Morales-Silva, Genesis N. Rodriguez Torres, Benjamin A. Dominguez Padovani, Lenin Godoy Muñoz, Marian T. Sepulveda-Orengo
Department of Basic Sciences, Ponce Health Sciences University-School of Medicine/Ponce Research Institute, Ponce, Puerto Rico 00732, USA
Abstract
Co-morbidity between cocaine use disorder (CUD) and trauma-related disorders has been shown frequently, demonstrating a strong relationship between trauma exposure and cocaine use. However, it is unknown how a traumatic event exposure prior to cocaine exposure can increase the risk of CUD development. Our research aims to assess how traumatic event exposure is a risk factor for developing a stronger CUD and its impact on cocaine-seeking behavior. We hypothesize that adult male and female Sprague Dawley rats exposed to a traumatic event (stressed group) in the form of fear conditioning (FC) will exhibit higher drug-seeking behavior compared to non-stressed rats. Rats were subjected to a single session of FC. Five days after FC, rats were subjected to 12 days of short-access cocaine self-administration (2 h/day), followed by 15 days of extinction training (2 h/day). Twenty-four hours after the last extinction session, rats were exposed to cue-primed and cocaine-primed reinstatements. Results show that stressed male rats had higher active level presses in cue-primed and cocaine-primed reinstatements compared to non-stressed male rats. In contrast, there was no difference in active lever presses between stressed and non-stressed female rats in cue- and cocaine-primed reinstatements. Our findings indicate that a traumatic event prior to cocaine exposure may influence the transition from the recreational use of cocaine to the development of CUD in a sex-dependent manner. As a future direction, we will investigate the effects of FC and cocaine exposure on synaptic changes in the PL-NAc core synapses in connection to cocaine-seeking behavior.

Disclosures: Y. Perez-Perez: None. **R.J. Morales-Silva:** None. **G.N. Rodriguez-Torres:** None. **B.A. Dominguez-Padovani:** None. **L. Godoy Muñoz:** None. **M.T. Sepulveda-Orengo:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.10/QQ20

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA038613
NIH Grant F32DA052966

Title: The impact of nicotine on nucleus accumbens and peripheral mitochondria

Authors: *C. A. CALARCO[1], I. WILLIAMSON[1], N. ZHANG[2], S. DOMINGUEZ-LOPEZ[1], B. M. POLSTER[3], M. LOBO[1];
[1]Neurobio., [2]Anesthesiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Anesthesiol., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD

Abstract: Exposure to stimulant drugs and subsequent chronic use profoundly impacts behavior, neuronal structure and firing, and gene expression through multiple brain regions. Some of these sweeping changes are mediated and supported by changes in cellular energy homeostasis and mitochondrial function. Recent work has shown that cocaine self-administration significantly alters mitochondrial size in nucleus accumbens (NAc) medium spiny neurons (MSNs), and that disruption of proper mitochondrial fission is sufficient to blunt responding for cocaine, but the impacts of other stimulant drugs have not been rigorously examined in the NAc. In other tissues or brain regions nicotine impacts cellular respiration, and can directly interact with mitochondria in reward circuits, although this has not been examined in the context of mediating the rewarding properties of nicotine itself. To this end we examined the impact of systemic nicotine administration on mitochondrial-related gene expression and respiration in NAc, as well as peripheral blood mitochondrial DNA copy number, which has been shown to correlate with both mood and drug exposure, including cigarette smoking. At the transcriptional level, qPCR of NAc tissue generated from mice that had undergone 7 days of IP nicotine exposure (0.5mg/kg or 1.0mg/kg) shows changes in mitochondrial related genes, with some differences by sex and dose. Broadly, the lower dose yielded more robust changes in mitochondrial related genes, with more genes being significantly altered in female mice. Notable upregulated genes in females include Nrf1, Tfam, and Mfn1 and 2. Downregulated genes include Egr3, Polg, and Tfb1. Interestingly, while Cycs was significantly upregulated in males, it was significantly downregulated in females. Functionally in a Seahorse-bioanalyzer, frozen-tissue assay, 7 days of nicotine decreases NAc complex I function, while increasing complex II at trend levels in males at the lower dose, with only subtle changes in females. The impacts of the higher nicotine dose on respiration are in progress. Peripherally, the higher dose of nicotine increases mitochondria DNA copy number in both males and females, while the lower dose only increases copy number is males. Future work will examine how these peripheral changes correlate with NAc mitochondrial changes, and how these mitochondrial changes impact cellular and mitochondrial morphology in NAc in a cell type selective manner between D1- and D2- MSN subtypes. Future work will be needed to understand the role of NAc mitochondria in regulating the cellular and behavioral responses to nicotine and how this relates to both chronic use and nicotine use disorders in humans.

Disclosures: C.A. Calarco: None. **I. Williamson:** None. **N. Zhang:** None. **S. Dominguez-Lopez:** None. **B.M. Polster:** None. **M. Lobo:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.11/QQ21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA038613
NIH Grant R01DA054905

Title: Mitochondrial OXPHOS signatures in the brain with perinatal fentanyl exposure

Authors: *S. L. HAJIRNIS[1], J. OLUSAKIN[1], G. KUMAR[1], J. MCLNERNEY[1], M. BASU[2], S. A. AMENT[2], M. LOBO[1];
[1]Dept. of Neurobio., Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Dept. of Psychiatry, Inst. for Genome Sciences, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: There is an emerging role formitochondria disruption in substance use disorder. While mitochondria have beenexamined in adult exposure to used substances in rodents, there is littleinformation about mitochondrial processes in conditions of drug exposure inutero. Previous studies from our lab developed a perinatal fentanyl exposure(PFE) model, in which C57BL/6 pregnant dam mice were singly housed and received10 μg/ml fentanyl in drinking water from embryonic day 0 (E0) until postnatalday 21 (P21). Transcriptomic analysis of reward brain regions in PFE adolescentmice (P35) identified differentially expressed genes of complex I mitochondrialoxidative phosphorylation (OXPHOS) in the nucleus accumbens (NAc), and ventraltegmental area (VTA) of both males and females as compared to the controlsubjects. Specifically, Ndufb1-ps in NAc (*FDR=0.013; p=0.0004*) and VTA (*FDR=0.0037;p=0.0004*); Ndufa5 in NAc (*FDR=0.0175; p=0.0006*) and VTA (*FDR=0.0009;p=0.0006*); Ndufa1 in NAc (*FDR=0.0146; p=0.0005*), VTA (*FDR=1.46E-05,p=1.94E-07*) of males. There were no changes seen in complex I genes infemales indicating sex-specific gene regulation of these genes in PFE mice. Theincrease of complex I gene transcripts suggests increasing demand for energy inNAc of males and not of females, whereas decreased expression of complex I genetranscripts implicates low energy demand in VTA of males. We are currentlyperforming Seahorse respirometry experiments in PFE-exposed mice to analyzecomplex 1 function. In parallel we are examining glycolysis and mitochondrialrespiration molecules, using Nanostring panels, in reward brain regions of PFEmice at various time points between P1 until P35. This will allow us toidentify the postnatal time course of disrupted mitochondrial signatures in PFEmice. Collectively, our studies are uncovering distinct mitochondrialsignatures occurring in the brain during postnatal development that occur withfentanyl exposure during the perinatal period.

Disclosures: S.L. Hajirnis: A. Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore. **J. Olusakin:** A. Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore. **G. Kumar:** A.

Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore. **J. Mclnerney:** A. Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore. **M. Basu:** A. Employment/Salary (full or part-time):; Department of Psychiatry, Institute for Genome Sciences, University of Maryland School of Medicine. **S.A. Ament:** A. Employment/Salary (full or part-time):; Department of Psychiatry, Institute for Genome Sciences, University of Maryland School of Medicine. **M. Lobo:** A. Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.12/QQ22

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R21DA052101

Title: Small but Mighty: CRISPR Epigenome Editing Using a Cas12f-derived System for Gene Expression Interference of Fentanyl Regulated Genes

Authors: *M. GREEN, N. HAJIRNIS, E. CHOI, R. CHANDRA, M. LOBO;
Dept. of Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: CRISPR epigenome editing, using CRISPR-activation or CRISPR-interference (CRISPRa/i) allows for more physiologically relevant manipulation of gene expression. Previously, our lab has used Cas9 derived CRISPRa/i epigenome editing tools in Neuro2A cells to manipulate expression of genes that were differentially expressed in D1 or D2 medium spiny neurons (MSNs) of mice following cocaine exposure, as well as developing light-inducible Opto-CRISPRa/i systems. Recent advances in CRISPR have paved the way for using a Cas12f derived system (CasMINI) instead of Cas9. The advantages of this system are twofold - for one dead (d)CasMINI is less than half the size of dCas9 allowing for packaging in adenosine-associated viruses (AAVs) instead of the previously used lentiviruses. Further, this construct allows the possibility of including multiple guide constructs for manipulation of multiple genes at the same time. The other advantage is the improved efficiency of Cas12 systems compared to Cas9 in mammalian cells. We are utilizing a CRISPRi system in which a KRAB domain is fused to dCasMINI in one construct, as well as a separate construct with guide RNA to guide the dCasMINI-KRAB fusion protein to endogenous gene targets. With this system, we target genes previously found to be upregulated in mouse nucleus accumbens (NAc) D1 MSNs following forced abstinence from fentanyl. We have validated that for the first time in Neuro2a cells that the dCasMINI-KRAB fusion gene can express at the mRNA and protein level. We are currently testing the ability of dCasMINI-KRAB to downregulate transcription of target genes, as well as validating a new light-inducible Opto-dCasMINI-KRAB system. The potential to use these tools

to alter transcription of specific gene(s) in a cell type-specific manner in the brain will allow routine use of AAV CRISPR epigenome editing tools.

Disclosures: **M. Green:** None. **N. Hajirnis:** None. **E. Choi:** None. **R. Chandra:** None. **M. Lobo:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.13/QQ23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA F32DA056191
NIDA R01DA047843
BSF 2017252

Title: Cocaine-induced Cellular and Molecular adaptations within VTA-projectiong ventral pallidum neurons in mice

Authors: ***R. CAMPBELL**[1], G. VIRATA[2], V. RHODES[2], S. KEY[2], R. CHANDRA[2], S. A. AMENT[3], M. LOBO[4];
[1]Univ. of Maryland Sch. of Medicin Program In Neurosci., Baltimore, MD; [2]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [3]Univ. of Maryland Sch. of Med., Univ. of Maryland Sch. of Med., Baltimore, MD; [4]Univ. of Maryland Sch. of Med., Univ. of Maryland SOM, Baltimore, MD

Abstract: Cocaine induces long-lasting changes within the reward circuitry that promote cocaine-seeking. The ventral pallidum (VP) is one node within the reward pathway known to receive input from and projects to several regions that mediate cocaine-seeking. This includes VP afferents to the ventral tegmental area (VTA-projecting VP) which are required for cocaine relapse. However, the cellular and molecular adaptations that occur within VTA-projecting VP neurons to promote cocaine-seeking are unknown. Here, we demonstrate that inhibition of VTA-projecting VP neurons is sufficient to reduce cue-induced reinstatement of cocaine-seeking in male and female mice following intravenous cocaine-self administration (IVSA). Furthermore, we found that *cFos* mRNA expression within the ventral pallidum is enhanced within male and female mice following cue-induced reinstatement of cocaine-seeking. Mice that underwent chronic cocaine-self administration have increased spine density within VTA-projecting VP neurons in comparison to saline controls. Collectively, this would suggest that VTA-projecting VP neurons are potentiated from cocaine and engaged during cocaine-seeking. Currently, we are examining whether VTA-projecting VP neurons exhibit changes in neural activity following cocaine use using fiber photometry. Altogether, these investigations aim to reveal the neural adaptations that occur within the VP circuitry to drive cocaine relapse.

Disclosures: **R. Campbell:** None. **G. Virata:** None. **V. Rhodes:** None. **S. Key:** None. **R. Chandra:** None. **S.A. Ament:** None. **M. Lobo:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.14/QQ24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R21DA052101

Title: CRISPR-CasMINI mediated combinatorial upregulation of hub genes identified in nucleus accumbens neuron subtypes with fentanyl abstinence

Authors: *N. HAJIRNIS, M. GREEN, E. CHOI, R. CHANDRA, M. LOBO;
Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD

Abstract: The nucleus accumbens is a major brain hub in the reward circuitry with distinct populations of D1- and D2- medium spiny neurons (MSNs) that are the projection neurons of this region. Gene co-expression analysis of actively transcribing mRNA from these neurons revealed distinct gene networks after fentanyl exposure and abstinence. Most of these genes were downregulated in the D1-MSNs, with fewer genes downregulated in the D2-MSNs. Hub genes were identified in the significant downregulated modules. Many of the downregulated hub genes in the D1-MSNs were nuclear factors, biosynthetic enzymes, or membrane receptors. While the majority of downregulated genes in the D2-MSNs are known to be responsible for membrane synthesis, secretory molecules, or Golgi processing. A major roadblock to systematically manipulate the transcription of these disrupted genes is the limitation of using the high molecular CRISPR-Cas9 system. We, therefore, deployed and developed a CRISPR-Cas12f-derived activation system called CasMINI-CRISPRa. We use the catalytically dead CasMINI fused with transcriptional activator (VP64) (CRISPRa) to manipulate the target genes. Our lab has previously developed dead-SaCas9 based CRISPRa, CRISPRi and opto-CRIPSR techniques with a major limitation of the size of dSaCas9 (~110 KDa). The CasMINI (~55 KDa) is half the size of SaCas9 and thus provides a robust genomic engineering platform to fuse with multiple domains of function. Additionally, the compactness of CasMINI provides advantage for incorporating multiple guides to target several genes together. We are using dCasMINI fused with VP64 (CRISPRa) to upregulate a combination of hub genes in the D1- and D2- MSN NAc subtypes. We will then determine how restoring these gene expression patterns impacts fentanyl abstinent gene modules. We also generated and are validating the opto-CasMINI-CRISPRa for light-inducible manipulation of the target genes. Overall, our results suggest a novel and efficient way of upregulating multiple genes at once, thereby allowing modular changes to the gene expression in specific neurons in the context of fentanyl exposure and abstinence.

Disclosures: **N. Hajirnis:** None. **M. Green:** A. Employment/Salary (full or part-time):; Department of Neurobiology, University of Maryland Baltimore. **E. Choi:** None. **R. Chandra:** A. Employment/Salary (full or part-time):; Dept of Neurobiology University of Maryland Baltimore. **M. Lobo:** A. Employment/Salary (full or part-time):; Dept of Neurobiology, University of Maryland Baltimore.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.15/QQ25

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant K01DA045295 to JRM

Title: Cocaine-seeking behavior is influenced by sex and a history of stress

Authors: ***S. S. ROLLINS**, A. GAULDEN, E. TEPE, E. SIA, J. R. MCREYNOLDS; Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Stress is an important contributing factor to addiction and is problematic as stress is unavoidable in daily life. Addiction can be characterized by a loss of control over drug intake that is modeled by escalating patterns of drug self-administration (SA). We have shown that a stressor, electric footshock stress, administered daily at the time of SA induces an escalation of cocaine intake in rats under short-access conditions (2-h/day). Stress-induced escalation of SA is likely the consequence of long-lasting neuroplastic changes that has long-term consequences for later cocaine-seeking behavior. There are also known sex differences in both substance use disorder and stress reactivity, therefore, we hypothesized that male and female rats may differ in how a history of stress impacts cocaine-seeking behavior. Male and female SD rats were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in 4 X 30 min SA sessions separated by 5-min drug-free periods. Some rats received shock in the SA chamber during the 5 min drug-free period over 14 days. Following SA, rats went through extinction training followed by reinstatement testing. A separate group of rats were tested for cue responding following 30 days of forced abstinence. Across sexes, a history of combined stress and cocaine SA resulted in delayed extinction of cocaine-seeking behavior, though this effect is greater in females. Rats who received shock during SA demonstrated augmented reinstatement to cocaine-priming injections (0, 2.5, 5, 10 mg/kg) and footshock stress. However, this effect was mostly present in male rats and the current extent of stress history facilitation of cocaine-seeking behavior in female rats is unclear. Interestingly, shock-induced reinstatement was present in no shock SA female, but not male, rats. In rats who underwent 30 days of forced abstinence, there was no incubation of cocaine craving and female rats responded more than male rats though there was no clear effect of stress history suggesting that incubation of craving may be the result of more intake-dependent neuroadaptations than extinction/reinstatement. Current studies are examining the impact of stress history on cue-induced reinstatement following extinction training. These data

suggest that stress-induced neuroplastic changes occur in regions of the brain that influence cocaine-seeking behavior and that these stress-induced neuroadaptations may be impacted by sex.

Disclosures: S.S. Rollins: None. **A. Gaulden:** None. **E. Tepe:** None. **E. Sia:** None. **J.R. McReynolds:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.16/QQ26

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant K01DA045295 to Jayme McReynolds

Title: Cannabinoid receptor 1 signaling regulates cocaine-taking and cocaine-seeking behavior following stress-induced escalation of cocaine self-administration.

Authors: *A. D. GAULDEN, S. S. ROLLINS, E. TEPE, K. CONRAD, J. R. MCREYNOLDS; Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Illicit substance use (e.g., cocaine use) is a major public health concern with no FDA-approved therapeutics making this a critical unmet need. Clinical literature investigating current cocaine users suggests that many dimensions of substance use are exacerbated by stress, including drug-taking behavior. Additionally, females show increased sensitivity/susceptibility to stress and many aspects of substance use disorder. However, to date, very few preclinical studies model the role of stress and sex in psychostimulant use. Using a rat cocaine self-administration model, we hypothesize that stress enhances cocaine-taking and cocaine-seeking behavior for male and female rats, and that female rats may show more dynamic stress outcomes. Here, we present a novel model of cocaine self-administration (SA) with a concurrent (but non-overlapping) homotypic chronic footshock stressor. Male and female rats both show chronic stress-induced increases in cocaine SA, though female rats in the stress group show greater "front-loading" behavior. Additionally, we investigate the role of endocannabinoid signaling as a potential mechanism which may underpin stress-induced changes in voluntary cocaine intake. Given the established role of endocannabinoid signaling in reward and stress, we hypothesize that endocannabinoid signaling may be upregulated by stress to augment cocaine-motivated behavior. Systemic administration of the cannabinoid type one receptor (CB1R) inverse agonist Rimonabant attenuated cocaine self-administration in both male and female stress rats though females showed a greater sensitivity to CB1R antagonism. Additionally, rats with a history of stress show greater cocaine-primed reinstatement and intra-prelimbic cortical administration of the CB1R antagonist attenuates cocaine-primed reinstatement only in rats with a history of stress. Finally, we have begun examining CB1R involvement in cocaine- and stress-induced nucleus accumbens shell dopamine signaling in male and female rats. We hypothesize that cocaine- and

footshock-evoked dopamine is modulated by CB1R signaling, and that this regulation may underpin our behavioral observations of CB1R regulation of stress-enhanced cocaine SA. Taken together, these data suggest that stress-induced adaptations in the endocannabinoid system regulate the effects of chronic stress on cocaine-taking and -seeking behavior.

Disclosures: A.D. Gaulden: None. **S.S. Rollins:** None. **E. Tepe:** None. **K. Conrad:** None. **J.R. McReynolds:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.17/QQ27

Topic: G.09. Drugs of Abuse and Addiction

Support: DA045764
U54MD007592-28

Title: Common Patterns of Neuronal Activity in the Prelimbic Cortex During Distress Tolerance and Impulsivity Predict Drug- and Water-Seeking Behavior.

Authors: *K. J. GALVAN[1], A. I. LOPEZ[2], D. E. CALVO[2], Y. A. VEGA[2], R. E. POWERS[2], A. Y. REYES[2], T. M. MOSCHAK[2];
[1]The Univ. of Texas At El Paso, El Paso, TX; [2]The Univ. of Texas at El Paso, El Paso, TX

Abstract: Impulsivity (IMP), distress tolerance (DT), and Pavlovian conditioned approach (PCA) are each behavior predictive of drug seeking and relapse. However, few studies have examined the behavioral and neural interactions among all of these tasks and drug-seeking. We hypothesize that there are certain common or unique neuronal activation patterns present that are involved between these behaviors and drug-seeking. Female (n=6) and male (n=6) Sprague Dawley rats were subjected to a viral infusion of GCAMP6s and lens implantation in the prelimbic cortex. After a recovery period, rodents went through a training period for each of the three tasks. Following training, they were implanted with intrajugular catheters and miniscope baseplates. Once recovered, we recorded PrL activity with in vivo calcium imaging during the behavioral tasks. Afterward, rats were placed into cocaine or water self-administration for two weeks, and on the 15th day, an extinction task was used to measure reward seeking. Subsequently, neurons were classified as ‘common’ or ‘unique’ depending on their shared activity patterns across the tasks. Low reward seekers for either cocaine or water had higher neuronal excitability in the DT and impulsivity tasks. This effect was driven by neurons with common activity patterns across both DT and impulsivity. Conversely, neurons with unique patterns of activity present solely in one or the other task did not differentiate low and high reward seekers. Understanding and targeting these common and unique neuronal populations could assist in specific therapeutics for individuals exhibiting one or more behaviors that predict drug seeking and relapse.

Disclosures: **K.J. Galvan:** None. **A.I. Lopez:** None. **D.E. Calvo:** None. **Y.A. Vega:** None. **R.E. Powers:** None. **A.Y. Reyes:** None. **T.M. Moschak:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.18/QQ28

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045764
NIH Grant U54MD007592-28

Title: Aversive Cues Drive both Escalation and Suppression of Cocaine Intake in Two Distinct Populations

Authors: R. E. POWERS[1], G. RAMIREZ[2], ***T. MOSCHAK**[1];
[1]Univ. of Texas at El Paso, El Paso, TX; [2]El Paso Community Col., El Paso, TX

Abstract: Drug use is often paired with a stimulus that is considered aversive in drug-naive individuals, such as smoke inhalation, injection, or drinking a bitter substance. Critically, the degree of aversion these stimuli elicit in a given individual and the ability of an individual to adapt or habituate to these aversive stimuli plays an important role in developing and maintaining drug use. However, existing preclinical models using aversive stimuli do not introduce the aversive stimulus until after several days of self-administration training, which does not mimic the experience of substance use in human populations. Here, we developed a novel model of aversive cue self-administration (ACSA) where an aversive stimulus (quinine infusion) was paired with cocaine from the very first day of training. Male and female Sprague Dawley rats were anesthetized and implanted with and intrajugular catheter and an intraoral cannula. Following recovery, animals were habituated to water infusions into their intraoral cannula. Thereafter, animals began the ACSA paradigm in a dedicated operant chamber. Here, animals could nosepoke for delivery of 0.8 mg/kg cocaine (i.v.) and 350 µl/kg 0.3M quinine (i.o.) for 2 hr/day. Self-administration lasted for 14 days and was followed by an extinction session where animals could self-administer quinine, but not cocaine. We found that pairing cocaine with quinine suppressed cocaine intake on day 1 compared to data from a previous published set of animals (n=13) self-administering cocaine paired with a neutral (tone-light) cue ($t(15) = 4.52$, $p < 0.001$). However, quinine-paired animals subsequently diverged in their response patterns ($F(13,65) = 5.85$, $p < 0.001$). Some (n=4) further decreased their intake ($F(13,39) = 2.90$, $p = 0.005$), while the remainder (n=3) increased their intake to the same level as that of animals with a light/tone cue ($F(13,26) = 3.38$, $p = 0.004$). When tested under extinction with cue (quinine or light/tone) but no cocaine, high cocaine + quinine animals had more nosepokes than low animals ($t(5) = 7.96$, $p < 0.001$) and were not different from light/tone animals ($t(14) = 0.24$, $p = 0.812$). Thus, our model both induces escalation of intake in

some subjects and completely prevents intake in others, which offers strong face validity to the first-time users that either cease or escalate consumption.

Disclosures: R.E. Powers: None. **G. Ramirez:** None. **T. Moschak:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.19/RR1

Topic: G.09. Drugs of Abuse and Addiction

Support: DA045764
U54MD007592-28

Title: Behavioral and Neuronal Activity Comparison Between Different Tasks that Predict Cocaine- and Water-Seeking Behaviors in the Prelimbic Cortex.

Authors: *Y. A. VEGA[1], A. Y. REYES[1], K. J. GALVAN[2], D. E. CALVO[1], A. I. LOPEZ[3], R. E. POWERS[1], T. M. MOSCHAK[1];
[1]The Univ. of Texas at El Paso, El Paso, TX; [2]The Univ. of Texas At El Paso, El Paso, TX; [3]Univ. of Texas at El Paso, El Paso, TX

Abstract: The prelimbic cortex is a brain region involved in different aspects of drug addiction such as reward processing, craving and relapse, inhibitory control as well as the regulation of drug-seeking behaviors. Extinction (EXT) and Progressive Ratio (PR) tasks have been utilized in previous studies to identify their relevance as measurements of drug-seeking. In EXT, rodents are stripped of their reward. Meanwhile, in PR, the requirement to receive a reward increases exponentially. The neuronal relationship between these behaviors are still unknown. We used male (n=2) and female (n=2) Sprague Dawley rats that received a viral infusion of the GCAMP6 virus and lens implantation into the Prelimbic Cortex (PrL). After a recovery period, the subjects underwent implantation of intrajugular catheters and miniscope baseplates, then were placed into cocaine or water self-administration for two weeks. Following a 30-day abstinence period, endoscopic in vivo calcium recording was conducted during the EXT and PR tasks. In EXT, there was no behavioral statistical difference between the cocaine or water groups nor across sexes. Meanwhile, animals had significantly higher breakpoints in the PR task for cocaine than water. In the prelimbic cortex during the EXT task there was a higher percentage of nonphasic cells in high and low reward seekers than during Progressive Ratio (chi-square = 4.87, p = 0.027). Identifying these behavioral patterns as well as the neuronal assemblies involved will allow deeper insight into drug-seeking behaviors, mechanisms of addiction and the development of potential therapeutics for drug addiction.

Disclosures: Y.A. Vega: None. **A.Y. Reyes:** None. **K.J. Galvan:** None. **D.E. Calvo:** None. **A.I. Lopez:** None. **R.E. Powers:** None. **T.M. Moschak:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.20/RR2

Topic: G.09. Drugs of Abuse and Addiction

Support: DA045764
U54MD007592-28

Title: Neuronal Activity Comparison Between Cocaine Self-Administration and Histamine-Cocaine Self Administration in the Prelimbic Cortex.

Authors: *A. LOPEZ[1], A. Y. REYES[2], K. J. GALVAN[3], D. E. CALVO[2], Y. VEGA[2], R. E. POWERS[2], T. M. MOSCHAK[2];
[1]Univ. of Texas at El Paso, El Paso, TX; [2]The Univ. of Texas at El Paso, El Paso, TX; [3]The Univ. of Texas At El Paso, El Paso, TX

Abstract: The pursuit of drugs in the face of adverse consequences is a key component of substance use disorders. One such model of persistent drug-taking uses histamine as a punisher that is paired with the drug of abuse. Histamine is a compound produced by the body and upon release can cause a number of symptoms, including inflammation and itching. However, few studies have investigated the neural substrates of drug-taking using histamine as a concurrent punisher. Here, we used endoscopic in-vivo calcium imaging in the prelimbic cortex (PrL) to compare changes in neural activity between unconditioned cocaine self-administration (SA) and histamine-cocaine self-administration (HSA). Sprague Dawley rats received a viral infusion of the GCaMP6s virus and lens implantation into the PrL. After a recovery period, the subjects underwent implantation of intrajugular catheters and miniscope baseplates, then were placed into cocaine self-administration for two weeks. Following a 30-day abstinence period, endoscopic in vivo calcium recording was conducted during the SA and HSA tasks. Our findings showcased that there was a significantly higher percentage of excited cells during the HSA task than during the SA task (chi-square = 12.03, $p < 0.001$). Identifying these neuronal assemblies involved will allow deeper insight into drug-seeking behaviors, mechanisms of addiction, and the development of potential therapeutics for drug addiction.

Disclosures: A. Lopez: None. **A.Y. Reyes:** None. **K.J. Galvan:** None. **D.E. Calvo:** None. **Y. Vega:** None. **R.E. Powers:** None. **T.M. Moschak:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.21/RR3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-NIDA K01DA054306
NIH-NIDA P01DA047233
NIH-NIDA R01DA007359

Title: Cellular and transcriptional correlates of drug-associated memories in the nucleus accumbens

Authors: *F. J. MARTINEZ-RIVERA[1], L. HOLT[2], R. DURAND-DE CUTTOLI[2], A. MINIER-TORIBIO[2], M. ESTILL[2], S. TOFANI[2], C. AZIZIAN[2], T. MARKOVIC[2], S.-Y. YEH[2], L. LI[2], R. FUTAMURA[2], A. GODINO[2], C. BROWNE[2], P. MEWS[2], H. ALEYASIN[2], S. J. RUSSO[2], L. SHEN[2], E. J. NESTLER[2];
[1]Neurosci., Mount Sinai Sch. of Med., New York, NY; [2]Neurosci., Nash Family Dept. of Neurosci. and Friedman Brain Institute, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Substance use disorders exemplify a maladaptive imbalance wherein drug seeking and taking persists despite negative consequences of drug use. Such imbalance is orchestrated by neurobiological adaptions linked to faulty cellular, epigenetic, and transcriptional modifications in brain reward regions such as the nucleus accumbens (NAc). However, while these events are commonly assigned to withdrawal, extinction, or renewal/relapse phases, there is a pressing need to characterize these alterations in a sex-, subregion-, and cell-specific manner. Here, we used cocaine self-administration (SA) in rats combined with RNA-sequencing (RNAseq) of NAc subregions (core and shell) to transcriptionally profile the impact of extinction learning on countering withdrawal- and renewal-associated drives. As expected, rats receiving extinction training in the original SA context (levers/cues) significantly reduced their seeking when compared with rats receiving forced abstinence in either their home cages or the original SA context. Further analysis showed that undergoing withdrawal in the original drug context promotes incubation of drug seeking. Consistent with this observation, subsequent bioinformatic analyses revealed distinct transcriptional patterns of this group when compared with home cage withdrawal or extinction training. Additional studies extend these findings by identifying the cellular and transcriptional basis of transferring extinction memories across contexts. These experiments involve rats acquiring and extinguishing in different contexts followed by transcriptomic analyses and patterns of opposing phenotypes (i.e., extinction vs. renewal). Complementary to these datasets, and with the goal of cell-specific characterizations, we are using chemogenetics, fiber photometry, and slice electrophysiology of NAc subregions and cell types (D1 vs. D2 medium spiny neurons) in a sex-dependent manner. Together, these approaches are providing unprecedented evidence of how extinction, withdrawal, and renewal reprogram cellular activity and transcriptomics of the NAc, insight which will guide identification of new ways of preventing relapse.

Disclosures: F.J. Martinez-Rivera: None. **L. Holt:** None. **R. Durand-De Cuttoli:** None. **A. Minier-Toribio:** None. **M. Estill:** None. **S. Tofani:** None. **C. Azizian:** None. **T. Markovic:** None. **S. Yeh:** None. **L. Li:** None. **R. Futamura:** None. **A. Godino:** None. **C. Browne:** None. **P. Mews:** None. **H. Aleyasin:** None. **S.J. Russo:** None. **L. Shen:** None. **E.J. Nestler:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.22/RR4

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045795
NIH Grant MH051399

Title: Investigating Transcription Factors that Mediate Responses to Chronic Cocaine or Stress Exposure: Insights into Stress-Induced Anhedonia and Addiction-Related Behaviors

Authors: *B. W. HUGHES, M. S. ESTILL, L. M. HOLT, A. R. LABANCA, A. TORRES-BERRIO, F. J. MARTINEZ, T. GYLES, A. MINIER-TORIBIO, C. J. BROWNE, P. MEWS, L. SHEN, E. J. NESTLER;
Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Understanding the molecular mechanisms underlying responses to stress and drug exposure is crucial for comprehending the pathophysiology of neuropsychiatric disorders. In this preliminary study, we employed the chromatin mapping technique, Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and sequencing analyses to investigate how exposure to chronic cocaine or stress influences genome-wide protein binding to DNA. We started by identifying DNA binding sites for the transcription factors CREB and ΔFOSB, which are known to regulate drug- and stress-induced behavioral and transcriptional responses. Our analysis revealed significant differences in protein binding patterns within the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) of mice exposed to chronic cocaine or social defeat stress. Both cocaine and stress altered the genome-wide binding of CREB and ΔFOSB, with distinct DNA binding site patterns observed across brain regions and treatment groups. These early findings underscore the unique alterations in protein-DNA interactions induced by cocaine and stress exposure within brain regions associated with reward and aversion. Moreover, these data have significant implications for elucidating the molecular underpinnings and distinct gene regulatory mechanisms that govern stress-induced anhedonia and addiction-related behaviors. Validation of these results, and examination of additional transcription factors and genes involved, will be pursued using our existing cocaine- and stress-based RNA-seq datasets. In conclusion, our study integrates CUT&RUN, differential binding analysis, gene annotation, and motif enrichment analysis to deepen our understanding of gene expression regulation in response to cocaine and chronic stress. These findings have significant implications for understanding molecular mechanisms underlying stress-induced anhedonia and addiction-related behaviors, while offering valuable insights into potential pharmacotherapeutic approaches for addressing the comorbidity between substance use disorders and other stress-related neuropsychiatric conditions.

Disclosures: **B.W. Hughes:** None. **M.S. Estill:** None. **L.M. Holt:** None. **A.R. LaBanca:** None. **A. Torres-Berrio:** None. **F.J. Martinez:** None. **T. Gyles:** None. **A. Minier-Toribio:** None. **C.J. Browne:** None. **P. Mews:** None. **L. Shen:** None. **E.J. Nestler:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.23/RR5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA K99AA027839
NIDA P01DA047233
Brain and Behavior Research Foundation

Title: Decoding the cellular consequences of cocaine exposure through epigenetic remodeling and transcriptional responses in striatal D1 and D2 medium spiny neurons

Authors: *P. MEWS[1], Y. VAN DER ZEE[1], A. MASON[1], H. KRONMAN[1], A. GURUNG[1], A. RAMAKRISHNAN[1], C. J. BROWNE[1], R. FUTAMURA[1], A. REYES[1], S. SIDOLI[2], L. SHEN[1], E. NESTLER[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Albert Einstein Col. of Med., New York, NY

Abstract: A hallmark of addiction is drug-induced disruption of gene programs across brain circuits that direct motivated behaviors, making individuals vulnerable to relapse even after prolonged abstinence. Chronic exposure to cocaine triggers enduring alterations in gene regulation within the nucleus accumbens (NAc), a critical hub for motivation and reward processing, which are linked to an increased risk of relapse. However, the molecular processes underpinning these maladaptive gene activities are not fully understood. We explored the cellular consequences of chronic cocaine exposure within the NAc, focusing on the epigenetic remodeling and heterogeneity of transcriptional responses within D1 and D2 medium spiny neurons (MSNs). We elucidate a previously unexplored epigenetic mechanism whereby sustained cocaine exposure instigates enduring transcriptional reprogramming in D1 MSNs. This reprogramming is associated with a marked diminution of the histone variant H2A.Z, enhanced genomic accessibility, and latent priming of gene programs involved in synaptic plasticity, which are rapidly induced upon cocaine relapse. The histone chaperone ANP32E facilitates the removal of H2A.Z from chromatin, and our findings reveal that selective Anp32e knockdown in D1 MSNs inhibits cocaine induced H2A.Z depletion and blocks the reinforcing effects of cocaine. However, we observed markedly different effects of cocaine exposure, withdrawal, and relapse in D2-MSNs. These findings underscore the significance of investigating cell-type-specific epigenome regulation to identify chromatin-based mechanisms that underpin the enduring impact of cocaine on the brain. Our study further posits histone variant exchange as a potential

therapeutic target to counteract the deleterious effects of drugs of abuse on brain function and behavior.

Disclosures: **P. Mews:** None. **Y. van der Zee:** None. **A. Mason:** None. **H. Kronman:** None. **A. Gurung:** None. **A. Ramakrishnan:** None. **C.J. Browne:** None. **R. Futamura:** None. **A. Reyes:** None. **S. Sidoli:** None. **L. Shen:** None. **E. Nestler:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.24/RR6

Topic: G.09. Drugs of Abuse and Addiction

Support: Supported by NIDA and NIMH

Title: Dopamine receptor type 1 (D1R) and dopamine receptor type 2 (D2R) expressing cells in the ventral hippocampus and their role in reinforcement

Authors: ***V. KONDEV**[1], A. GODINO[1], A. MINIER-TORIBIO[1], E. J. NESTLER[2]; [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: The ventral hippocampus (vHPC) has been implicated in regulating emotional processing and negative affect. We have recently demonstrated that the vHPC contains distinct populations of dopamine receptor type 1 (D1R)- and dopamine receptor type 2 (D2R)-expressing neurons that modulate approach-avoidance behavior in mice. Here, we map functional inputs onto, as well as projection targets of, these vHPC D1R- and D2R-expressing cells. Furthermore, using fiber photometry, we describe how these D1R and D2R cells within the vHPC respond to both natural and drug rewards. These studies further characterize these novel cell types in the vHPC and provide a new circuit mechanism by which drugs of abuse may promote reinforcement.

Disclosures: **V. Kondev:** None. **A. Godino:** None. **A. Minier-Toribio:** None. **E.J. Nestler:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.25/RR7

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01DA014133
NIH P01DA047233
NIH P30DA018343

Title: Sex- and Withdrawal-Dependent Proteomic Changes in Nucleus Accumbens and Prefrontal Cortex: Insights into Synaptic Adaptations in Substance Use Disorders

Authors: *Y. YIM[1], A. GODINO[2], T. T. LAM[3], E. J. NESTLER[4];
[2]Icahn Sch. of Med. at Mount Sinai, [1]Icahn Sch. of Med. at Mount Sinai, New York, NY;
[3]Director, Keck MS & Proteomics Resource, Yale Sch. of Med., New Haven, CT; [4]Icahn Sch. of Med. At Mount Sinai, Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Substance use disorders (SUD) continue to be a significant public health problem in the United States, presenting a challenge for effective treatment due to their complex neurobiological mechanisms. Dysregulated signaling within reward-processing brain regions, such as the nucleus accumbens (NAc) and prefrontal cortex (PFC), plays a critical role in promoting drug-seeking behavior and relapse. Relative to our understanding of transcriptional responses to drugs of abuse, our knowledge about changes in the proteomic landscape of synapses is limited. Identifying these changes could reveal more effective targets for SUD treatment. In this study, we build upon previous research on cocaine-mediated proteomic adaptations by focusing on the discovery of sex- and withdrawal (WD)-dependent changes in the synaptic proteome of the NAc and PFC. Adult male and female C57BL/6J mice (12 weeks old) were subjected to daily cocaine (20 mg/kg) or saline (vehicle) intraperitoneal (I.P.) injections for 7 days, followed by 24 hours or 30 days of forced abstinence (withdrawal; WD). After WD, the NAc and PFC were extracted, and synaptosomes were purified and analyzed using lipid chromatography-tandem mass spectrometry (LC-MS/MS) followed by label-free quantification at the Yale/NIDA neuroproteomics core. Through this analysis, we identified multiple synapse-enriched proteins that were either induced or repressed in a brain region-, sex-, and WD-time dependent manner. While most of these cocaine-regulated proteins are expressed by neurons, a significant subset is enriched in astrocytes or microglia, consistent with the involvement of these cell types in synaptic processes. Interestingly, in the NAc after 30 days of WD, female mice exhibited ~2.5 times more significant proteome changes than male mice. In the PFC after 24 hours of WD, male mice displayed ~2.5 times more significant proteome changes (primarily repression) than female mice. We are currently validating these findings to identify candidate synaptic proteins that directly contribute to drug-seeking behavior in a brain region-, sex-, and WD-time dependent manner. This study aims to enhance our understanding of the molecular restructuring of synapses in the reward circuitry, which ultimately influences susceptibility to relapse.

Disclosures: Y. Yim: None. **A. Godino:** None. **T.T. Lam:** None. **E.J. Nestler:** None.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.26/RR8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA R01 grant DA046457

Title: Noncontingent footshock does not suppress cocaine self-administration but causes lasting effects in a sex-dependent manner

Authors: *P. KAHANEK, A. M. CRUZ, M. PALADINO, A. STARNES, R. J. SMITH; Texas A&M Univ., College Station, TX

Abstract: A defining characteristic of addiction is compulsive drug use, or continued drug use despite negative consequences. In an animal model of compulsive drug seeking, a subset of animals continue to seek cocaine despite footshock consequences, while another subset reduces cocaine seeking to avoid footshock. Previously we found that noncontingent footshock, unlike contingent footshock, did not reduce self-administration of cocaine in male rats. Here, we wanted to investigate the effects of noncontingent footshock in female rats, and to explore potential lasting effects of noncontingent footshock in both males and females. Male and female Sprague Dawley rats were trained to self-administer intravenous cocaine via a seeking-taking chained schedule of reinforcement during daily 2-h sessions. After ~3 weeks of self-administration, rats were given 4 days of testing with either contingent footshock (0.4 mA, 0.3 sec, randomly 1⁄3 trials, delivered after completion of seeking) or noncontingent footshock (same parameters and average number of shocks, but independent of behavior). Similar to our previous studies in males, we found that noncontingent footshock did not reduce cocaine self-administration in females, whereas contingent footshock resulted in reduced cocaine self-administration on average, with some rats more sensitive than others. Even at higher intensities of footshock (1 mA, 1 sec), noncontingent footshock did not reduce cocaine seeking in males or females. We then exposed rats to the opposite footshock condition, such that rats first exposed to contingent footshock were now given noncontingent footshock, and vice versa. In both males and females, noncontingent footshock still resulted in no change in self-administration. In males, contingent footshock suppressed self-administration to a similar degree as rats receiving contingent footshock for the first time. However, in females, contingent footshock did not suppress self-administration as much as rats receiving contingent footshock for the first time, indicating that a history of noncontingent footshock led to increased punishment resistance. These data indicate that contingency plays a key role in the suppressing effects of footshock in both male and female rats, and that females show lasting effects of noncontingent footshock. Further work is necessary to determine the underlying mechanisms of these sex differences, and whether exposure to noncontingent footshock impairs future detection of contingency in this paradigm.

Disclosures: P. Kahanek: None. **A.M. Cruz:** None. **M. Paladino:** None. **A. Starnes:** None. **R.J. Smith:** A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

PSTR497. Addictive Substances, Plasticity, and Stress

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR497.27/RR9

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA037744
NIH Grant F99 NS130870

Title: Effects of modulating acetylcholine activity in dorsomedial striatum on punishment of cocaine seeking

Authors: *A. CRUZ, R. SMITH;
Texas A&M Univ., College Station, TX

Abstract: Compulsive drug seeking that continues despite negative consequences may result from a loss of behavioral flexibility, which is the ability to update behavior in response to changes in environmental conditions. Striatal cholinergic interneurons (CINs) have been shown to drive behavioral flexibility via acetylcholine (ACh) release in the dorsomedial striatum (DMS). Specifically, previous work has shown that blocking behavioral evoked ACh release in the DMS via a muscarinic M2/M4 receptor agonist oxotremorine-sesquifumarate (oxo-s) impairs behavioral flexibility (Ragozzino et al., 2009). Therefore, we hypothesized that oxo-s will reduce behavioral flexibility and lead to persistent cocaine seeking despite negative consequences. We trained male Sprague Dawley rats to self-administer cocaine on a seeking-taking chained schedule of reinforcement. To assess cocaine seeking in response to negative consequences, rats were exposed to 2 sessions of footshock (0.7 mA, 0.3 s) that occurred randomly on 1/3 of trials after completion of seeking. Prior to each footshock session, rats received microinjections in DMS (0.5 ul/side) of oxo-s (1.6 mM) or vehicle (PBS). We found similar levels of punishment sensitivity in rats treated with oxo-s or vehicle, indicating that DMS cholinergic signaling is not required for suppression of cocaine seeking in response to negative consequences. When we gave oxo-s prior to a cocaine self-administration session without footshock, we found similar levels of self-administration in rats treated with oxo-s or vehicle, indicating that DMS cholinergic signaling is not required for cocaine self-administration. We are currently investigating whether increasing ACh activity in DMS will enhance punishment sensitivity.

Disclosures: A. Cruz: None. **R. Smith:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.01/RR10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA006214

Title: A rodent model for prescription opioid-associated opioid use disorder: Role of the orexin system

Authors: *K. A. NEWMAN[1], C. THOMAS-SERRANO[2], G. S. ASTON-JONES[3];
[1]Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ; [3]Brain Hlth. Inst., [2]Rutgers Univ., Piscataway, NJ

Abstract: The opioid crisis is an urgent public health issue for which there are few effective medications. Abuse of prescription opioids may lead to the development of opioid use disorder (OUD). Important facets of OUD are increased drug demand and adverse withdrawal states, which consist of physical and affective symptoms. It is thought that following OUD development, negative affect in withdrawal contributes to increased opioid demand via negative reinforcement mechanisms. Orexins, hypothalamic neuropeptides, promote both opioid seeking and withdrawal states, representing a promising pharmacological target for OUD. We therefore sought to establish a rodent model of prescription opioid-associated OUD, and determine whether antagonism of orexin signaling could attenuate OUD phenotypes. Adult male Long Evans rats were given 21d of twice-daily injections of saline (n=10, 1ml/kg; i.p.) or oxycodone (n=10, 3mg/kg; i.p.) and tested for signs of negative affect during acute abstinence. We found that compared to saline-treated controls, oxycodone-treated rats displayed behaviors indicative of increased negative affect, including decreased body weight gain ($p<0.0001$), increased anhedonia ($p<0.05$), and increased hyperalgesia ($p=0.08$). One week following this treatment, rats were trained to self-administer fentanyl on an FR-1 schedule for 10d. Then, rats were trained and tested on a within-session behavioral economics (BE) procedure to assess demand for fentanyl. In the initial days of acquisition, rats pretreated with oxycodone had higher fentanyl intake than saline-pretreated controls ($p<0.05$). Interestingly, although we observed individual differences in fentanyl consumption across groups, oxycodone-pretreated “high takers” self-administered significantly more fentanyl than saline-pretreated “high takers” ($p<0.05$). We observed that demand elasticity (inverse motivation) did not differ between the highest-motivated saline- and oxycodone-pretreated rats. However, within lower-motivated rats, oxycodone-pretreated rats were more motivated for fentanyl than saline-pretreated rats ($p=0.0513$). Finally, the orexin receptor-1 antagonist SB-334867 was administered 30min prior to BE testing (30mg/kg, i.p.). In highly motivated oxycodone-pretreated rats, SB more effectively reduced motivation for fentanyl than in saline-pretreated rats. Together, these results indicate that prescription opioid-associated OUD can be modeled in rodents and that oxycodone consumption can increase opioid demand. In addition, the orexin system may be important for OUD-like phenotypes and represent a promising pharmacological target for OUD.

Disclosures: K.A. Newman: None. **C. Thomas-Serrano:** None. **G.S. Aston-Jones:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.02/RR11

Topic: G.09. Drugs of Abuse and Addiction

Support: ERC-2019-ADG - ERC Advanced Grant

Title: Cellular determinant of positive and negative reinforcement in opioid addiction

Authors: *F. CHAUDUN[1], L. PYTHON[1], Y. LIU[1], J. CAND[1], A. HIVER[1], B. KIEFFER[2], E. VALJENT[3], C. LUSCHER[1];
[1]Ctr. Médical Universitaire Genève, Geneve, Switzerland; [2]Univ. of Strasbourg Inst. for Advanced Study, INSERM U1114, Strasbourg, France; [3]Inst. de genomique fonctionnelle, Montpellier, France

Abstract: Opioids have been used over the centuries as painkillers. When used chronically, opioids cause addiction in more than a third of users, a fraction exceeding the one observed with psychostimulants (Anthony et al.,1994). On top of its rewarding properties, opioids also induce strong dependence, defined by a stereotypical withdrawal syndrome (Comer and Cahill, 2018). Here we test whether negative reinforcement, i.e. self-administration to alleviate the unpleasant withdrawal syndrome may add to positive reinforcement, to enhance addiction liabity. To address this question, we use genetically modified mouse lines, in vivo recording and operant behavior during precipitation of withdrawal. We first demonstrate that fentanyl inhibits GABA neurons in the ventral tegmental area (VTA) via μ-opioid receptors (μORs), causing disinhibition of dopamine (DA) neurons that elicits a striatal DA transient underlying positive reinforcement. Furthermore, deleting μORs in the VTA abolished the reinforcing DA transients but had no impact on precipitated withdrawal. Mapping cFos and neuronal activities during precipitated withdrawal revealed strong activation of μOR-positive neurons in the central amygdala (CeA) and deleting μORs in CeA abolished escape jumps, a cardinal withdrawal symptom. Finally, optogenetic activation of CeA μOR expressing neurons was sufficient to elicit negative reinforcement ; mice quickly learned to press a lever to stop the optogenetic activation. To conclude, our work demonstrates that μOR-expressing neurons of VTA control positive reinforcement while μOR-expressing neurons in the CeA are the cellular determinant of induction for negative reinforcement. Subsequent work will strive to identify the locus where positive and negative reinforcement circuits converge to yield compulsive behavior.

Disclosures: F. Chaudun: None. **L. Python:** None. **Y. Liu:** None. **J. Cand:** None. **A. Hiver:** None. **B. Kieffer:** None. **E. Valjent:** None. **C. Luscher:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.03/RR12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA009813
UG3 Grant DA050325
Gift from Dr. Edward and Mrs. Janis Saylor

Title: Effect of acute treatment with the glucagon-like peptide-1 receptor agonist, liraglutide, and estrus phase on cue- and drug-induced fentanyl seeking in female rats.

Authors: *L. URBANIK, J. BOOTH, N. ACHARYA, P. GRIGSON;
Penn State Col. of Med., Hershey, PA

Abstract: Opioid Use Disorder (OUD) is a crisis in the United States. Despite positive advancements with medication assisted therapies, overdose deaths in the US have continued to rise with an unprecedented 107,000 recorded overdose deaths in 2021. These overdose deaths are largely driven by the potent synthetic opioid, fentanyl, which is now being mixed with illicit drugs. We have previously demonstrated that male rats readily self-administer fentanyl, with evident individual differences in fentanyl taking, seeking, and reinstatement behaviors. We also have shown that acute treatment with the glucagon-like peptide-1 receptor (GLP-1R) agonist, liraglutide, can reduce fentanyl taking and seeking behavior in male rats. However, given that females are significantly more vulnerable to drug-related cues, drug cravings, and the rapid development of OUD compared to males, it is imperative that we investigate the biological risk factors on fentanyl dependence and fentanyl use disorder. Further, preclinical models have reported that females in the estrus phase of the estrous cycle have increased fentanyl intake, more rapid development of OUD, and enhanced relapse vulnerability compared to those in a non-estrus phase. Thus, here we aimed to better understand the effect of estrus phase in our model of OUD and on the effectiveness of acute liraglutide treatment. Herein, we demonstrate that female rats readily self-administer fentanyl (1.85 ug/infusion) intravenously, with marked individual differences in fentanyl taking behavior. Additionally, rats in the estrus phase exhibited greater fentanyl intake compared with those in a non-estrus phase, greater cue-induced fentanyl seeking, and greater drug-induced reinstatement of fentanyl-seeking. Finally, acute treatment with the GLP-1R agonist, liraglutide (0.3 mg/kg s.c.), reduced cue-induced fentanyl seeking and potently blocked drug-induced reinstatement of fentanyl seeking, whether tested in estrus or in non-estrus. Overall, these data support the broad effectiveness of acute GLP-1R agonists as a promising non-opioid treatment for OUD.

Disclosures: L. Urbanik: None. **J. Booth:** None. **N. Acharya:** None. **P. Grigson:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.04/RR13

Topic: G.09. Drugs of Abuse and Addiction

Support: UG3DA050325
Dr. Ed Saylor and his wife Janis Saylor

Title: Acute treatment with the glucagon-like peptide-1 receptor agonist, liraglutide, reduces cue-induced fentanyl seeking and associated c-Fos and tyrosine hydroxylase activation patterns in brain in rats

Authors: *B. EVANS[1], N. ACHARYA[2], K. T. NEWMASTER[2], Y. KIM[2], P. S. GRIGSON[2]; [1]Penn State Col. of Med. Neurosci. Grad. Program, Hershey, PA; [2]Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA

Abstract: Background. Although there are three pharmacological treatments for opioid use disorder (OUD) including methadone, buprenorphine, and naltrexone, relapse rates remain high. Over 36,000 people overdosed on synthetic opioids in 2019, and this number increased by 30% in 2020 due to the COVID-19 pandemic. New treatments, then, are essential. Recently, we have proposed that substance use disorder involves a hijacking of not only reward circuits, but also of circuits involved in physiological need as well. In accordance, treatment with glucagon-like peptide-1 receptor agonists (GLP-1RAs), known satiety agents, reduce responding for ethanol, nicotine, cocaine and opioids. But, where in the brain are these GLP-1RAs working? **Methods.** Here, we used fentanyl self-administration, immunohistochemistry (IHC), and light sheet microscopy to measure fentanyl seeking, the associated brain activation patterns (i.e., c-Fos and tyrosine hydroxylase, TH), and reversal of both by treatment with the GLP-1RA, liraglutide, in male Sprague-Dawley rats. Specifically, rats were given 6 h to self-administer fentanyl (1.85 ug/infusion) across 14 daily trials. On day 15, all rats were injected with saline or liraglutide (0.3 mg/kg sc) and 6 h later placed in the drug self-administration chamber for a 90 min extinction test where cues were presented, but no drug was delivered. Thirty min later, the rats were deeply anesthetized and brains prepared for IHC and light sheet microscopy. **Results: Behavior.** As is typical for other drugs of abuse tested, about half of the rats, referred to as the high drug takers, self-administered more fentanyl/6h across trials 1 - 14 than did the other half, the low drug takers. During the 90 min extinction test, all rats pretreated with saline exhibited high seeking for fentanyl; this seeking behavior was blocked by treatment with the GLP-1RA. **Brain activation.** High taking/seeking was associated with an increase in expression of c-Fos and TH in the medial prefrontal cortex (mPFC), nucleus accumbens (NAc) core, NAc shell, ventral tegmental area (VTA), locus coeruleus (LC), and cingulate cortex (CC); these activation patterns were reversed by treatment with the GLP-1RA, liraglutide. **Conclusion.** Together, these data show that fentanyl taking and seeking in the most vulnerable drive neuronal activity in substrates involved in drug need (i.e., aversion/withdrawal, LC and CC) and those involved in reward/seeking (mPFC, NAc core and shell, and VTA), and that treatment with the satiety agent, liraglutide, reverses both drug-seeking behavior and these associated patterns of brain activation.

Disclosures: B. Evans: None. **N. Acharya:** None. **K.T. Newmaster:** None. **Y. Kim:** None. **P.S. Grigson:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.05/RR14

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse DA048336
National Institute on Alcohol Abuse and Alcoholism T32: AA007583

Title: Behavioral economics of polysubstance use: a role for orexin signaling in the augmentation of opioid consumption by nicotine exposure

Authors: *S. HONEYCUTT, D. D. LICHTE, E. A. GILLES-THOMAS, S. L. MCSAIN, A. MUKHERJEE, G. C. LONEY;
Univ. at Buffalo, Buffalo, NY

Abstract: Both ongoing adult nicotine administration (ANA) and former adolescent nicotine exposure (ANE) are associated with liability for developing opioid use disorder (OUD). Both ANA and ANE increase intravenous self-administration (IVSA) of opioids, elevate breakpoints in progressive ratio tests, and enhance punishment-resistant responding for opioids. Here, we have applied behavioral economics (BE) analyses to generate demand curves for IVSA of remifentanil (RMF) to compare various behavioral indices of opioid consumption across ANA and ANE administration procedures. Furthermore, we also sought to explore the role orexins play in the motivational enhancement by nicotine across age of exposure. We examined opioid IVSA behaviors within a BE model with and without pharmacological blockade of orexin-1 receptors (OxR1) by SB-334867 (SB). Adolescent male and female Long-Evans rats received nicotine (0.4 mg/kg, SC) or saline twice daily for 10 days (PND 34-43). Later (~PND 75), rats were operantly trained for IVSA of RMF (3.2 µg/kg/inf), followed by BE demand curve tests where the RMF dose was systematically decreased (3.2-0.006 µg/kg) within session to assess motivation to respond as a function of cost to obtain RMF. Curve-fits of responding provided motivational indices of RMF consumption at low price (Q0), normalized elasticity (α), and maximal responding (Omax). After baseline was established, rats were treated with SB, or vehicle, to determine the effects of Ox1R antagonism on RMF consumption in this model. Baselines and tests were conducted following ANE and again following reinstatement of nicotine for the ANA condition. There, rats were given nicotine or saline injections prior to behavioral sessions. We found both ANE and ANA significantly increased consumption of RMF relative to controls. Analyses of the BE factors revealed that Q0 differed between ANE rats and controls while in ANA rats Q0 and Omax were significantly higher, and α significantly lower, relative to controls. These findings suggest that ANE is sufficient to increase demand for opioids but ongoing nicotine administration exacerbates this increase in demand. Regardless of age of nicotine exposure, SB produced significantly greater suppression of opioid motivation in nicotine-treated rats, relative to controls, suggesting that the orexin system may play a mechanistic role in nicotine-enhancement of opioid consumption. These data support that cessation of nicotine use may be particularly efficacious in curbing opioid misuse and that orexin treatment may benefit smokers in treatment for OUD.

Disclosures: S. Honeycutt: None. **D.D. Lichte:** None. **E.A. Gilles-Thomas:** None. **S.L. Mcsain:** None. **A. Mukherjee:** None. **G.C. Loney:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.06/RR15

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant P01DA047233

Title: Identification of Mbd3 as a Key Hub Gene in Nucleus Accumbens After Heroin Self-Administration and Relapse in Mice

Authors: *R. FUTAMURA[1], C. BROWNE[1], X. ZHOU[2], A. MINIER-TORIBIO[1], R. PAN[4], X. CHEN[5], A. RAMAKRISHNAN[1], Y. YIM[1], M. SALERY[1], A. GODINO[3], L. SHEN[1], Y. HURD[1], B. ZHANG[1], S. LIU[4], E. J. NESTLER[6];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Genet. and Genomics, [3]Icahn Sch. of Med., New York, NY; [5]Inst. for Genomic Med., [4]Columbia Univ. Program In Neurobio. And Behavior, New York, NY; [6]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: Repeated opioid exposure causes epigenetic changes throughout the brain reward circuitry, which are hypothesized to promote relapse susceptibility. Of note, shifts in the DNA methylation landscape within the nucleus accumbens (NAc)—a brain region involved in mediating motivation and reward processing—have been linked to compulsive drug-seeking and drug-taking behaviors characterized by relapse upon re-exposure to the drug or context related-cues. Recently, our laboratory established broad patterns of transcriptional regulation across six brain reward regions that are driven by volitional drug-taking and -seeking behaviors using intravenous heroin self-administration (SA) in mice (Browne et al, Sci Adv, PMID: 37294757). To identify gene networks regulating relapse in this model, we utilized multiscale embedded gene co-expression network analysis (MEGENA) of this RNA-sequencing (RNA-seq) dataset, which revealed a gene network in the NAc highly enriched with genes upregulated by heroin-primed drug-seeking. Within this network, we found methyl-CpG binding domain protein 3 (*Mbd3*) to be the strongest hub gene; *Mbd3* was not only one of the most upregulated genes in this condition but also positively associated with addiction-like behavior from exploratory factor analysis. Preliminary data show that viral manipulation of *Mbd3* in all NAc neurons controls rewarding responses to opioid exposure. Ongoing efforts include determining the cell-type-specificity of *Mbd3* regulation in D1- or D2- medium spiny neurons (MSNs) in the NAc and using viral approaches to assess how *Mbd3* in each cell type influences drug-seeking behavior in relapse. Further, whole genome bisulfite sequencing (WGBS) is underway to examine how opioid exposure shifts the DNA methylation landscape within the NAc which will then allow us to correlate transcriptomic and methylomic data to ultimiately determine convergent regulatory mechanisms implicated in opioid use disorder (OUD) pathogenesis.

Disclosures: R. Futamura: None. **C. Browne:** None. **X. Zhou:** None. **A. Minier-Toribio:** None. **R. Pan:** None. **X. Chen:** None. **A. Ramakrishnan:** None. **Y. Yim:** None. **M. Salery:**

None. **A. Godino:** None. **L. Shen:** None. **Y. Hurd:** None. **B. Zhang:** None. **S. Liu:** None. **E.J. Nestler:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.07/RR16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA048946
NIH Grant K99 DA052624

Title: Nucleus accumbens fast spiking interneurons regulate opioid reward

Authors: *E. A. GAUTHIER[1], A. BOLDS[2], P. E. ROTHWELL[2], E. M. LEFEVRE[2];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Opioid use disorder is a public health crisis that is characterized by compulsive drug seeking behavior, as well as lifelong vulnerability to relapse. A better understanding of how opioids affect the intrinsic reward circuitry is crucial to generate therapeutic interventions. The nucleus accumbens is a central hub in the reward circuitry. Interneurons that express parvalbumin (PV; also known as fast-spiking interneurons) form one of the most prominent sources of synaptic inhibition in the NAc, and receive stronger excitatory inputs in contrast to the other neuronal populations in the NAc. Increasing evidence suggests NAc PV interneuron circuitry is altered in drug addiction, leading to a shift in motivational drive towards drug seeking and relapse. In this series of experiments, we hypothesize that opioids act on the PV interneurons directly through the mu opioid receptor, and that chemogenetically inhibiting these interneurons will blunt fentanyl reward behavior. We carried out in-situ hybridization experiments to confirm that NAc PV interneurons express the mu opioid receptor, as well as ex-vivo slice electrophysiology experiments to confirm that washing on mu opioid receptor agonist decreases both the intrinsic firing rate and inhibitory output of the PV interneurons. For the behavioral experiments, male and female PV-Cre mice underwent stereotaxic surgery for injection of the Cre-dependent inhibitory DREADD (and control) virus in the nucleus accumbens shell. Subsequently, fentanyl psychomotor sensitization, conditioned place preference, and multi-stage intravenous self administration were used to measure behavioral responses to opioids. DREADD inhibition of fast-spiking interneurons significantly attenuated the induction of fentanyl-evoked locomotor sensitization ($p<0.05$). DREADD inhibition of these cells tended to decrease preference for the fentanyl-paired side in conditioned place preference, although this result was not statistically significant. In self-administration experiments, DREADD inhibition of fast-spiking interneurons tended to increase the breakpoint during the progressive ratio test, but this is not statistically significant. However, DREADD inhibition of these cells significantly decreased cue-induced reinstatement ($p<0.05$). Altogether, these results suggest that fast-spiking

interneurons in the NAc play a role in behavioral responses to opioid administration, and have potential to be a therapeutic target in the treatment of opioid use disorders.

Disclosures: E.A. Gauthier: None. **A. Bolds:** None. **P.E. Rothwell:** None. **E.M. Lefevre:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.08/RR17

Topic: G.09. Drugs of Abuse and Addiction

Support: Istituto Pasteur-Fondazione Cenci Bolognetti Grant"Anna Tramontano"
Sapienza University of Rome Grant AR122181634AA6CD
Sapienza University of Rome Grant RM11715C457665A1
Sapienza University of Rome Grant RM11916B0E316F23
National Institute on Drug Abuse, Grant DA047976

Title: The impact of discrete versus continuous dimensions strategies in heroin and cocaine self-administration on drug-taking patterns and social interaction

Authors: *G. D'OTTAVIO[1,2], S. PEZZA[1,2], I. REVERTE[1,2], S. F. ZENONI[2], C. MARCHETTI[1,2], D. MAFTEI[1], C. FABRIZIO[2], A. TERMINE[2], M. S. MILELLA[3], D. RAGOZZINO[1,2], M. VENNIRO[4], A. BADIANI[1], F. BOIX[5], D. CAPRIOLI[1,2];
[1]Sapienza Univ. of Rome, Rome, Italy; [2]IRCSS Santa Lucia Fndn. CERC, Rome, Italy; [3]Toxicology Unit, Policlinico Umberto I Univ. Hosp., Rome, Italy; [4]Univ. of Maryland Sch. of Med., Baltimore, MD; [5]Section for Drug Abuse Research, Dept. of Forensic Sci., Oslo Univ. Hosp., Oslo, Norway

Abstract: Background: During their drug-use history, cocaine and heroin users gain mastery and control over their drug consumption. Indeed, they self-regulate the dosage, route, speed, and frequency of administration as a function of the expected effects (e.g., avoid withdrawal, experiencing euphoria, etc.). Counterintuitively, most preclinical self-administration and choice procedures use discrete dimension strategies, featured by experimenter-imposed unit-doses interspersed by time-outs, which prevent the experimental animal to self-select the appropriate dose-time relationship of administration. Here, we contrasted discrete to continuous dimension strategies (i.e., self-selected doses without timeout) on drug-related behaviors. **Methods:** We analyzed the drug-taking patterns and modeled drug-brain levels (PK profiling) under distinct self-administration training conditions, featured by the presence of time-out between consecutive drug injections (discrete) or its absence (continuous). We further assessed the motivation to take and seek drugs across training conditions and in the context of drug-vs-social choice procedures. **Results:** The drug-taking patterns, and related PK profiling, were profoundly different across both training conditions and drug under examination. Notably, we did not observe overdoses in

rats trained without a time-out, contrary to what the literature would have anticipated. Rather, the lack of time-out was associated with stronger motivation to take and seek drugs. Finally, heroin, but not cocaine, continuous self-administration induced social withdrawal in rats in drug-vs-social choice procedures. **Conclusions:** Here, we provide evidence advocating for the implementation of continuous, rather than discrete dimension strategies in self-administration and choice procedures because it mirrors more accurately human-drug-related behaviors (and likely the neural adaptations).

Disclosures: G. D'Ottavio: None. **S. Pezza:** None. **I. Reverte:** None. **S.F. Zenoni:** None. **C. Marchetti:** None. **D. Maftei:** None. **C. Fabrizio:** None. **A. Termine:** None. **M.S. Milella:** None. **D. Ragozzino:** None. **M. Venniro:** None. **A. Badiani:** None. **F. Boix:** None. **D. Caprioli:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.09/RR18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant K00DA053527
NIH Grant P30DA048742
NIH Grant R01DA041808

Title: The beta-lactam derivative MC-100093 decreases cue-induced reinstatement following extinction from intravenous self-administration of oxycodone in male and female rats

Authors: *D. W. HART[1], Y. ALONSO-CARABALLO[1], M. A. BRICKNER[1], M. ESGUERRA[1], W. E. CHILDERS[2], M. ABOU-GHARBIA[2], M. J. THOMAS[1];
[1]Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Temple Univ. Sch. of Pharm., Philadelphia, PA

Abstract: Despite being a major public health challenge, long-lasting treatments for opioid use disorder are scarce. Studies have previously demonstrated that ceftriaxone, a β-lactam antibiotic that also increases expression of the astrocytic glutamate transporter 1 (GLT-1), is able to decrease cue-induced and cocaine-induced reinstatement of drug-seeking following extinction, and cue-induced relapse after long term withdrawal in rats who previously self-administered cocaine. Since ceftriaxone was first identified as a modulator of GLT-1, another beta-lactam derivative MC-100093 (093) has been developed, lacking antimicrobial properties while still increasing GLT-1 expression, along with the ability to cross the blood-brain barrier when administered by intraperitoneal injection. While 093 has also been shown to reduce cue-induced reinstatement following extinction from cocaine self-administration, it is unknown if this effect is specific to cocaine or also occurs for opioids. In this study, we hypothesized that 093 would decrease cue-induced reinstatement following extinction from oxycodone self-administration, a

highly addicting and commonly prescribed opioid. Recent reports suggest sex-specific modulation of GLT-1 expression in the nucleus accumbens of rats following cocaine self-administration. However, the majority of the research on ceftriaxone and 093 has been done only in male rats. Thus, our experiments were designed to include both male and female rats to reveal any potential sex difference. We first recapitulated previous studies, showing that 093 treatment (vs. saline) during extinction from cocaine self-administration decreased cue-induced reinstatement. We then performed the same experiment with oxycodone, and again found that treatment with 093 (vs. saline) decreased cue-induced reinstatement. These results suggest that 093's ability to decrease reinstatement is not specific to cocaine. Tissue punches from the nucleus accumbens were collected to confirm 093 increases expression of GLT-1. Our study shows the potential of 093 to decrease relapsing behaviors across multiple drug classes. Future studies examining 093 with other self-administration routes and paradigms, such as intermittent access protocol, oral self-administration, or relapse following forced abstinence will better elucidate 093's potential as a treatment for opioid use disorder.

Disclosures: D.W. Hart: None. **Y. Alonso-Caraballo:** None. **M.A. Brickner:** None. **M. Esguerra:** None. **W.E. Childers:** None. **M. Abou-Gharbia:** None. **M.J. Thomas:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.10/RR19

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC discovery grant
Concordia University Bridge Fund

Title: The effect of chemogenetic activation of the anterior paraventricular nucleus of the thalamus on heroin seeking in abstinent male and female rats.

Authors: *E. G. AH-YEN[1], F. WU[1], P. RODRIGUEZ[1], V. DOUAN[1], C. BORGES[2], U. SHALEV[1];
[2]Concordia Univ., [1]Concordia Univ., Montreal, QC, Canada

Abstract: Drug addiction is a chronic disorder characterized by a cycle of excessive drug use, abstinence, and relapse. Relapse poses a substantial obstacle to addiction treatment, and the risk for relapse is exacerbated by chronic food restriction. However, the underlying neuronal mechanisms are unknown. The paraventricular nucleus of the thalamus (PVT) has been shown to have an important role in drug seeking, but the literature shows conflicting results. A possible explanation for this discrepancy could be the two distinct subregions: the anterior (a) PVT and posterior (p)PVT. Our laboratory has demonstrated a role for the pPVT in food restriction-induced augmentation of heroin seeking in abstinent rats. We then examined the effect of chemogenetic inhibition of the aPVT in male rats and found no statistically significant effect on

heroin seeking in the food restricted or sated groups. Due to the lack of significant findings, we validated our results by pharmacologically inhibiting the aPVT. Pharmacological inhibition of the aPVT confirmed these findings in males. However, female rats showed an increase in heroin-seeking behaviour after pharmacological inhibition of the aPVT. This may suggest an inhibitory role for the aPVT, at least in females. Additionally, it would suggest that the aPVT and pPVT do not differ under our conditions. In order to confirm our findings and further understand the role of the aPVT in heroin seeking, this study aimed to chemogenetically activate the aPVT in male and female rats under the same conditions. Here, male and female Long Evans rats were injected with a viral vector carrying an inhibitory Designer Receptor Exclusively Activated by Designer Drug (DREADD) into the aPVT. Rats were then trained to self-administer heroin (0.1mg/kg/infusion) for 10 days, followed by a forced abstinence period of 16 days. During forced abstinence, rats were either sated or food restricted (FDR) to reach ~ 90% baseline body weight. On day 15 of food restriction, rats were either injected with J60 to chemogenetically activate the aPVT or with vehicle (VEH) and underwent a 3-hour heroin-seeking test under extinction conditions. Data are currently being analyzed. The findings will help us further understand the involvement of the aPVT in heron seeking in abstinent male and female rats.

Disclosures: **E.G. Ah-Yen:** None. **F. Wu:** None. **P. Rodriguez:** None. **V. Douan:** None. **C. Borges:** None. **U. Shalev:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.11/RR20

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA/NIAAA, NIH

Title: The glucagon-like peptide-1 (GLP-1) analogue semaglutide decreases fentanyl vapor self-administration in mice

Authors: *E. H. TRESSLER[1,2], V. P. ACOSTA[1,2], K. E. WHITING[1,2], G. F. KOOB[1], L. F. VENDRUSCOLO[1,2], L. LEGGIO[1,2];
[1]NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD; [2]Natl. Inst. on Alcohol Abuse and Alcoholism (NIAAA), Bethesda, MD

Abstract: GLP-1 stimulates insulin secretion, inhibits glucagon release, decreases appetite, and delays gastric emptying. GLP-1 receptors (GLP-1R) are expressed peripherally in areas such as the pancreas, small intestine, and vagus nerve and centrally within forebrain regions associated with reinforcement. Evidence supports a role for the GLP-1R in the reinforcing properties of addictive drugs (e.g., alcohol, stimulants, opioids, and nicotine). However, the effect of a GLP-1R analogue on opioid-related behaviors in opioid dependent rodents remains to be tested. The objective of this study was to test the effect of semaglutide, a long-lasting GLP-1R analogue, on

fentanyl vapor self-administration (SA) in opioid nondependent and dependent male and female C57BL/6J mice. Nondependent mice that were allowed short-access (ShA; 1 h/day sessions, n = 15, 8 F) exhibited stable fentanyl vapor SA, whereas dependent mice that were allowed long-access (LgA; 6 h/day sessions, n = 15, 8 F) escalated fentanyl vapor SA and showed signs of opioid dependence. Semaglutide decreased fentanyl vapor SA in the ShA group at a dose of 0.1 mg/kg and in the LgA group at doses of 0.05 mg/kg and 0.1 mg/kg. Semaglutide also decreased the mice motivation to obtain fentanyl in the ShA group at a dose of 0.1 mg/kg and in the LgA group at doses of 0.05 mg/kg and 0.1 mg/kg. We did not find sex differences in fentanyl vapor SA. Fentanyl injections increased locomotion in both ShA and LgA groups regardless of semaglutide treatment. This increase in locomotion in the LgA group was greater in males than females. In the ShA group, semaglutide decreased locomotion regardless of sex and fentanyl treatment. Semaglutide did not change opioid-induced analgesia or opioid withdrawal-induced hyperalgesia. These results suggest that semaglutide decreased fentanyl vapor SA in mice of both sexes with increased effects in opioid dependent mice without altering analgesia or withdrawal. These results support further investigation of GLP-1R agonism as a potential pharmacotherapy for the treatment of opioid use disorder.

Disclosures: E.H. Tressler: None. **V.P. Acosta:** None. **K.E. Whiting:** None. **G.F. Koob:** None. **L.F. Vendruscolo:** None. **L. Leggio:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.12/SS1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA007278
NIH Grant MH112355

Title: Investigating the neurobiological basis of persistent reward-seeking: a validated oral fentanyl self-administration task in mice.

Authors: *P. DAVIS[1], K. GIRVEN[1,2], K. MOTOVILOV[1], E. JUDD[3], A. SUKO[1], R. PALMITER[3,2], L. DE LECEA[4], L. ZWEIFEL[3,2], M. BRUCHAS[1,3,2];
[1]Dept. of Anesthesiol. & Pain Med., [2]Dept. of Pharmacol., [3]Program in Neurosci., Univ. of Washington, Seattle, WA; [4]Dept. of Psychiatry and Behavioral Sci., Univ. of Stanford, Stanford, CA

Abstract: Research demonstrates that substance-use disorder affects one's decision-making and reward processing resulting in impaired goal-directed behaviors. There has been a united effort to identify the neural correlates involved, as well as the systems-level processes that govern chronic opiate use disorders, a severe problem in the US. However, there remains a lack of understanding of the intrinsic neurobiology that underlies persistent reward-seeking despite value

changes that would normally alter goal-directed behavior. Addressing this gap in knowledge requires approaches that combine increased resolution with the measurement of behavioral phenotypes associated with persistent reward-seeking. This proposal seeks to establish an oral fentanyl self-administration task that can be combined with various techniques such as fiber photometry or used in a head-fixed setting for single-cell imaging using a two-photon microscope. During each session, a fixed ratio one (FR1) task is delivered to mice by way of an active nose poke with 10 seconds of access to a 10ug/ml fentanyl solution. It has been found that after a period of familiarization with the given task, mice will reliably self-administer fentanyl during each session. Now, in an effort to further validate the designed task, mice who consistently self-administer fentanyl during sessions will undergo a series of assays designed to measure common physiological and behavioral alterations caused by the intake of fentanyl including tail flick, rectal body temperature, open field locomotor assays, elevated zero maze anxiety assays, and sampling for blood opiate levels. These various assays will be collected after a brief period of FR1 fentanyl self-administration and compared to a naïve WT cohort in both male and female mice. Establishing a stringently-validated oral fentanyl self-administration task will further our understanding of the neurobiology underlying persistent reward-seeking behavior, addressing a critical knowledge gap in substance-use disorder research.

Disclosures: **P. Davis:** None. **K. Girven:** None. **K. Motovilov:** None. **E. Judd:** None. **A. Suko:** None. **R. Palmiter:** None. **L. de Lecea:** None. **L. Zweifel:** None. **M. Bruchas:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.13/SS2

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Drug Abuse DA048336

Title: Adolescent nicotine exposure escalates both seeking and taking of fentanyl and augments punishment resistance in a heterogenous seeking-taking chain schedule

Authors: *A. MUKHERJEE, D. D. LICHTE, E. A. GILLES-THOMAS, M. T. CRAIG, S. C. HONEYCUTT, H. KWOK, E. M. EICHNER, D. P. NINNASOPHA, G. C. LONEY; Behavioral Neurosci., Univ. at Buffalo, Buffalo, NY

Abstract: Overdose deaths resulting from synthetic opioids remain a significant public health concern. The vast majority of individuals in treatment for opioid use disorders (OUD) report concurrent nicotine use and the rate of OUDs in smokers has continued to increase while there is a trend for a levelling off of OUDs in non-smokers. Approximately 90% of adult smokers report that their nicotine use started in adolescence which represents a critical period for neural development. A wealth of preclinical work has shown that nicotine administration in preclinical models increases opioid intake and reduces sensitivity to the aversive effects of opioids. In the

present study, we investigated the effect of adolescent nicotine exposure (ANE) on self-administration of the long-acting synthetic opioid fentanyl HCl in adulthood in male and female Long-Evans rats. First, rats were exposed to systemic nicotine (0.4 mg/kg) twice a day for 10 days during adolescence. Next, upon reaching adulthood (PND 75), we trained these rats, in the absence of further nicotine administration, on a two-link heterogenous seeking-taking cycle to determine whether ANE affects either the appetitive seeking response or consummatory taking response for intravenous infusions of fentanyl (0.75 µg/kg/inf). Additionally, following acquisition of the seeking-taking operant, we implemented foot-shock punishment to examine the degree to which ANE results in punishment resistant self-administration of fentanyl that persists despite aversive consequences. We found that ANE significantly increased consumption of fentanyl across all phases of the experiment. Additionally, ANE rats had significantly higher seeking responses per min. Furthermore, these significant differences in fentanyl seeking and taking behaviors persisted following aversive footshock implementation contingent on fentanyl seeking. Overall, nicotine-treated rats showed greater self-administration of fentanyl and contingent footshock punishment was significantly less efficacious in limiting fentanyl seeking in ANE rats, compared to saline controls.

Disclosures: **A. Mukherjee:** None. **D.D. Lichte:** None. **E.A. Gilles-Thomas:** None. **M.T. Craig:** None. **S.C. Honeycutt:** None. **H. Kwok:** None. **E.M. Eichner:** None. **D.P. Ninnasopha:** None. **G.C. Loney:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.14/SS3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P50DA044121

Title: Long access to fentanyl vapor leads to escalation of consumption.

Authors: *M. CENTENO, L. PÉREZ-CERVERA, R. JABAKHANJI, L. FANG, J. COX, A. APKARIAN;
Northwestern Univ., Chicago, IL

Abstract: Fentanyl vaping (Moussawi et al, 2021) provides a powerful context for the study of opioid self-administration in chronic pain. It is well known that mice escalate heroin and fentanyl intake across 6h self-administration sessions to a greater extent than in short access paradigms (1h; Towers et al 2019). However, how extended access to fentanyl vapor self-administration leads to addiction-like behaviors in mice with pain has not been studied. To address this question, we investigated how fentanyl vapor self-administration for different durations affected drug motivation during training and following a period of abstinence. We also studied how the fentanyl intake affected the locomotion and anxiety of mice and considered the impact of sex.

Mice were placed in a chamber with two nosepokes, located on opposite walls: one active and another inactive. Importantly, active nosepokes resulted in fentanyl delivery of 1.5 seconds paired with a light cue presentation while inactive nose poking had no consequence. Mice were exposed to short-access (2h) fentanyl sessions for six days and then started a long-access (12h) for ten days. After that, they remained abstinent for 1 week and underwent a cue-seeking session. An open field assay was performed immediately following the last short access session, the last long access session, the cue-seeking session and on the following days. Compared to groups of mice exposed to only short access self-administration paradigms, our results show that duration of each session of fentanyl exposure affects opioid self-administration and drug seeking behavior. In particular, long-access improved both discrimination between active and inactive nosepokes compared to short-access and drug escalation. Additionally, males discriminate better between active and inactive nosepokes at the beginning of the long-access, with females having a later but steeper slope. Regarding mobility, mice were hyperactive at the end of fentanyl self-administration compared to one day later, and there were no differences in anxiety -defined as the amount of time spent in the periphery during the open field assay- after long-access compared to baseline. This suggests that the details of the self-administration protocol affect drug craving and that it is therefore important to take them into account when generating our preclinical models.

Disclosures: M. Centeno: None. **L. Pérez-Cervera:** None. **R. Jabakhanji:** None. **L. Fang:** None. **J. Cox:** None. **A. Apkarian:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.15/SS4

Topic: G.09. Drugs of Abuse and Addiction

Support: This Work was Supported by NIDA/NIH

Title: Effect of a Toll-Like Receptor 3 Antagonist on Heroin Related Behaviors in Mice

Authors: *H. MILLS[1], N. SAID[2], L. VENDRUSCOLO[2], G. KOOB[3];
[1]NIH/NIDA, Baltimore, MD; [2]NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD; [3]NIH, Natl. Inst. on Alcohol Abuse & Alcohol NIAAA, Bethesda, MD

Abstract: With more than 75,000 opioid overdose deaths in the U.S. between 2020 and 2021, understanding the biological mechanisms that contribute to opioid use disorder (OUD) is critical for the development of strategies of prevention and treatment of OUD. Although opioids are potent analgesics when taken acutely, chronic use results in the development of hyperalgesia during withdrawal, which may contribute to continued drug taking. Increasing evidence points to the role of the neuroimmune system in opioid dependence. Opioid use activates neuroimmune pathways that promote the expression of inflammatory mediators, which in turn can lead to

increased nociceptive states. Toll-Like-Receptor 3 (TLR3) is a component of one such pathway, and its potential role in opioid-related changes in nociceptive states remains unknown. In the present study, we utilized a novel TLR3 antagonist, CuCPT4a, to investigate the role of TLR3 in a mouse model of heroin-induced analgesia and hyperalgesia during spontaneous withdrawal. We also tested CuCPT4a on fentanyl vapor self-administration. First, we performed a pharmacokinetic study and determined that CuCPT4a crosses the blood-brain barrier and that its half-life is about 2 h. Acute administration of CuCPT4a had no effect on heroin-induced analgesia as assessed using a hotplate test. Similarly, an acute CuCPT4a administration did not cause an effect on thermal hyperalgesia during heroin withdrawal as measured by a coldplate test. Using a schedule of repeated CuCPT4a administration or continuous CuCPT4a administration via minipumps, we found no significant effects on hyperalgesia. Finally, in a preliminary experiment, acute administration of CuCPT4a demonstrated a trend in reducing progressive ratio responding for fentanyl in a prolonged access fentanyl self-administration procedure. Ongoing work is focused on replicating these findings, as well as exploring potential mechanisms and molecular markers of these effects.

Disclosures: **H. Mills:** None. **N. Said:** None. **L. Vendruscolo:** None. **G. Koob:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.16/SS5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant NIDA R21DA052568
NIH Grant T32 MH115886
University of Minnesota's MNDrive (Minnesota's Discovery, Research, and Innovation Economy) Initiative Fellowship for 2023-2024

Title: The effects of electrical stimulation on reinstatement of morphine conditioned place preference in rats

Authors: *L. DAVIS[1], F. IACOBUCCI[2], M. ESGUERRA[3], M. THOMAS[2], A. S. WIDGE[2]; [1]Psychiatry, [2]Univ. of Minnesota, Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Minnesota Dept. of Neurosci., Univ. of Minnesota Dept. of Neurosci., Minneapolis, MN

Abstract: Relapse is a critical phase of the addiction cycle. Drugs of abuse result in potent and persistent synaptic plasticity that perpetuates drug reseeking behavior. Corticostriatal pathways are affected by and contribute to the addiction cycle, predominantly through their associations with reward and craving. In mouse models, direct manipulation of these pathways through the use of optogenetics has removed drug reseeking after chronic exposure to morphine. However, optogenetics cannot be directly translated to use in humans. Electrical stimulation is safe to use in humans and poses high translational potential from animal models. The purpose of this study

was to determine whether electrical stimulation of the IL could replicate the results from optogenetic stimulation. Four long-evans rats (2M, 2F, 350-550 g) were implanted with a single stimulating electrode in the IL and a recording electrode within the nucleus accumbens shell (NAcshell). The rats then ran through a ~2.5 week CPP protocol where they were conditioned to morphine. Prior to drug reinstatement, the IL was stimulated. The outcome measures were the input/output evoked response potential curves and video behavioral data. Repeated measures ANOVA used to determine significance. We anticipate that the stimulation will cause long-term depression of the IL-NAcshell pathway and this will cause a reversal of drug re-seeking behavior.

Disclosures: L. Davis: None. **F. Iacobucci:** None. **M. Esguerra:** None. **M. Thomas:** None. **A.S. Widge:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.17/SS6

Topic: G.09. Drugs of Abuse and Addiction

Title: Involvement of the norepinephrine system in oxycodone-motivated behaviors

Authors: A. TAYLOR[1], A. AGARWAL[1], T. BUCK[1], A. MOORE[1], M. CRISSMAN[1], S. PARI[1], G.-H. BI[1], G. TREISMAN[2], Z.-B. YOU[1], *E. GARDNER[1];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Psychiatry and Behavioral Sci., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Clonidine, an alpha-2 noradrenergic receptor agonist, significantly attenuates stress-induced relapse to self-administration of heroin and psychostimulants in laboratory animals and is frequently used to treat acute opioid withdrawal syndromes in the clinical environment. The role of clonidine in oxycodone reward has not been explored preclinically. We show in the present study that pretreatment with clonidine (0, 25, 50 µg/kg, i.p.) dose-dependently reduced oxycodone self-administration tested under either 0.1 or 0.025 mg/kg/infusion. Clonidine pretreatment also significantly decreased the breakpoint for oxycodone self-administration tested under progressive ratio reinforcement. Clonidine pretreatment (0, 5, 25, 50 µg/kg, i.p.) significantly accelerated the extinction of oxycodone-seeking behavior in laboratory rats (saline substitution for oxycodone). Coadministration of clonidine (50 µg/kg) with oxycodone (1 mg/kg) significantly potentiated and prolonged oxycodone's antinociceptive effects in a tail flick test. Clonidine pretreatment (0, 5, 25, 50, 100 µg/kg, i.p.) inhibited brain stimulation reward maintained by optogenetic stimulation of dopamine neurons in the ventral tegmental area only at the highest dose in DAT-Cre mice. Clonidine (0, 25, 50 µg/kg, i.p.) did not produce either conditioned place preference (CPP) or conditioned place aversion (CPA) in either oxycodone-naïve rats or in oxycodone self-administration trained rats that were exposed to CPP/CPA testing immediately following the completion of self-administration (acute withdrawal). Daily clonidine

pretreatment (5, 25 µg/kg, i.p.) significantly accelerated extinction of drug-seeking behaviors in oxycodone trained rats. The lack of clonidine-induced CPP effects seen in the present study indicates that clonidine lacks intrinsic rewarding properties. Clonidine reduced opioid withdrawal symptoms in the present animal experiments, congruent with current use for treating opioid withdrawal in human patients. Thus, our findings suggest that clonidine may be an effective agent to reduce opioid use and motivation for opioid use, without altering opioid analgesia.

Disclosures: **A. Taylor:** None. **A. Agarwal:** None. **T. Buck:** None. **A. Moore:** None. **M. Crissman:** None. **S. Pari:** None. **G. Bi:** None. **G. Treisman:** None. **Z. You:** None. **E. Gardner:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.18/SS7

Topic: G.09. Drugs of Abuse and Addiction

Title: Fentanyl and morphine cross-sensitization in adult and adolescent rats

Authors: D. L. SANCHEZ, A. MARTINEZ, M. AROCHE, J. A. TAYLOR, A. LUJAN, *C. CRAWFORD;
Psychology, California State University, San Bernardino, San Bernardino, CA

Abstract: Behavioral sensitization, which is manifested as an augmented behavioral response to repeated drug treatment, is a useful procedure for studying differences in the addictive potential of psychoactive compounds. Because of the rapid increase in the misuse of synthetic opioids, we recently assessed the abuse liability of fentanyl in adult and adolescent rats using a one-trial behavioral sensitization paradigm. Interestingly, while fentanyl does induce an enhanced behavioral response after prior exposure similar to other abused drugs (i.e, morphine, cocaine, amphetamine), it also causes a substantial suppressive effect on locomotor activity on initial administration. This reduction in activity makes the augmented behavioral response with repeated administration difficult to interpret. The goal of the present study, therefore, was to clarify whether fentanyl produces behavioral sensitization by assessing whether fentanyl would cross sensitize with another mu agonist known to induce a sensitized response. Thus, we evaluated whether prior exposure to fentanyl would cause an enhanced locomotor response in morphine-treated rats. In this experiment, adult and adolescent male and female rats were injected once with fentanyl (0 or 200 µg/kg, sc) and placed immediately in locomotor activity chambers for 60 min. After a 48-h abstinence period, all rats were injected with fentanyl (100 µg/kg, sc) or morphine (5 mg/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 fentanyl injected rats 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. This increase in activity was found for rats treated with fentanyl or morphine on the test day; however, sex did modify this increase. Specifically, female rats treated with fentanyl on the pre-exposure day exhibited greater levels of activity when treated with morphine than fentanyl on the test day, while morphine and fentanyl produced similar levels of activity in fentanyl pretreated male rats. Regardless of pretreatment conditions, adult female rats exhibited more activity than male rats on the test day. Our results suggest that despite the activity suppressing effects of fentanyl, repeated exposure leads to behavioral sensitization, consistent with its high rate of abuse.

Disclosures: D.L. Sanchez: None. **A. Martinez:** None. **M. Aroche:** None. **J.A. Taylor:** None. **A. Lujan:** None. **C. Crawford:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.19/SS8

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Discovery grant
Concordia University Bridge Fund

Title: Brain areas involved in acute food deprivation-induced heroin seeking test, after punishment-imposed abstinence, in male rats.

Authors: *C. BORGES, B. GIULIANO, E. G. AH-YEN, J. L. CHARLES, U. SHALEV; Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: The biggest challenge in the treatment of drug addiction is preventing relapse. One of the reasons for lacking a reliable treatment for relapse is the incomplete understanding of the neuronal mechanisms underlying it. A common trigger to relapse is stress, and an environmental stressor in addicts is acute food deprivation. Addicts often choose to allocate money and time towards drugs over food. Relapse to the drug often happens after a period of voluntary abstinence in which addicts choose to abstain from drug use due to the associated negative consequences. Using our recently developed animal model of food deprivation stress-induced relapse to heroin after punishment-imposed abstinence, we aim to identify the brain regions involved in stress-induced relapse after voluntary abstinence. Rats (male Long Evans, N=30) underwent heroin self-administration training (0.1 mg/kg/infusion) for 2 weeks using the seek-take chain procedure (i.e., completing a VI60 schedule of reinforcement on the seek lever allowed access the drug-paired lever - take lever, under FR1). After self-administration, rats went through 4 days of punishment-imposed abstinence, in which 30% of the completed seek links resulted in a mild footshock instead of access to the take lever. The footshock lasted 0.5 s and it gradually increased at 0.1 mA/day from 0.2 to 0.4 mA and remained fixed at 0.4 mA for one more day. Before heroin-seeking tests, rats were exposed to a 24 h food deprivation (FD) or

Sated period. Tests were done under extinction conditions for 1 h. Brains were collected during self-administration (Baseline, N=6), punishment (N=4), FD/sated day without test (FD: N=3, Sated: N=3) and FD-induced heroin seeking tests (FD: N=7, Sated: N=7). Fos expression, indicating neuronal activation, was assessed through immunohistochemistry, focusing on brain areas related to stress [central nucleus of the amygdala (CeA), basolateral amygdala (BLA), bed nucleus of the stria terminalis (BNST)], reward [prefrontal cortex (PFC), nucleus accumbens (NAc)], and feeding and conflict [paraventricular nucleus of the thalamus (PVT)]. Data analysis is in progress.

Disclosures: C. Borges: None. **B. Giuliano:** None. **E.G. Ah-Yen:** None. **J.L. Charles:** None. **U. Shalev:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.20/SS9

Topic: G.09. Drugs of Abuse and Addiction

Support: NARSAD YIA 26771
Tufts Substance Use Initiative
Tufts Seed Grant

Title: Micro RNA regulation of genes related to wnt signaling in the nucleus accumbens in the development of oxycodone addiction-like behaviors in rats

Authors: D. OLIVER[1], K. E. BUDGE[2], S. ISGATE[3], E. BYRNES[4], ***F. M. VASSOLER**[5]; [1]HSS, New York, NY; [2]Cummings Sch. of Vet. Med. at Tufts Univ., Grafton, MA; [3]s, Grafton, MA; [4]Tufts Univ. Cummings Sch. Vet Med., North Grafton, MA; [5]Tufts Univ. Cummings Sch. of Vet. Med., North Grafton, MA

Abstract: Opioid addiction is a lifelong disease resulting from long-lasting molecular and behavioral changes. Micro RNAs (miRNAs) are key modulators of gene regulation and are known to be involved in substance use disorder and addiction. It is the goal of the present studies to measure miRNA expression within the nucleus accumbens (NAc) and correlate those expression changes with mRNA changes in the same region in rats that were trained to self-administer oxycodone to determine miRNA and mRNA targets that may be implicated in the development of addiction-like behaviors. To develop targets, male rats were trained to self-administer oxycodone (0.1 mg/kg/infusion, i.v.) during 2h daily sessions for 15 days. Each animal had a yoked saline control animal that received a saline infusion every time the leader received oxycodone. All animals were euthanized 1 hour following the last self-administration session. Total RNA was extracted from the NAc. Both miRNA and mRNA were sequenced from the NAc and computational analysis was utilized to determine upregulated miRNAs and putative downregulated mRNA targets and downregulated miRNAs and putative upregulated mRNA

targets. Several miRNAs (miR-182, miR320) were identified as well as mRNA targets related to wnt signaling (Wnt-3, Tle4, Cacna2D, PIPPR4, Pou313). These targets were tested using qrtPCR in both the short access animals as well as in a separate group of animals trained utilizing a long-access paradigm that more closely resembles addiction-like behaviors. These long-access animals naturally separated into two groups: high and low responders. Preliminary evidence suggests that wnt signaling and genes related to neuroplasticity are related to miRNA expression and may be involved in the development of addiction-like behaviors.

Disclosures: D. Oliver: None. **K.E. Budge:** None. **S. Isgate:** None. **E. Byrnes:** None. **F.M. Vassoler:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.21/SS10

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA025634-13
NIH-NIAAA T32AA026577

Title: Dose-dependent increase in VTA dopamine neural activity by acute morphine administration

Authors: *R. M. DONKA[1], M. LOH[2], M. F. ROITMAN[3], J. D. ROITMAN[3];
[1]Psychology, Univ. of Illinios, Chicago, Chicago, IL; [3]Psychology, [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: In the United States, opioid abuse remains a significant burden to public health despite reduction efforts in part due to their highly addictive nature. The potent reinforcing effects of opioids contribute to the transition from initial use to dependence. Withdrawal from persistent use is accompanied by a behavioral reward deficit or anhedonia, often resulting in increased drug seeking and relapse. Morphine modulates the mesolimbic dopamine system, a critical pathway for reward processing and substance use disorders, however the temporal dynamics of dopaminergic cell activity are not well defined. Here, we measured ventral tegmental area (VTA) dopamine neural activity in response to acute morphine treatment using *in vivo* fiber photometry. To measure activity of dopamine neurons in the VTA, a Cre-dependent GcAMP6f was injected and a fiber optic cannula was implanted in the VTA (AP −5.4, ML−0.7, DV −8.15 mm relative to bregma) of Long Evans rats (TH:Cre^{+}; N = 12). Experimentation began four weeks post-surgery to allow for recovery and viral expression. We conducted two recording sessions per week across four consecutive weeks. Each week, a control session occurred the day prior to the session with morphine exposure. Each session consisted of a 15-minute baseline and 60-minute post injection recording period. One dose of morphine was administered each week in escalating order (2.5, 5.0, 7.5, and 10 mg/kg, i.p.). Calcium transient analysis was conducted to identify

changes in the frequency, amplitude, and half-width of transients relative to control sessions and between doses. Data were normalized to the pre injection baseline, and peaks were detected using a criterion of a 3 SD increase above the local minimum value. Overall, all doses of morphine resulted in a significant increase in the frequency and amplitude of transients relative to saline control and across escalating doses. With the lowest dose of 2.5 mg/kg, the increase in frequency and amplitude diminished by 45 minutes post injection, but no decrease was observed in the higher doses. These experiments demonstrate with high temporal precision the dynamic changes exhibited by VTA dopamine neurons in response to escalating doses of morphine. Future studies will seek to elucidate shifts in response to chronic morphine administration and subsequent withdrawal which may help define neural processes underlying both the rewarding effects of opioid use with emphasis on negative affective aspects of withdrawal.

Disclosures: R.M. Donka: None. **M. Loh:** None. **M.F. Roitman:** None. **J.D. Roitman:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.22/SS11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 1R01AA029674

Title: Effects of novel β-lactam, MC100093, in respiratory exchange in hydrocodone overdosed mouse model using comprehensive laboratory animal monitoring systems

Authors: W. WONG[1], M. ABOUGHARBIA[2], W. CHILDERS[3], *Y. SARI[4];
[1]Pharmacol. and Exptl. Therapeut., Univ. of Toledo, Toledo, OH; [2]Dept. of Pharmaceut. Sci., [3]Pharmaceut. Sci., Temple Univ., Philadelphia, PA; [4]Pharmacol. and Exptl. Therapeut., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

Abstract: Opioid overdose has been a major health problem in the United States due to their misuse and abuse. Studies from our lab and others demonstrated that chronic exposure to opioids alter glutamate transport and transmission. Recent study from our laboratory showed that treatment with a novel β-lactam, MC-100093, reduced ethanol consumption and normalized the expression of a major glutamate transporter 1 (GLT-1) in reward brain regions. In the current study, we investigated the effects of MC-100093 and ceftriaxone in mice exposed to escalating doses of hydrocodone using comprehensive laboratory animal monitoring systems (CLAMs) to measure mice's respiratory frequency, locomotor activity, O_2 consumption, and CO_2 production. Male C57BL/6 mice (8 weeks) were injected with hydrocodone (20 mg/kg, i.p.) every other day for 13 days, and on day 15, mice received a higher dose of hydrocodone (40 mg /kg, i.p.). Control group received saline i.p. injection every other day. MC-100093 group received similar hydrocodone dosing, and mice received MC-100093 (50 mg/kg, i.p.) for the last seven days or ceftriaxone (200 mg/kg, i.p.) for last five days of this study. Data analyses revealed that exposure

to escalating doses of hydrocodone was associated with increased O_2 consumption and CO_2 production, and these effects were attenuated by MC-100093 and ceftriaxone treatment. Importantly, MC-100093 was more effective in attenuating hydrocodone-induced reduction in respiratory exchange ratio . Furthermore, ceftriaxone treatment reduced hydrocodone-induced hyperactivity in mice. These findings suggest that MC-100093 and ceftriaxone might be a therapeutic drug for attenuating hydrocodone-induced respiratory alteration.

Disclosures: **W. Wong:** None. **M. AbouGharbia:** None. **W. Childers:** None. **Y. Sari:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.23/SS12

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA035943
F32 DA054767

Title: Prolonged history of fentanyl reinforcement leads to habitual decision-making in rats

Authors: *E. GARR[1], Y. CHENG[2], P. H. JANAK[2];
[2]Johns Hopkins Univ., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Drug-seeking behaviors are colloquially referred to as “habits”. The term “habit” has a specific definition which means an action that is insensitive to its future consequences. This definition has found an application in drug addiction research, but there are several limitations. One is that the effect of drugs on habit formation is usually measured in the context of seeking nondrug rewards, which precludes the study of a drug habit per se. It is thus uncertain whether a drug-procuring action would develop into a habit. The other limitation is that no study to date has examined the effect of exogeneous opioids on habit formation, even though opioids account for most overdose deaths in the United States. Both issues are problematic for the translational validity of the habit model of drug seeking. To determine whether an action reinforced with opioid reward shows habitual control, Long Evans rats (4 male, 4 female) were trained to earn liquid fentanyl rewards while thirsty. Rats were first trained to lever press for fentanyl (0.05 ml, 50 µg/ml) on a fixed ratio 1 schedule. Responding and intake increased across sessions. Next, rats were trained to press for either fentanyl or 10% sucrose on a fixed ratio 5 schedule after IP injections of naltrexone (0.1 mg/kg) or saline. Naltrexone injections increased lever pressing for fentanyl, but there was no effect on pressing for sucrose (injection x reward interaction, $p = .03$). These results suggest that liquid fentanyl activated opioid receptors and was reinforcing. Next, rats were trained to perform a two-step task to earn fentanyl (0.05 ml, 25 µg/ml). The two-step task can diagnose the degree of habitual and goal-directed control by determining whether actions are controlled by an anticipation of future consequences or just the long-run estimate of reward without regard for the route connecting action and reward. Rats were trained on the task

for 32 sessions, after which they earned fentanyl or sucrose rewards in alternating sessions. Sucrose sessions took place in a context with altered scent and floor texture. An analysis of the stay probabilities—the probability of repeating an action as a function of the previous trial's outcome (reward or omission) and transition type (common or rare)—indicated that choice behavior was habitual for both reward types (main effect of trial outcome, $p = .023$). Overall, these results indicate that a prolonged history of fentanyl reinforcement encourages habitual choice for both fentanyl and food rewards.

Disclosures: **E. Garr:** None. **Y. Cheng:** None. **P.H. Janak:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.24/SS13

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA037897
T32 DA028874
F31 DA058451

Title: A novel role for interpeduncular GLP-1Rs and projections to the laterodorsal tegmental nucleus in the reinstatement of fentanyl-seeking behavior

Authors: *R. HERMAN[1], V. CHINAKA[1], A. POTHIKAMJORN[1], H. D. SCHMIDT[2]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania Sch. of Med., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: The interpeduncular nucleus (IPN) densely expresses mu opioid receptors (MORs) and regulates mesolimbic reward signaling. While emerging studies indicate that the IPN plays an important role in opioid-related behaviors including withdrawal, its role in opioid seeking has not been explored. Given that the IPN also expresses glucagon-like peptide 1 receptors (GLP-1Rs) at high levels, we hypothesized that activation of GLP-1Rs in the IPN would attenuate fentanyl reinstatement. We trained male and female rats to self-administer intravenous fentanyl (1.25 μg/kg/infusion) for 21 days. Once fentanyl taking was extinguished, rats were pretreated with intra-IPN infusions of vehicle or the GLP-1R agonist Ex-4 (0.01 or 0.1 μg) before drug- + cue-induced reinstatement tests. We showed that intra-IPN infusions of Ex-4 dose-dependently decreased drug- + cue-induced reinstatement of fentanyl seeking at doses that did not affect body weight, chow intake, or pica. Additionally, we found that GLP-1Rs and MORs are both expressed on GABAergic IPN neurons that project to the laterodorsal tegmental nucleus (LDTg). Given that GLP-1Rs are excitatory, these results support a circuit whereby IPN GLP-1R activation attenuates fentanyl reinstatement by activating GABAergic IPN projections to the LDTg. Therefore, we hypothesized that chemogenetic activation of the IPN->LDTg pathway would be sufficient to attenuate fentanyl reinstatement. We bilaterally infused an AAV

expressing the neural activating DREADD hM3D(Gq) into the IPN and implanted a guide cannula directly above the LDTg to selectively activate the IPN->LDTg projection during reinstatement tests. We showed that intra-LDTg infusions of clozapine-N-oxide (CNO) attenuated fentanyl seeking in rats infused with an hM3D(Gq)-expressing virus but not mCherry controls, confirming a role of the IPN->LDTg projection in fentanyl-seeking behavior. We are currently using an AAV expressing the Cre-dependent inhibitory DREADD hM4D(Gi) in GAD:Cre rats to examine the effects of cell-type specific inhibition of the GABAergic IPN->LDTg projection on fentanyl reinstatement and Ex-4 efficacy. We are also investigating the effects of IPN GLP-1R activation and chemogenetic activation of the IPN->LDTg pathway on measures of aversion during fentanyl abstinence to further characterize the mechanisms by which these manipulations may impact fentanyl-seeking behavior. This work identifies a novel pathway underlying fentanyl-seeking behavior, provides new insight into the complex role of the IPN in regulating reward and aversion, and supports the use of GLP-1R agonists as a potential treatment for fentanyl use disorder.

Disclosures: **R. Herman:** None. **V. Chinaka:** None. **A. Pothikamjorn:** None. **H.D. Schmidt:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.25/SS14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Diversity Supplement Grant
NIH DA036657

Title: The Role of D1 and D2 Medium Spiny Neurons During Hippocampal Replay Events in a Drug-Context Association Model

Authors: *A. FRYC[1], K. CLEMENZA[2], L. L. SJULSON[3];
[1]Albert Einstein Col. of Med., The Bronx, NY; [2]Neurosci., [3]Albert Einstein Col. of Med., Bronx, NY

Abstract: The opioid epidemic is a long standing public health crisis recently exacerbated by COVID-19, and was last reported to kill over 80,000 people in the United States. An obstacle to overcoming the epidemic are high relapse rates associated with opioid use disorder. Drug-context associations act as triggers for relapse, and are thought to be reconsolidated, or maintained, with every re-exposure. D1 and D2 medium spiny neurons (MSNs) in the nucleus accumbens are known to update and encode the reward value associated with a context. This has been shown to occur during hippocampal CA1 “replay” events, which are known to encode spatial contexts. A well established relapse model used to investigate drug-context associations is the Conditioned Place Preference (CPP) paradigm. Here, we aim to simultaneously record

hippocampal CA1 replay events and D1 and D2 MSN activity during the acquisition, extinction, and reinstatement phases of CPP. The results of this study will provide clarity on the progression of the reward value associated with an originally neutral spatial context throughout these phases, and distinguish between the roles of this circuit during learning and performance of reward related behavior.

Disclosures: A. Fryc: None. **K. Clemenza:** None. **L.L. Sjulson:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.26/SS15

Topic: G.09. Drugs of Abuse and Addiction

Support: Departmental startup

Title: Role of projections from orbitofrontal cortex to dorsal striatum in incubation of oxycodone craving

Authors: *H. LIN[1], A. OLANIRAN[3], S. GARMCHI[1], X. LI[2];
[2]Univ. of Maryland Col. Park, [1]Univ. of Maryland, College Park, MD; [3]Psychology, Univ. of Maryland, Col. Park Neurosci. and Cognitive Sci. Program, College Park, MD

Abstract: Prescription opioids are the main driver of opioid epidemic that involves drug misuse, addiction, and even overdose death. The high relapse rate is a major challenge in treating drug addiction, including oxycodone. In rats, oxycodone seeking progressively increases during abstinence and maintains for an extended period, a phenomenon termed incubation of oxycodone craving. We previously found that the orbitofrontal cortex (OFC) plays a causal role in this incubation. Here, we aimed to identify critical downstream regions of OFC in incubation of oxycodone craving by focusing on the central to medial portion of the dorsal striatum (DS), based on previous anatomical evidence. In Exp.1, we first injected fluorescence-conjugated cholera toxin subunit B (CTb-555) into DS. Next, we trained male Sprague-Dawley rats to self-administer oxycodone (0.1 mg/kg/infusion, 6 h/d) for 10 days. We then either tested (Seeking-test) or did not test (No-test) rats for oxycodone seeking on abstinence day 15. Immediately after the test, we perfused the rats for immunohistochemistry to label Fos (a neural activity marker) in OFC. We found that the number of Fos + CTb double-labeled cells in OFC was significantly higher in Seeking-test group than No-test group on abstinence day 15. Based on these results, in Exp.2, we assessed the effect of pharmacological inactivation of DS on incubated oxycodone seeking. We found that injections of SCH23390 (0.75 µg/0.5 µl/side) into DS significantly decreased oxycodone seeking on abstinence day 15. In Exp.3, we used an anatomical disconnection procedure (muscimol + baclofen, 50 + 50 ng/ 0.5 µl/side in OFC; SCH23390, 0.75 µg in 0.5 µl/side in DS) to examine the causal role of OFC◊DS projections in incubated oxycodone seeking. We found that contralateral disconnections of OFC◊DS projections

significantly decreased oxycodone seeking on abstinence day 15. Taken together, our data showed that the activation of OFC◊DS projections was associated with oxycodone seeking on abstinence day 15, and both DS and OFC◊DS projections play critical roles in incubated oxycodone seeking. Ongoing studies are assessing the effect of ipsilateral disconnections on incubated oxycodone seeking and whether the role of OFC◊DS projections in oxycodone seeking is time-dependent during abstinence.

Disclosures: H. Lin: None. **A. Olaniran:** None. **S. Garmchi:** None. **X. Li:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.27/SS16

Topic: G.09. Drugs of Abuse and Addiction

Title: Xylazine reduces intravenous fentanyl consumption and induces a sex-specific withdrawal syndrome that is not altered by naloxone in rats

Authors: *S. KHATRI[1], S. SADEK[1], P. T. KENDRICK, Jr[1], M. HONG[2], J. BECKMANN[3], T. E. PRISINZANO[1], K. DUNN[5], C. D. GIPSON[4];
[2]Dept. of Pharmacol. and Nutritional Sci., [3]Psychology, [4]Pharmacol. and Nutritional Sci., [1]Univ. of Kentucky, Lexington, KY; [5]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Opioid use disorder is a leading public health crisis in the US. The recent surge in overdose cases is attributed to the rise of fentanyl use, a synthetic μ-opioid receptor agonist. Urine screens of fentanyl using individuals were positive for a veterinary anesthetic, xylazine. Individuals using xylazine with fentanyl report that xylazine prolongs the “high” of fentanyl as well as the onset of fentanyl withdrawal, and display naloxone resistance. To date, there is no preclinical model of fentanyl self-administration (SA) with xylazine exposure. Thus, here we established a rat model of fentanyl/xylazine co-SA and withdrawal. Male and female Long Evans rats underwent fentanyl (2.5 μg/kg/infusion, FR1, 2 hrs) or saline SA for 10 sessions, followed by an additional 8 session of pretreatment with xylazine (2.5 mg/kg, i.p.) or vehicle (saline). In a separate cohort, fentanyl was adulterated with increasing doses of xylazine (3 sessions each of 0.05. 0.10, and 0.5 mg/kg/infusion; intravenous xylazine dose was determined based on a preliminary pharmacokinetics study). Somatic signs of withdrawal were then evaluated with or without naloxone treatment (0.1 mg/kg, SC). Lastly, a cohort underwent chronic xylazine treatment (same i.p. doses), and somatic signs of withdrawal were evaluated post-xylazine or saline pretreatment. All rats acquired fentanyl SA. Fentanyl consumption was decreased in animals receiving experimenter-delivered xylazine as compared to vehicle. Intravenous xylazine dose-dependently reduced fentanyl consumption compared to fentanyl SA alone, which was more robust in females than males. Naloxone did not increase somatic signs of fentanyl withdrawal in either sex; however, somatic signs of withdrawal were higher across timepoints in

females following xylazine/fentanyl co-SA as compared to females following fentanyl SA alone. Xylazine exposure alone also induced a withdrawal syndrome only in females. Together, adulteration of intravenous fentanyl with xylazine dose-dependently suppressed fentanyl consumption in both sexes, and induced a unique withdrawal syndrome in females which was not altered by acute naloxone administration. These studies lay a foundation upon which neurobiological studies can evaluate mechanisms of xylazine/fentanyl co-use.

Disclosures: **S. Khatri:** None. **S. Sadek:** None. **P.T. Kendrick:** None. **M. Hong:** None. **J. Beckmann:** None. **T.E. Prisinzano:** None. **K. Dunn:** None. **C.D. Gipson:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.28/SS17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA25267
NIDA Grant DA048353

Title: Exploring the Potential for Abuse Liability and Respiratory Depression Induced by Mitragynine and 7-Hydroxymitragynine in Rats

Authors: *J. D. ZUARTH GONZALEZ[1], A. K. RAGSDALE[1], S. MUKHOPADHYAY[2], C. R. MCCURDY[2], L. R. MCMAHON[1], J. L. WILKERSON[1];
[1]Texas Tech. Univ. Hlth. Sci. Ctr., Amarillo, TX; [2]Univ. of Florida, Gainesville, FL

Abstract: Kratom (*Mitragyna speciosa*) is a natural product widely available in most US states with little to no regulation. Many use kratom to self-treat pain and opioid dependence, yet scientific evidence is lagging. Between 2011 and 2017, there were 1807 kratom exposures reported to poison control centers in the United States. Out of these, 65% occurred in the last two years (2016 and 2017), and 51.9% resulted in a serious medical outcome. Given that 40+ alkaloids are present within kratom, there is a pressing need to identify and further evaluate the safety of the active components in the plants. Among these alkaloids, mitragynine is the most prevalent in the plant, while 7-hydroxymitragynine, a minor alkaloid with a strong affinity for the mu-opioid receptor, has also been identified. This study aims to evaluate both the potential for abuse and the impact on respiratory parameters of mitragynine and 7-hydroxymitragynine. In jugular catheter-implanted male and female Sprague Dawley rats, intravenous (iv) self-administration for the opioid agonist remifentanil (1 µg/kg/infusion) was established, and animals were trained on a multi-component paradigm in which different doses were available at each component. Once remifentanil responding was stable, remifentanil was substituted for the test compounds (mitragynine, 7-hydroxymitragynine, and fentanyl). Respiratory experiments were conducted in a separate cohort of males and females with jugular catheters, using a within-subjects design, and included a 7-day drug washout period between test sessions. Respiratory

frequency, tidal volume, and minute ventilation were measured pre-and post-drug administration using whole-body plethysmography in unrestrained animals. When mitragynine was substituted for remifentanil, mitragynine suppressed remifentanil-associated lever responses. However, either 7-hydroxymitragynine or fentanyl substitution maintained responding on the remifentanil-associated lever. This same profile was observed in respiration experiments in which mitragynine failed to produce respiratory depression, but 7-hydroxymitragynine resulted in respiratory depression similar to fentanyl. These results align with in-vitro and in-vivo literature, indicating mitragynine has low abuse potential and does not result in respiratory depression. However, 7-hydroxymitragynine may be subject to abuse liability and respiratory depression.

Disclosures: J.D. Zuarth Gonzalez: None. **A.K. Ragsdale:** None. **S. Mukhopadhyay:** None. **C.R. McCurdy:** None. **L.R. McMahon:** None. **J.L. Wilkerson:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.29/SS18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant U01DA045300
NIDA Grant T32DA007288-30

Title: Multi-symptomatic approach reveals distinct behavioral profiles and neuronal correlates of heroin vulnerability versus resiliency

Authors: *B. KUHN[1], N. CANNELLA[2], A. T. CROW[1], V. LUNERTI[3], C. ALLEN[4], S. WALTERHOUSE[1], R. CHALHOUB[1], A. GUPTA[4], G. HARDIMAN[5], L. SOLBERG WOODS[6], D. CHUNG[4], R. CICCOCIOPPO[3], P. W. KALIVAS[7];
[1]Med. Univ. of South Carolina, Charleston, SC; [2]INSERM U1215, Bordeaux Cedex, France; [3]Univ. Camerino, Camerino, Italy; [4]Ohio State Univ., Columbus, OH; [5]Queen's Univ. Belfast, Belfast, Ireland; [6]Psychiatry, Wake Forest Univ., Winston-Salem, NC; [7]Dept Physiol, Med. Univ. S Carolina, Charleston, SC

Abstract: There has been a significant rise in opioid use disorder (OUD) worldwide, making it imperative to gain a better understanding of the behavioral and neurobiological traits underlying vulnerability and resiliency. Current rodent models focus on how one or few traits interact in a linear manner to predict substance use disorder, however, OUD consists of several symptoms that interact with one another and can vary across individuals. In this study, male and female heterogeneous stock rats (n=926) were assessed across several measures of heroin taking, refraining and seeking behaviors, and using a Bayesian stochastic block model (SBM) separated into OUD vulnerable, resilient, and intermediate subpopulation. Relative to resilient rats, vulnerable rats exhibit potentiated compulsive heroin-taking behavior following forced abstinence, withdrawal-induced ultrasonic vocalizations and heroin-taking in the presence of an

adverse stimuli. Furthermore, using a hierarchical analysis, vulnerable rats are comprised of distinct sub-clusters, exhibiting heterogeneity in salient traits conferring overall vulnerability with differences between male and female rats. Phenotypes also engage distinct neural circuitry following cued-reinstatement, with sexual dimorphism present within the vulnerable subpopulation. Together, these findings highlight distinct behavioral and neuronal adaptations associated with OUD vulnerability and resiliency using a model akin to human OUD. Current analyses are focused on assessing genetic and epigenetic differences between these distinct subpopulations

Disclosures: **B. Kuhn:** None. **N. Cannella:** None. **A.T. Crow:** None. **V. Lunerti:** None. **C. Allen:** None. **S. Walterhouse:** None. **R. Chalhoub:** None. **A. Gupta:** None. **G. Hardiman:** None. **L. Solberg Woods:** None. **D. Chung:** None. **R. Ciccocioppo:** None. **P.W. Kalivas:** None.

Poster

PSTR498. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR498.30/SS19

Topic: G.09. Drugs of Abuse and Addiction

Support: 5F31DA053774-02

Title: A novel gene therapy-based approach for treating opioid use disorder

Authors: *K. CLEMENZA[1], A. FRYC[1], L. L. SJULSON[2];
[1]Neurosci., [2]Neuroscience, Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Opioid use disorder is a debilitating condition that poses a substantial, and growing, public health burden. Although effective medications exist, they typically require strict lifelong adherence, which is unrealistic for many people suffering from this disorder. There is thus an urgent need to develop new interventions that confer long-term protection from a short-term administration. To this end, we have developed a novel gene therapy-based strategy using a mutant Low-Affinity Mu Opioid Receptor (LAMuOR) that can be conceptualized as an inhibitory DREADD (designer receptor exclusively activated by designer drugs) that is activated by high-affinity exogenous opioids of abuse. We use adeno-associated viral vectors (AAVs) to express LAMuOR in dopaminergic neurons of the ventral tegmental area in mice to test the hypothesis that LAMuOR suppresses opioid-induced dopamine release and opioid use disorder-related behaviors. We find that LAMuOR-expressing mice exhibit reductions in fentanyl-induced dopamine release in the nucleus accumbens, with the highest LAMuOR-AAV dose group exhibiting suppression below baseline levels. Alternatively, LAMuOR expression was not found to impact cocaine-evoked dopamine elevations in most AAV dose groups, indicating that baseline dopamine signaling remains intact in those groups. LAMuOR-expressing mice also exhibited reductions in fentanyl-induced hyperlocomotion, while opioid drug-independent

activities like drug-naive and cocaine-induced open field locomotion and sucrose preference were unaffected. Further, preliminary results indicate that mice in the highest LAMuOR-AAV group engage in significantly reduced oxycodone self-administration in both free and forced-choiced conditions. Together, these results suggest that LAMuOR may be a promising treatment strategy for opioid use disorder through which a single intervention confers a lifelong therapeutic effect.

Disclosures: K. Clemenza: None. **A. Fryc:** None. **L.L. Sjulson:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.01/SS20

Topic: H.07. Long-Term Memory

Support: T.A.G., A.B., A.S.-B., and L.K. were supported by the Federal Ministry of Education and Research (BMBF; 01GQ1705A) and by NIH/NINDS grant U01 NS113198-01.
T.A.G. was supported by a stipend of the German National Academic Foundation (Studienstiftung des deutschen Volkes).
L.K. received funding via the German Research Foundation (DFG; KU 4060/1-1; Projektnummer 447634521).
J.J. was supported by NIH grant MH104606 and the National Science Foundation (NSF).
P.C.R. received research grants from the Fraunhofer Society (Munich, Germany) and from the Else Kröner-Fresenius Foundation (Bad Homburg, Germany) and personal fees, travel support, and honoraria from Boston Scientific, Brainlab, and Inomed.

Title: Human single neurons lock to theta phases during memory encoding and retrieval

Authors: *T. A. GUTH[1,2], J. JACOBS[4,5], A. BRANDT[2], P. C. REINACHER[3,6], A. SCHULZE-BONHAGE[2], L. KUNZ[1];
[1]Dept. of Epileptology, Univ. Hosp. Bonn, Bonn, Germany; [2]Epilepsy Center, Med. Ctr., [3]Dept. of Stereotactic and Functional Neurosurgery, Med. Ctr., Univ. of Freiburg, Freiburg, Germany; [4]Dept. of Biomed. Engin., Columbia Univ., New York, NY; [5]Dept. of Neurolog. Surgery, Columbia Univ. Med. Ctr., New York, NY; [6]Fraunhofer Insitute for Laser Technol., Aachen, Germany

Abstract: The electrophysiological mechanisms of human memory encoding and recall are incompletely understood. Precise interactions between single-neuron spiking and brain oscillations presumably play a critical role in this process. Here, we used human single-neuron recordings from an object-location memory task with separate periods for encoding and retrieval

(Kunz et al., Neuron, 2021) to investigate the timing of single-neuron activity in the medial temporal lobe relative to the local theta rhythm (1-10 Hz) while epilepsy patients encoded and retrieved object-location memories in a virtual environment. 18 epilepsy patients participated in this task and contributed a total of 27 experimental sessions. We extracted single-neuron action potentials from hybrid depth electrode recordings (1025 neurons in total) using previously established spike-sorting algorithms (Chaure et al., Journal of Neurophysiology, 2018) and computed the Hilbert transform of the local field potential to estimate the instantaneous theta phase of each spike. For each neuron, we then examined its phase locking to the local theta rhythm, tested how this theta-phase locking varied as a function of theta power and task variables, and compared the phase distributions of action potentials between encoding and retrieval. We found that a significant portion of the recorded neurons phase locked to the theta rhythm, which was similarly strong during the encoding and retrieval of object-location memories. Neurons generally locked to the trough of theta oscillations, replicating previous observations (Jacobs et al., Journal of Neuroscience, 2007). Theta-phase locking was most prevalent during periods with high theta power. In some of the phase-locked neurons, we furthermore observed small but significant shifts in the preferred phases between encoding and retrieval, which is in line with theoretical models suggesting separate phases for encoding and retrieval (Hasselmo et al., Neural Computation, 2002). Overall, our results are consistent with the idea that human memory involves specific timing relationships between single-neuron action potentials of individual neurons and the theta rhythm during both memory encoding and retrieval.

Disclosures: T.A. Guth: None. **J. Jacobs:** None. **A. Brandt:** None. **P.C. Reinacher:** None. **A. Schulze-Bonhage:** None. **L. Kunz:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.02/SS21

Topic: H.07. Long-Term Memory

Support: NYU Internal Funds

Title: The Effects of Novelty and Abstract Reward on Memory Performance

Authors: *B. J. CARONE[1,2,3], E. B. ABRAMS[1,2,3], P. RIPOLLES[1,2,3];
[1]Psychology, New York Univ., New York, NY; [2]Ctr. for Language, Music, and Emotion (CLaME), New York, NY; [3]Music and Audio Res. Lab. (MARL), New York, NY

Abstract: Novelty and reward have been shown to affect memory performance via the release of dopamine. This process is thought to be supported, among others, by a brain circuit in the service of memory composed by the ventral striatum, the substantia nigra/ventral tegmental area, and the hippocampus (the SN/VTA-HP loop). While non-human animal research has focused on primary

and secondary rewards, recent work in humans has demonstrated that abstract rewards such as music modulate memory formation and that this process is dopamine-dependent. In the current project, we use music as a tool to better understand how reward and novelty interact in facilitating both recognition and recollection memory formation.
Seventy five adults completed the behavioral memory paradigm. On Day 1, participants listened to 48 20-second excerpts of unfamiliar classical music and provided ratings of pleasure, arousal, emotional valence, and familiarity for each. The next day, they were presented with the same excerpts shuffled with 48 new excerpts, and completed an old-new and remember/know/guess task. In this memory test participants indicated whether they heard the excerpt on the previous day and, if so, whether they remember something specific about the excerpt (recollection), know the excerpt (recognition), or guessed. In addition, novelty was calculated as surprisal for each excerpt using a computational model, the Dynamic Regularity Extraction model (D-REX). Data collection for the fMRI version of this experiment is ongoing.
Generalized linear mixed effects models were used to examine whether reward (pleasure ratings) and/or novelty (D-REX surprisal output) predicted recognition (hits vs. misses) and recollection (remembered or known vs. guessed). For recognition, we found that both reward and novelty significantly predicted recognition independently. For recollection, we found an interaction between reward and novelty, suggesting that the probability of recollection increases when songs are both novel and highly rewarding.
The present study shows that reward and novelty independently contribute to recognition, but that an interaction between both modulates recollection. Our results suggest that the interaction between reward and novelty creates a synergistic effect, whereby the combined impact is greater than the sum of its parts. These results build on the established model of the SN/VTA-HP loop, demonstrating separable but integrative effects of novelty and reward on long-term memory consolidation. Neuroimaging results will clarify whether the ventral striatum is responsible for this integration and shed light on how prefrontal regions facilitate recollection.

Disclosures: **B.J. Carone:** None. **E.B. Abrams:** None. **P. Ripolles:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.03/SS22

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant RGPIN-04933-2018
Canada Foundation for Innovation JELF
Ontario Research Fund 36876
NSERC Postgraduate Doctoral Scholarship

Title: Developmental differences in parietal cortex and hippocampal engagement during precise memory formation

Authors: *S. VIJAYARAJAH, M. L. SCHLICHTING;
Univ. of Toronto, Toronto, ON, Canada

Abstract: Developmental refinements to memory precision continue throughout childhood, which may suggest that memories are more abstract in children. Yet, how memories of different qualities are formed in the developing brain is not well understood. Here, we used fMRI to examine how neural engagement during learning supports the formation of memories that contain event-specific details (precise memories) and general themes (gist memories). Children (N=42; 7-9 years) and adults (N=42; 24-35 years) studied scene photographs presented consecutively. After, they performed a memory test for the scenes that included highly similar new scenes (lures) yoked to each studied scene. For studied scenes participants remembered, we considered responses to the associated lures to classify memories as either precise or gist: Precise memories were associated with correct rejections of lures, while gist memories were associated with false alarms to lures. Both children and adults showed more precise than gist memories. The prevalence of these memory types also varied between age groups, such that adults had more precise memories yet fewer gist memories than children. We identified neural engagement during encoding that was associated with different subsequent memory outcomes (gist, precise, or forgotten memories). Lateral occipital cortex, superior parietal cortex, and posterior hippocampus were all sensitive to these memory outcomes but differed in the specific nature of this effect. Lateral occipital cortex was more engaged for subsequently remembered (irrespective of precision) than forgotten scenes in both age groups. Superior parietal cortex engagement also differed between remembered and forgotten scenes in both age groups, and moreover distinguished between precise and gist memories only among adults. Lastly, posterior hippocampal engagement in adults differed according to memory success but not precision (remembered, irrespective of precision > forgotten), yet children showed this effect for gist memories only. Therefore, while early maturing visual regions support successful encoding similarly in children and adults, posterior hippocampus and parietal cortex differently support memory quality over development. Specifically, posterior hippocampus may support gist memories in childhood and only later come to support the emergence of precise memories. By contrast, parietal cortex may differentiate levels of memory precision in adults and support the extraction of abstract themes shared across experiences. In sum, these results suggest that different neural mechanisms may underlie precise and gist memory formation in children and adults.

Disclosures: S. Vijayarajah: None. **M.L. Schlichting:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.04/SS23

Topic: H.07. Long-Term Memory

Title: Stimulation of Medial Prefrontal Cortex modulates inhibitory control during memory retrieval

Authors: *A. KHAN, R.-Y. TONG;
The Chinese Univ. of Hong Kong, Ma Liu Shui, Hong Kong

Abstract: The prefrontal cortex utilizes inhibitory control processes to detect and stop interfering stimuli, with various brain regions being involved. Brain stimulation presents an intriguing prospect for affecting these processes by specifically targeting the implicated brain regions. In this study, we investigated the effects of transcranial direct current stimulation on the medial prefrontal cortex (MPC) during memory retrieval within a retrieval-induced forgetting (RIF) paradigm. RIF is a phenomenon where recalling target memories results in forgetting related non-target memories during later recall that reflects inhibitory control. Results from the study showed that the stimulation led to a selective decrease in the retrieval induced forgetting in the final test recall, but no difference was observed in facilitation of the items. Additionally, the stimulation decreased alpha/beta synchrony in the experimental group when compared to the sham group which was localized to the precuneus cortex. Overall, these findings suggest that direct current stimulation of the MPC can modulate the precuneus through network-level modulation, thereby weakening inhibitory control during memory retrieval.

Disclosures: A. Khan: None. **R. Tong:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.05/SS24

Topic: H.07. Long-Term Memory

Support: R01AG065255
NSF GRF DGE-2146755

Title: Dynamic neural and pupil-linked responses to mnemonic prediction errors

Authors: *A. M. XUE[1], M. KAESTNER[2], A. M. NORCIA[2], A. D. WAGNER[1];
[1]Dept. of Psychology, [2]Wu Tsai Neurosciences Inst., Stanford Univ., Stanford, CA

Abstract: When ongoing experience diverges from memory-based predictions, it is adaptive for the brain to prioritize processing the external world in service of encoding novel, incoming information into memory. These “mnemonic prediction errors” may consequently evoke changes in neural activity and arousal to support learning of new information. Moreover, the extent to which these errors promote memory encoding may depend on the strength of the initial memory prediction, such that stronger prediction errors may promote better encoding. We recorded scalp EEG and pupillometry as young, healthy human adults performed an associative novelty task, wherein they encoded strong (studied 4x) and weak (studied 1x) verb-image pairings and then

performed a cued associative match/mismatch retrieval task. First, analysis of the encoding strength manipulation revealed that memory performance was higher for strong compared to weak associations. Second, in a surprise subsequent recognition memory test, memory for expectation-violating stimuli (i.e., mismatch items) was above chance; the magnitude of the mnemonic prediction errors had variable effects across participants, with more showing better memory for items that violated weak compared to strong predictions. Third, initial analyses of the effects of mnemonic prediction errors on neural markers of top-down attention, cognitive control, and overall arousal suggest that prediction errors evoke immediate decreases in posterior alpha, signifying more top-down attention; strong prediction errors may evoke immediate increases in frontal theta, indicating enhanced cognitive control. Pupil diameter exhibited more sustained dilation (i.e., greater arousal) following strong, but not weak, prediction errors. Furthermore, multivariate pattern analyses indicate that stimuli violating strong predictions can be decoded with higher accuracy than those violating weak predictions. Finally, we examined whether EEG measures of memory reinstatement fluctuate at a theta rhythm, as predicted by computational models and revealed by recent extant research; initial evidence indicates that classifier evidence of retrieved content fluctuates at a theta frequency (~5-10Hz). As participants dynamically switch between memory states following a prediction error, classifier evidence of predicted and unpredicted information fluctuates at similar frequencies. Altogether, this research reveals the temporal dynamics of cognitive and neural responses to violations of memory-based predictions. In future work, we will examine how these changes relate to subsequent memory for expectation-violating information.

Disclosures: **A.M. Xue:** None. **M. Kaestner:** None. **A.M. Norcia:** None. **A.D. Wagner:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.06/SS25

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grants (RGPIN-2017-06753)
CIHR Grant (PJT-178337)
Brain Canada Foundation Grant
Vanier Canada Graduate Scholarship

Title: Learning regularities and exceptions are supported by distinct hippocampal pathways as revealed by diffusion-weighted functional footprints

Authors: *M. GUMUS[1], M. L. MACK[2];
[2]Univ. of Toronto, [1]Univ. of Toronto, Toronto, ON, Canada

Abstract: Flexible learning relies on reconciling prior knowledge with new experiences. Successfully navigating the challenges of learning is thought to be supported by the

complementary functions of the hippocampus (HPC) and its two central pathways of information flow (Schapiro et al., 2017): the trisynaptic pathway (TSP), which rapidly encodes specific experiences into distinct memory traces; and the monosynaptic pathway (MSP), which slowly extracts regularities over many experiences. Emerging evidence in humans supports this proposed circuitry; for example, neural activation and representations in MSP-related subfields relate to learning regularities (Schapiro et al., 2016) and white matter integrity in TSP-related connections is associated with learning category exceptions (Schlichting et al., 2021). Yet, empirical characterization of these two distinct pathway-specific functions in the same learning task remains a key open question. Participants (N=37) completed a rule-plus-exception visual category learning task during fMRI scanning. We leveraged diffusion-weighted and high-resolution anatomical MRI to identify pathway-specific endpoints within entorhinal cortex (ERC) and HPC subfields. We hypothesized that these "pathway footprints" may better target distinct subregions of ERC and HPC that underlie the pathway-specific operations supporting regularity extraction (MSP: ERC - CA1) and distinct encoding (TSP: ERC - DG - CA2/3 - CA1). We assessed the relationship between univariate activation within MSP and TSP footprints during learning and behavioural learning outcomes for category regularities and exceptions. Consistent with our predictions, we found that individuals' performance in learning category regularities was associated with higher MSP than TSP activation. But the reverse was true of learning category exceptions—higher TSP than MSP footprint activation led to better exception learning. Notably, these learning-specific activation signatures were not found within typical full-volume ERC and HPC subfields regions of interest. These findings (1) provide novel evidence that learning category regularities and exceptions is supported by complementary hippocampal functions related to its central pathways and (2) suggest the "pathway footprint" method may offer an insightful window into the functional dynamics of hippocampal circuitry.

Disclosures: M. Gumus: None. **M.L. Mack:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.07/TT1

Topic: H.07. Long-Term Memory

Support: NIH/NINDS grant U01 NS113198-01
BMBF; 01GQ1705A and 01GQ1705B
NSF grant BCS-1724243

Title: Single-neuron representations of temporal order in the human medial temporal lobe during encoding and retrieval

Authors: *M. F. KHAZALI[1], A. BRANDT[1], P. C. REINACHER[1], J. JACOBS[2], A. SCHULZE-BONHAGE[1], L. KUNZ[3];

[1]Freiburg Univ., Freiburg, Germany; [2]Columbia Univ., New York, NY; [3]Univ. of Bonn, University of Bonn, Germany

Abstract: Episodic memory consists of several single events that are temporally organized. Cellular activations in the medial temporal lobe (MTL) contribute to the neural representation of episodic memory. Here, using invasive single-neuron recordings in human epilepsy patients (n = 17), we examined whether human MTL neurons represent the serial position of events that are sequentially organized during encoding and retrieval periods of episodic memory task. The patients freely navigated a virtual environment in order to explore and remember the locations and the serial positions of different objects (Kunz et al., Neuron, 2021). During each exploration period, the patients sequentially encountered two or three different objects, placed in different locations. This allowed us to examine single-neuron firing rates as a function of the serial position in which the objects were presented. Our results show that a significant number of single neurons in the human MTL are tuned to the serial position of the objects during the exploration period—for example, by activating most strongly whenever the subject is presented with the first object, independent of the identity of the object itself. Overall, about 10% (n=95 out of 945) of human MTL neurons showed serial position selectivity (SPS) with percentages higher than 10% for entorhinal cortex, hippocampus and parahippocampus, and lower than 6% for amygdala and temporal pole. We performed further preliminary analysis of the firing rate of SPS neurons during serial position retrieval period. The results of this analysis might suggest that SPS neurons preserve their serial position preference across encoding and retrieval periods, a hint of their potential involvement in recalling the temporal structure of episodic memory.

Disclosures: M.F. Khazali: None. **A. Brandt:** None. **P.C. Reinacher:** None. **J. Jacobs:** None. **A. Schulze-bonhage:** None. **L. Kunz:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.08/TT2

Topic: H.07. Long-Term Memory

Support: Natural Science and Engineering Research Council of Canada Discovery Grant

Title: Item manipulability affects memory task performance: An EEG oscillation investigation

Authors: *K. LAMBERT[1], Y. Y. CHEN[2], C. R. MADAN[3], A. SINGHAL[1];
[1]Univ. of Alberta, Edmonton, AB, Canada; [2]Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA; [3]Psychology, Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Item manipulability affects memory task performance: An EEG oscillation investigation
Certain object properties may render an item more memorable than others. One such property is

manipulability, or the extent to which an object can be interacted with using our hands. Here we investigate if the manipulability of an item modulates memory task performance on both a behavioural and neural level. In particular, we recorded electroencephalography (EEG) from a large sample of individuals (N = 53) during a visual item recognition memory task. Items were rated as either high or low manipulability. Retrieval involved asking participants to judge if the presented stimulus was also presented at the encoding stage. Our data analysis focused on activity in the theta (3.5-7 Hz) and alpha (8-14 Hz) rhythms, both of which have been implicated in attentional and memory processes. Activity in the theta rhythm over the central region was greater for high manipulability stimuli at both encoding and retrieval. At retrieval, participants were significantly slower when responding to new as opposed to old high manipulability items. This effect was not observed for low manipulability items. Additionally, new high manipulability items were accompanied by increased alpha activity while low manipulability items were accompanied by decreased activity in the rhythm. Together, these findings provide further evidence that manipulability affects the processing of visual stimuli during memory tasks.

Disclosures: **K. Lambert:** None. **Y.Y. Chen:** None. **C.R. Madan:** None. **A. Singhal:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.09/TT3

Topic: H.07. Long-Term Memory

Support: NIH-NINDS 1R01 NS107727
NIH-NINDS 2R01 NS089729

Title: Repulsion of similar memories measured via Natural Language Processing of verbal recall

Authors: *A. BABU[1], Z. YE[1], B. A. KUHL[2];
[2]Dept. of Psychology, [1]Univ. of Oregon, Eugene, OR

Abstract: Human fMRI work investigating the role of the hippocampus in episodic memory has revealed a “repulsion” effect where hippocampal representations for highly similar memories become separated from one another with experience (Chanales et al., 2017; Favila et al., 2016; Wanjia et al., 2021). More recently, behavioral studies have revealed a parallel effect where overlapping features of highly similar memories become remembered as more different (Chanales et al., 2021; Drascher & Kuhl, 2022). However, these behavioral studies have been limited to artificially generated stimuli and measurement along specific feature dimensions (i.e., color or facial features). In the current study, we leveraged Natural Language Processing algorithms to quantify verbal recall of complex naturalistic scene images within a 768-dimension semantic feature space. This allowed us to test for a similar behavioral repulsion effect when recalling more complex and naturalistic stimuli. N=120 participants learned associations between faces (cues) and scene images (associates). Critically, half of the subjects were assigned to a

competitive condition and half to a non-competitive condition. Participants in the competitive condition studied six images from a single category (e.g., six pools), while participants in the non-competitive condition studied one image from each of six different categories (e.g., one pool, one library, etc.). Same-category images in the competitive condition were intended to cause competition and drive a behavioral repulsion effect. After extensive learning of the face-scene pairs, participants were cued with each face and asked to type a description of the corresponding scene image. These descriptions were then transformed into text embeddings in the semantic feature space. Text embeddings for images in both conditions were then correlated with category-matched text embeddings from the non-competitive condition to determine whether within-category similarity of embeddings differed for images recalled from the competitive vs. non-competitive conditions. We found that within-category similarity was significantly lower in the competitive condition than the non-competitive condition, Welch's $t(75.31) = 3.87$, $p < 0.001$. These results indicate that memory content for similar images becomes more distinct with competition, mirroring behavioral repulsion effects observed in prior work. In a preliminary fMRI study, we investigate how changes in hippocampal representations across learning relate to behavioral measures of verbal recall.

Disclosures: **A. Babu:** None. **Z. Ye:** None. **B.A. Kuhl:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.10/TT4

Topic: H.07. Long-Term Memory

Support: IBS-R015-D1
NRF-2019M3E5D2A01060299
NRF-2019R1A2C1085566

Title: Neural correlates of context transitions in continuous internal thoughts

Authors: *K. HEO[1,2,3], D. KWON[1,2,3], W. SHIM[1,2,3,4];
[1]Sungkwunkwan Univ., Suwon, Korea, Republic of; [2]Ctr. for Neurosci. Imaging Research, Inst. for Basic Sci. (IBS), Suwon, Korea, Republic of; [3]Dept. of Intelligent Precision Healthcare Convergence, Suwon, Korea, Republic of; [4]Dept. of Biomed. Engin., Suwon, Korea, Republic of

Abstract: Continuous narratives can be organized into discrete events as they unfold over time. Prior research has shown that the boundaries of these events, which represent transitions in narrative context, elicit neural responses in the default mode network (DMN) and hippocampus (HC) during movie watching or story listening. However, it remains unclear how the brain organizes internally produced continuous thoughts in the absence of external input. To investigate how internally generated thoughts are represented as discrete structures, we conducted an fMRI study where participants freely spoke their thoughts. In the scanner,

independent groups of participants engaged in three tasks: recalling a previously watched movie (movie-recall), sharing their opinions on given topics (topic), and speaking about their spontaneous thoughts (think-aloud). After scanning, participants' speech transcriptions were segmented into coherent chunks, corresponding to events in the movie for movie-recall and aligning with context transitions for the topic and think-aloud tasks. We found increased neural responses in subregions of the DMN, including the retrosplenial cortex (RSC) and posterior cingulate cortex (PCC), at transition points between events or context, regardless of the speech type. However, neural responses in the angular gyrus (AG) and HC showed no significant changes at context transitions during topic and think-aloud tasks but exhibited increased responses at event boundaries during movie recall. These results indicated that the RSC and PCC play a crucial role in transitioning between different situational contexts, consistent across all three speech tasks, while the AG and HC may be more involved in demarcating event boundaries within a clear narrative structure. Furthermore, when participants listened to audio clips of actors performing the topic and think-aloud tasks, we observed similar increases in neural responses in the RSC and PCC at context transitions, albeit with a delayed peak compared to speech tasks. In conclusion, our findings suggest the distinct functional roles of the DMN and HC in structuring continuous experiences: the medial posterior DMN regions track changes in both external and internal situational context, while the HC and AG are more likely to be engaged in processing narratives, whether by observing existing narratives or self-generating them, which involves memory retrieval and integration.

Disclosures: **K. Heo:** None. **D. Kwon:** None. **W. Shim:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.11/TT5

Topic: H.07. Long-Term Memory

Support: Czech Science Foundation project n. 21-44843L

Title: Middle temporal gyrus and hippocampus are key functional connectivity hubs for verbal memory encoding

Authors: ***B. MATOUSKOVA**[1,2,3], J. CIMBALNIK[1,4], M. KUCEWICZ[4,5], P. KLIMES[2,1]; [1]Intl. Clin. Res. Ctr., St. Anne's Univ. Hosp. Brno, Brno, Czech Republic; [2]Inst. of Scientific Instruments of the Czech Acad. of Sci., Brno, Czech Republic; [3]Fac. of Med., Masaryk Univ., Brno, Czech Republic; [4]Mayo Clin., Rochester, MN; [5]Gdansk Univ. of Technol., Gdansk, Poland

Abstract: Understanding the neural mechanisms behind successful memory encoding is crucial for advancing our knowledge of cognition and memory processes, as well as for developing targeted interventions to improve memory function. In this study, we utilized several functional connectivity metrics to identify hubs responsible for successful or unsuccessful memory

encoding in a free recall task. We recorded intracranial EEG data with sampling frequency 5000 Hz from eleven epileptic patients undergoing presurgical evaluation. The chosen functional connectivity features included coherence, linear correlation, phase synchrony, relative entropy and spectra multiplication. Firstly, functional connectivity was calculated in standard frequency bands, ripples and fast ripples for each patient, feature, encoded word and time window (we have used 500 ms windows with 100 ms steps from 1000 ms before to 2000 ms after word presentation) for all possible pairs of contacts. Next, whole-brain connectivity maps were calculated for recalled and forgotten words using the median feature values and then Mann-Whitney U test was performed to identify connections exhibiting differential connectivity during encoding of recalled and forgotten words. Using the significance threshold of p-value 0.01 we calculated the number of significantly different connections for each contact. Global hubs were identified using a three-sigma limit, and their corresponding brain structures were determined by MNI-AAL3 atlas. Finally, for each feature, frequency band and time window the percentage of patients for whom each structure served as a global connectivity hub was calculated. We focused on structures implanted in at least 50 % of patients and time windows from 300 ms before and after word presentation. Our findings revealed the middle temporal gyrus as the most significant global connectivity hub for distinguishing between recalled and forgotten words with the spectra multiplication feature across all time windows. The same anatomical hub was identified with coherence feature within the low gamma band (20-45 Hz) from 400 to 1800 ms after word presentation. Another hub was found in the hippocampus with the linear correlation feature in beta band (12-20 Hz) around 800 ms, which preceded the middle temporal gyrus hub in the delta band (1-4 Hz) around 400 ms after word presentation. These results provide a mechanistic insight into the roles of the middle temporal gyrus and hippocampus as global network hubs of human memory encoding and confirm the recently reported effects of direct electrical stimulation in these regions.

Disclosures: **B. Matouskova:** None. **J. Cimbalnik:** None. **M. Kucewicz:** None. **P. Klimes:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.12/TT6

Topic: H.07. Long-Term Memory

Support: ONR MURI N00014-23-1-2086

Title: Hierarchically aligned neural state changes relate to perceived event boundary strength

Authors: *Y. LEE, J. CHEN;
Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: In the human brain, cortical areas are hierarchically organized according to their intrinsic processing timescales; studies suggest that default mode network (DMN) regions may integrate information over windows as long as one minute, while the processing timescales of low-level areas (e.g., primary sensory areas) are much shorter (Hasson et al., 2015). The cortical processing hierarchy framework posits that information is transmitted from lower-level to higher-level areas during naturalistic movie-viewing (Chang et al., 2022). It has also been shown that when people view naturalistic movies, neural state boundaries (transitions between states) in DMN areas match human-identified event boundaries; furthermore, these DMN neural state boundaries coincided with boundaries observed in cortical regions with shorter processing timescales (Baldassano et al., 2017; Geerligs et al., 2022). We hypothesized that when neural state changes align across multiple levels of the cortical hierarchy, perceived "strength" of event boundaries would be higher. In order to measure the hierarchical level of each brain region, we calculated temporal receptive windows (TRW; Hasson et al., 2008; Lerner et al., 2011) using fMRI data in which participants watched an audiovisual movie temporally scrambled at three different timescales (Chen et al. 2016). We localized six TRW levels along the cortical hierarchy, from sensory areas (level 1: the *shortest* timescales) up to default mode network areas (level 6: the *longest* timescales). For each cortical level, we then obtained neural state boundaries in a separate audiovisual movie-viewing fMRI dataset (N = 20; Lee & Chen, 2022). In preliminary analyses examining boundary alignment across levels 4-6, we identified two groups of level-6 boundaries: those that were aligned with both level-5 and level-4 boundaries (21 *cortically aligned* boundaries) and those that were not (32 *non-aligned* boundaries). Two human observers rated the prominence (strength) of the level-6 boundaries. Boundary strength judgment was significantly consistent across people (r = 0.67; P < .001), and non-aligned boundaries were rated as numerically (but not significantly) less prominent than aligned boundaries (aligned: z = -0.10; non-aligned: z = -0.17).

Disclosures: **Y. Lee:** None. **J. Chen:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.13/TT7

Topic: H.07. Long-Term Memory

Support: NIH-NINDS 2R01 NS089729
F31NS126016

Title: Dissociable roles for CA3 and CA1 in differentiating overlapping spatial memories

Authors: *W. GUO[1], S. HAN[1], B. A. KUHL[2];
[2]Dept. of Psychology, [1]Univ. of Oregon, Eugene, OR

Abstract: The hippocampus is believed to play a critical role in disambiguating memories for similar events (Yassa & Stark, 2011). Recent fMRI studies have found that event similarity triggers a "repulsion" of hippocampal activity patterns (Chanales et al., 2017; Favila et al., 2016; Hulbert and Norman, 2014)—an effect that is thought to specifically occur within hippocampal subfields CA3 and dentate gyrus (Dimsdale-Zucker et al., 2018; Wanjia et al., 2021). However, an important question is whether repulsion is driven by attention to diagnostic visual features or by internal beliefs (even in the absence of diagnostic visual features). In the current study, we used high-resolution fMRI to test for repulsion effects within hippocampal subfields during a spatial route-learning task in which we experimentally manipulated route similarity and internal beliefs. Participants (N = 40) learned and repeatedly travelled (virtually) along 4 routes within the University of Oregon campus. Critically, the 4 routes were comprised of 2 pairs of overlapping routes. Overlapping routes were initially identical before becoming subtly different and ultimately diverging to terminate at unique destinations. Specifically, overlapping routes contained 3 segments: 'same,' 'similar,' and 'different.' During the 'same' segment, overlapping routes contained identical images (and travelled identical paths). During the 'similar' segment, images were similar but non-identical yet paths were identical. During the different segment, the images and paths were distinct. Critically, each trial was preceded by a cue indicating the likely destination. These cues were intended to manipulate subjects' beliefs about the destination even when visual information was not diagnostic. Pattern similarity analyses revealed repulsion of overlapping route representations within CA3 and dentate gyrus (CA3DG) that were primarily driven by subjects' beliefs about the likely destinations. In fact, repulsion in CA3DG was greatest when visual information was most ambiguous. Representations in CA1 markedly differed from those in CA3DG: namely, CA1 exhibited a transient repulsion effect that selectively occurred when subjects' beliefs about the route destinations were violated by salient visual information. Representations in early visual cortex and parahippocampal place were overwhelmingly driven by stimulus similarity, as opposed to beliefs or violations of beliefs. These findings provide new evidence for dissociable, but complementary, roles for hippocampal subfields in differentiating overlapping spatial memories.

Disclosures: **W. Guo:** None. **S. Han:** None. **B.A. Kuhl:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.14/TT8

Topic: H.07. Long-Term Memory

Support: National Natural Science Foundation of China (31730038 and 31971000)
Young Top Notch Talents of Ten Thousand Talent Program

Title: Unique contributions of encoding-retrieval neural global pattern similarity to individual differences in semantic false memory

Authors: *X. SHAO[1], C. CHEN[2], E. F. LOFTUS[2], G. XUE[1], **B. ZHU**[1];
[1]Beijing Normal Univ., Beijing, China; [2]Univ. of California, Irvine, Irvine, CA

Abstract: In the Deese-Roediger-McDermott (DRM) task, after hearing semantically related words (e.g., dream, awake, and bed), some people are more likely than others to falsely recognize unstudied but semantically related lures (e.g., sleep) rather than unstudied and unrelated foils (e.g., pen). Previous studies showed that semantic false memory was influenced by both neural global semantic representations in the temporal pole during encoding and neural global pattern similarity between encoding and retrieval (ER-nGPS) in the frontoparietal cortex (Chadwick et al., 2016; Ye et al., 2016; Zhu et al., 2019). However, such previous results were based on group-level analysis, so it is unknown whether an individual differences approach would confirm those results and/or reveal new brain regions that may play a role in false memory. This study collected functional magnetic imaging (fMRI) data from 80 participants while they performed the DRM task, and used an individual differences approach to identify brain regions whose ER-nGPS would be associated with false memories, even after controlling for neural representations at encoding. First, consistent with previous group-level analysis showing the importance of neural representations in the temporal pole during encoding, we found that participants with greater semantic representations in the temporal pole during encoding showed more false memory. Second, although a group-level analysis of our study confirmed previous analysis implicating ER-nGPS in the left inferior frontal gyrus for false memory, our individual differences analysis further found that, even after controlling for encoding neural representations, participants with greater ER-nGPS in the right inferior parietal lobe showed more false memory. In sum, our group-level analyses confirmed previous results and our individual differences approach led to a new discovery that ER-nGPS in the right inferior parietal lobe plays a role in semantic false memory.

Disclosures: X. Shao: None. **C. Chen:** None. **E.F. Loftus:** None. **G. Xue:** None. **B. Zhu:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.15/TT9

Topic: H.07. Long-Term Memory

Support: NIH/NINDS R01NS114913

Title: Neural correlates of high-precision memory retrieval as identified by fMRI

Authors: *M. HOU[1], A. N. Z. AKTAS[1], P. F. HILL[2], A. D. EKSTROM[2], M. D. RUGG[1];
[1]Ctr. For Vital Longevity, Utdallas, Richardson, TX; [2]Dept. of Psychology, Univ. of Arizona, Tucson, AZ

Abstract: It has been proposed that a distinction can be drawn between the likelihood that a retrieval cue will elicit recollection of a prior episode (retrieval success) and the specificity or level of episodic detail of the retrieved memory (precision). On the basis of prior fMRI findings it has been proposed that retrieval-related neural activity in the hippocampus and angular gyrus (AG) is differentially sensitive to success and precision respectively. Here, we examined retrieval-related neural activity in the hippocampus and AG during a memory test, similar to those employed previously, that permitted test trials to be classified according to whether or not they elicited successful retrieval and, if so, to quantify the precision of the retrieved memory. A sample of young human adults (N = 24, mean age = 24, age range = 18-30, 13 female) underwent fMRI scanning during a single study-test cycle. At study, participants viewed 102 object images. Each image was placed at a randomly selected location on an imaginary circle, and the task was to move a character, which was located elsewhere on the circle, until it overlapped the image. Test items comprised a random sequence of the studied images intermixed with 34 unstudied images. The images were presented at fixation, which was located at the center of a circle with the same radius as the imaginary one at study. The requirement was to move a character, which was randomly located on the circle, to the location of studied image, guessing if necessary. Thus, location memory accuracy could be quantified in terms of the angle between the correct location and the location selected by the participant. Participants then signaled whether they judged the presented object to have been studied. Trials associated with a correct recognition judgment were classified as eliciting high-precision or 'guess' location judgments according to an analysis based on a mixture model. A contrast between the two trial types identified robustly greater activity for high precision memories in left AG along with a weaker effect in the hippocampus. Crucially, activity in the AG was also robustly elevated for guess trials relative to correct rejections (unstudied items correctly judged as such). These findings call into question the proposal that, in this paradigm, the AG is sensitive to the precision of a recollected memory. An alternative possibility is that the region is responding to the visuomotor demands of the test task, which are low for correct rejection trials, intermediate for guesses, and highest for high precision memory judgments.

Disclosures: M. Hou: None. **A.N.Z. Aktas:** None. **P.F. Hill:** None. **A.D. Ekstrom:** None. **M.D. Rugg:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.16/TT10

Topic: H.07. Long-Term Memory

Title: Specificity of recollection-related activity for different memory dimensions

Authors: *F. PROCIDA[1], M. FRISONI[1], M. TULLO[1], A. TOSONI[1,3], M. PERRUCCI[1,3], P. CHIACCHIARETTA[2], R. GUIDOTTI[1], C. SESTIERI[1,3];
[1]Dept. of Neuroscience, Imaging and Clin. Sci., [2]Dept. of Innovative Technologies in Med. and

Dent., Univ. G. d'Annunzio of Chieti-Pescara, Chieti, Italy; [3]Inst. for Advanced Biomed. Technologies, Chieti, Italy

Abstract: The mnemonic representation of complex events is multidimensional. While previous research has identified a network of brain regions involved in several forms of episodic memory retrieval, the degree of specificity for different memory dimensions of this recollection-related activity is still largely unknown. In the present functional Magnetic Resonance Imaging (fMRI) study, we asked human participants to perform a cued recollection task that requires to retrieve specific information about a previously encoded TV show (the first episode from the series "Sherlock", BBC) along four dimensions (objects/character's details, spatial layouts, temporal sequences, verbal dialogues). In particular, participants were asked to provide a true/false judgment for statements regarding a particular movie scene, previously identified through a segmentation procedure in an independent group of subjects. The paradigm distinguished the BOLD activity associated with memory retrieval from that related to sentence reading and response preparation/execution. Retrieval-related activity was further divided according to the four memory dimensions (details, spatial, temporal, verbal). Common activity for all dimensions was observed in a largely left-lateralized network of regions including lateral prefrontal, lateral superior parietal, and lateral temporal cortex. However, a large degree of specificity for memory dimensions was observed especially in the posterior nodes of the recollection network, i.e. the lateral temporo-parietal and the medial parietal cortex. The majority of these dimension-specific activations exhibited a leftward lateralization. These results support the view that different memory information is processed by a mosaic of regions within large portions of associative cortex involved in higher-order mnemonic functions.

Disclosures: F. Procida: None. **M. Frisoni:** None. **M. Tullo:** None. **A. Tosoni:** None. **M. Perrucci:** None. **P. Chiacchiaretta:** None. **R. Guidotti:** None. **C. Sestieri:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.17/TT11

Topic: H.07. Long-Term Memory

Support: NIH Grant 1R21AG071231-01

Title: Fmri correlates of retrieval gating of scene and object information

Authors: *A. KIDWAI[1], S. SROKOVA[2], M. D. RUGG[3];
[1]Brain and Behavioral Sci., UT Dallas, Richardson, TX; [2]The Univ. of Texas at Dallas, Univ. of Texas at Dallas, Richardson, TX; [3]Univ. of Texas at Dallas, Univ. of Texas at Dallas, Dallas, TX

Abstract: A neural correlate of episodic memory retrieval is ‘cortical reinstatement’, which refers to patterns of activity that were initially elicited during the encoding of an event and that are reinstated at retrieval. Stronger cortical reinstatement has been reported to be associated with

more accurate memory judgements. This has led to the suggestion that cortical reinstatement may be an objective indicator of the retrieval of episodic information. The term 'retrieval gating' refers to the phenomenon by which individuals seemingly attenuate or 'gate' the retrieval of information belonging to an encoded episode that is irrelevant to a retrieval goal in order to optimize retrieval of goal-relevant information. In three prior fMRI studies, retrieval gating was operationalized in terms of the attenuation of scene reinstatement effects when the retrieval task required memory for non-scene rather than scene-related information. In the present study we addressed the question of whether retrieval gating could be identified for the reinstatement of object as well as scene information. Participants (24 male and female cognitively healthy young adults) encoded a series of concrete words that, on different trials, were superimposed on images of scenes, objects, or pixelated backgrounds. The word-image pairs were presented at one of two locations. In a scanned test task that employed words as the retrieval cues, the participants performed two different retrieval tests, which were organized into 8 blocks (4 blocks for each test). In the background task, the requirement was to first judge whether each test item was studied or unstudied, and if studied, whether it had been paired with an object, a scene, or a pixelated image. In the location task, the old/new judgment was followed by the requirement to signal the location (left/right) of the word when it was studied. Retrieval gating of scene information (lower scene reinstatement in the location than the background task) was evident in the scene-selective parahippocampal place area and retrosplenial complex, replicating prior findings. We were however unable to identify reinstatement effects for objects, even in the background task, and were therefore unable to determine whether gating extends to items other than scenes. Nonetheless, these findings significantly extend the range of experimental contexts within which retrieval gating effects can be identified.

Disclosures: **A. Kidwai:** None. **S. Srokova:** None. **M.D. Rugg:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.18/TT12

Topic: H.07. Long-Term Memory

Support: ONR N00014-22-1-2123

Title: An effect of memory set size on ERP components associated with retrieval

Authors: *I. UTOCHKIN, C. ZHAO, E. VOGEL;
Univ. of Chicago, Chicago, IL

Abstract: Previous research concerning event-related potential (ERP) correlates of human recognition memory has established two ERP components associated with discrimination between previously studied (old) and non-studied (new) items used for memory test: mid-frontal negativity, FN400, and late posterior component, LPC (Rugg & Curran, 2007). A lot of studies

have addressed which aspects of memory retrieval these components might correlate with (e.g., familiarity vs. recollection, decision making, confidence, etc.). However, the previous research almost has not addressed the roles of basic characteristics of memory lists, such as memory set size (MSS), on the recognition ERP components. Although people are very accurate at recognition of even very large lists (Brady et al., 2008), there is still a temporal cost of MSS to recognition (Wolfe, 2012). Here, we tested MSS effects on the ERP components associated with visual recognition. In each block of our experiment, participants first studied a set of 8, 32, or 64 serially presented images, each item was presented for 800 ms. Each study stage was followed by a test stage, when old images were randomly intermixed with the same number of new images. Observers had to manually respond whether each image has been old old or new. Each test image was presented for 800 ms before the observers were allowed to respond. The ERP components of interest were defined as the differences between ERP to correctly classified old and new images. We found substantial MSS effects in both mid-central (F3, F4, Fz, Fc1, Fc2, Fc5, Fc6) and posterior (P3, P7, P4, P8, Cp1, Cp2, Pz) clusters of electrodes. Specifically, the smaller MSS caused earlier and larger differences between the ERPs to old and new test items. Recognition was also more accurate at smaller MSS. Therefore, our findings suggest that FN400 and LPC can be sensitive to the strength and accessibility of encoded material at retrieval.

Disclosures: I. Utochkin: None. **C. Zhao:** None. **E. Vogel:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.19/TT13

Topic: H.07. Long-Term Memory

Support: NIH Grant MH129436

Title: Integrating new and old memories during sleep: Testing the effects of interleaved memory reactivation

Authors: *B. E. SHERMAN, E. M. SIEFERT, A. C. SCHAPIRO;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: As we encounter new people, places, and concepts, we must update our existing knowledge structures to account for new information. Neural network models simulating learning over time have suggested that this integration may be achieved by interleaving the reactivation of newly learned and previously learned information, especially during offline periods, such as sleep. In the current study, we provide an empirical test of this hypothesis in humans. Participants first came into the lab for three 'prior knowledge training' sessions (on three consecutive days), during which they learned about a set of novel objects from three different categories. Each object comprised multiple visual features, some of which were unique to a given object and some of which were shared with other members of the category. In addition

to the visual features, participants also learned the name of each object (presented auditorily). One to two weeks later, participants returned for a final experimental session. After testing participants on their memory for the previously learned objects, we introduced them to a set of new objects from the same three categories. Following this 'new learning' phase, participants took a nap in the lab while undergoing EEG. To bias memory reactivation during the nap, we performed Targeted Memory Reactivation (TMR) time-locked to the peaks of slow oscillations, using the object names as cues. For one category, we interleaved the cueing of prior learned and newly learned objects; for another category, we cued only the newly learned objects (to bias reactivation away from interleaving); for the third category, we did not cue any objects. After the nap, participants were tested on their memory for all objects and underwent several additional tests aimed at measuring the integration of new and old memories. Preliminary analyses (data collection ongoing) provide validation of several aspects of the design. For example, participants exhibited high fidelity memories for the previously learned objects, even after the 1-2 week delay, suggesting that our paradigm was successful in instantiating stable "prior knowledge." Additionally, we observed characteristic event-related potentials following the playing of sounds, suggesting that the brain processed the TMR cues during sleep. Future analyses will focus on the role of interleaved cueing on memory integration, aiming to shed light on the mechanisms by which the brain integrates information over time to build up stable knowledge structures.

Disclosures: B.E. Sherman: None. **E.M. Siefert:** None. **A.C. Schapiro:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.20/TT14

Topic: H.07. Long-Term Memory

Support: NIH Grant SC3GM121192
NIH Grant 5R25NS114326

Title: Effects of HD tDCS on Encoding and Judgments of Learning

Authors: *J. REJOUIS[1], M. A. GARCIA[2], A. M. GAYNOR[3], E. F. CHUA[1,2];
[1]Psychology, Brooklyn Col., Brooklyn, NY; [2]Grad. Ctr. of the City Univ. of New York, New York, NY; [3]Cognitive Neurosci. Division, Dept. of Neurol., Columbia Univesity Med. Ctr., New York, NY

Abstract: As individuals acquire new knowledge, they also evaluate their own learning. Our research aims to investigate the effects of brain stimulation over the prefrontal cortex on encoding and judgments-of-learning. Prior work using conventional 1x1 transcranial direct current stimulation (tDCS) over the frontal cortex in healthy young adults showed stimulation impaired associative encoding, but surprisingly did not show any effects on judgments-of-learning. One potential explanation for the lack of effects on judgments-of-learning and

surprising direction of the effects on encoding is that the low spatial resolution of conventional tDCS obscured the specific roles of prefrontal sub-regions in encoding and judgments-of-learning. The current experiment used High Definition-tDCS to test the roles of the anterior prefrontal cortex (aPFC) versus the dorsolateral prefrontal cortex (DLPFC) in encoding and judgments-of-learning, and to test whether they can be improved with brain stimulation. Participants studied novel word pairs, while receiving active HD-tDCS over the aPFC or DLPFC, or sham HD-tDCS. After each word pair, participants made a judgment-of-learning indicating their confidence in their ability to recognize those word pairs 24 hours later. In a subsequent memory test, participants viewed intact, rearranged, and new word pairs, and were asked to judge each as "intact", "rearranged", or "new". Data collection is ongoing, but repeated measures ANOVAs on preliminary data (N=26) showed fewer false alarms to new word pairs (i.e., new items called "intact") for the aPFC and DLPFC conditions compared to sham, which may reflect better encoding. There were no significant effects of stimulation on judgments-of-learning. Overall, preliminary data indicate that HD-tDCS over different frontal regions improves encoding, but does not affect judgments-of-learning.

Disclosures: J. rejouis: None. **M.A. Garcia:** None. **A.M. Gaynor:** None. **E.F. Chua:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.21/TT16

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant RGPIN-04933-2018
Canada Foundation for Innovation JELF
Ontario Research Fund 36876
NSERC Postgraduate Doctoral Scholarship

Title: Attention to general versus specific aspects of visual experience differently engages lateral occipital and parietal cortex in children and adults

Authors: *D. HUANG, S. VIJAYARAJAH, M. L. SCHLICHTING;
Univ. of Toronto, Toronto, ON, Canada

Abstract: How we attend to our experiences can have a substantial influence on what is later remembered. However, the nature of this influence is likely not stable from early in life but rather refined across childhood, as adults are relatively better at both selectively attending to and remembering their experiences compared with children. Yet little is known about the neural mechanisms by which attention might influence children's memory. Here, we characterized developmental differences in neural engagement during attention to general (category) versus specific (item details) aspects of scenes, and how they relate to later memory quality. Children (N=42; 7-9 years) and young adults (N=42; 24-35) performed an incidental encoding task during

fMRI scanning, followed by a surprise memory test. At encoding, participants were cued to attend to the general (scene category; e.g., beach) or specific (picture; e.g., particular beach) aspects of scene photographs across blocks. After some blocks, participants indicated which of two scenes matched a scene from the preceding block on the cued dimension (scene category or picture), which allowed us to determine whether they successfully oriented to the cued dimension. In the memory test, participants indicated whether studied scenes and similar yoked scenes (lures) were old or new. Performance on the attention task was above chance and similar for general and specific in both age groups, suggesting that children and adults successfully modulated their attention to the cued dimensions. Despite this similar performance, memory quality nonetheless differed by the attention cue: item-specific attention was associated with better subsequent memory for studied scenes in both age groups, and greater false alarms to lures in adults. In terms of neural engagement during memory formation, we found that adults engaged frontoparietal regions during category attention and ventral visual and parietal regions during item-specific attention. Moreover, the degree of attentional modulation in activation of lateral occipital (item-specific) and parietal (category) cortex was reliably greater in adults than children. Neural modulation also tracked across adults (but not children) according to their level of behavioural memory modulation. Therefore, despite exhibiting memory differences according to attention and adequate attention task performance, children showed overall less evidence of consistent neural modulation during attention than adults. Broadly, these findings begin to show how age-related changes in visual and parietal engagement may contribute to developmental refinements in how attention is deployed in children.

Disclosures: D. Huang: None. **S. Vijayarajah:** None. **M.L. Schlichting:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.22/TT17

Topic: H.07. Long-Term Memory

Support: Lumen Prize Elon University

Title: Evaluating the self-reference effect as an encoding strategy for individuals displaying autistic traits: An eye tracking study

Authors: *L. H. FEINTUCH[1], E. J. HUNTER[2], A. A. OVERMAN[2];
[2]Psychology, [1]Elon Univ., Elon, NC

Abstract: Individuals with autism spectrum disorder (ASD) notably display diminished episodic recollection, however, the neurocognitive basis of these observations remains unclear (Cooper & Simons, 2018). Research in young adults with ASD and those who report autistic traits has shown inconsistencies in the success of using self-referential encoding strategies to enhance episodic recollection (Grisdale et al., 2014; Williams et al., 2017). The present study compared

the effectiveness of self-referential versus semantic encoding strategies in adults between the ages of 18 and 25 with varying degrees of autistic traits as measured by the Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001). 31 participants completed a memory task (based on Leshikar et al., 2015) in which they encoded adjectives by judging whether each word described themselves ("Self" task), or was commonly used ("Common" task). Participants then completed a recognition task in which they indicated (via "Remember", "Familiar", or "New" response) whether they had studied each word (item memory), and if so, which study task was used (source memory). Eye-tracking data were also collected during the encoding phase, based on prior findings that eye movements may reflect hippocampal activity (e.g., Hannula & Ranganath, 2009). On average, participants exhibited significantly better item and source memory for words encoded with the Self task than with the Common task (self-reference effect), particularly for positively-valenced words. The self-reference effect for item memory was significantly positively correlated with AQ; this correlation was particularly strong for subjective recollection (i.e., "Remember") responses. Eye-tracking measures indicated greater dwell time proportion and number of fixations on word stimuli for low-frequency and positively-valenced words compared to high-frequency and negatively-valenced words, respectively, but did not differ by encoding task, and were not correlated with AQ. Overall, the findings suggest that self-referential encoding enhances both memory accuracy and the subjective experience of recollection to a greater degree for individuals with high AQ than low AQ.

Disclosures: L.H. Feintuch: None. **E.J. Hunter:** None. **A.A. Overman:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.23/TT18

Topic: H.07. Long-Term Memory

Support: NRF of Korea, Basic Science Research Program (2020R1A2C2007770)
NRF of Korea, Neurological Disorder Research Program (2020M3E5D9079913)
SNU, New Faculty Startup Fund

Title: Goal-dependent population dynamics during episodic memory retrieval

Authors: *M. KWON[1], G. KIM[1], S.-H. LEE[2];
[1]Dept. of Bio and Brain Engineering, Col. of Engin., KAIST, Daejeon, Korea, Republic of;
[2]Dept. of Psychology, Col. of Social Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The retrieval of specific information pertinent to the current goal from memory is crucial for guiding our behaviors. Previous studies have reported the diverse roles played by multiple areas, including the prefrontal cortex and hippocampus, in memory retrieval. Moreover, depending on the contents of memory, different information processing can take place in each of

these areas. Despite the intricacy of these processes, behavioral goals or demands can provide a framework for overall information processing, exerting influence on and shaping the neuronal responses in each region involved. To test this possibility at the neural population-level, we conducted a functional magnetic resonance imaging (fMRI) experiment using natural movie clips. Before the scan, participants watched six short movie clips and were asked to memorize them. On the following day, they performed two retrieval tasks: an action retrieval task and a place retrieval task. In the action retrieval task, participants focused on retrieving the actor's action from the cued episode, while in the place retrieval task, they were asked to recall the location information from the episode. By applying principal components analysis (PCA) to the neural responses during the retrieval period, we identified two components (PC1 and PC2) for each participant. We examined how distinct representations were plotted in the PC1-PC2 space, depending on the tasks. Remarkably, significantly distinct representations between the tasks were observed in the superior prefrontal cortex, lateral prefrontal cortex, and hippocampus from the early phase of retrieval, while there was a tendency for a gradual increase in distinction between the tasks during retrieval in the visual cortex. Additionally, in the ventral prefrontal cortex, such distinction was not observed. These results suggest that behavioral goals provide a framework for population-level representations in the superior and lateral prefrontal cortex, hippocampus, and visual cortex, while eliciting different temporal dynamics of the representations in these regions.

Disclosures: **M. Kwon:** None. **G. Kim:** None. **S. Lee:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.24/Web Only

Topic: H.07. Long-Term Memory

Title: Memory Retrieval Effects as a Function of Differences in Phenomenal Experience

Authors: *A. SCHMIDT[1], B. KIRWAN[2];
[1]Neurosci. Ctr., Brigham Young Univ., PROVO, UT; [2]Neurosci. Ctr., Brigham Young Univ., Provo, UT

Abstract: Conscious experience and perception are by definition restricted to a single perspective. There is evidence to suggest differences in phenomenal experience can produce observable differences in behavior, however how these differences can influence memory is not well reported. We tested n=49 participants who completed the Internal Representations Questionnaire (IRQ) and who underwent fMRI during encoding and a recognition memory test for faces and words. We calculated a cognitive bias reflecting individual participants' propensity toward either Visual Imagery or Internal Verbalization. There were weak positive correlations between memory performance for faces and a bias toward visual imagery and between memory performance for words and bias toward internal verbalization. We found a typical pattern of

activation for words vs. faces during both encoding and retrieval. There was no effect of internal representation bias on fMRI activation during encoding. At retrieval, visualization bias was positively correlated with memory-related activation for both words and faces in inferior occipital gyri. There was a crossover interaction in a network of brain regions such that visualization bias was associated with greater activation for words and verbalization bias was associated with greater activation for faces, consistent with increased effort for non-preferred stimulus retrieval. These findings suggest that individual differences in cognitive representations affect neural activation across different types of stimuli potentially affecting memory retrieval performance.

Disclosures: A. Schmidt: None. **B. Kirwan:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.25/TT19

Topic: H.07. Long-Term Memory

Support: NIH Grant MH055687

Title: Covert reinstatement during encoding predicts recall probability

Authors: *D. HALPERN[1], M. J. KAHANA[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Psychol, Univ. Pennsylvania, Philadelphia, PA

Abstract: Psychological theories of memory suggest that latent rehearsal or reactivation of previously seen items during an initial encoding experience affects subsequent remembering. Preliminary evidence for this hypothesis came from the primacy effect in the serial position curve and subsequent behavioral studies using overt rehearsal and articulatory suppression show that the amount and timing of item rehearsal affects which items are recalled and their output order. In this work, we make the prediction that this latent rehearsal may be correlate with reinstatement of item-specific neural activity. However, past analyses of neural reinstatement in memory tasks have been largely motivated by consolidation theory which primarily predicts that the most important time period of reinstatement for subsequent memory occurs during rest or sleep. Here, we leverage the high temporal precision of intracranial EEG to investigate the relationship between neural reinstatement that occurs between presentations of to-be-memorized items and its relationship to subsequent recall. We analyzed electrophysiological recordings from 220 neurosurgical patients performing 463 sessions of a categorized free-recall task. Lists comprised 12 words (four exemplars from each of three taxonomic categories, drawn from a set of 25 categories). Items appeared on the screen for 1.6 seconds each with 500-750ms blank inter-stimulus intervals (ISIs) in between. Following an arithmetic distractor task, subjects freely recalled as many words as possible. To investigate reinstatement, we compare the pattern similarity of oscillatory neural signals across all electrodes and 8 frequency bands during the

initial encoding presentation of a word and subsequent ISIs. Using a mixed effects model with varying intercepts and slopes for subjects, sessions, lists and serial positions, we find that the neural activity during ISIs is more similar to items on the list that are subsequently recalled than those that are not. To rule out potential confounds of similar memory processing or semantic similarity, we show that this result does not depend on whether the item before the ISI was itself subsequently recalled or whether it was from the same category as the initial item. However, unlike in psychological studies of rehearsal, the degree of neural reinstatement did not predict whether a specific item would be recalled first. Overall, these results suggest that neural reinstatement shares some but not all properties of behavioral measures of rehearsal and point to a new role for reinstatement in memory encoding.

Disclosures: D. Halpern: None. **M.J. Kahana:** None.

Poster

PSTR499. Neural Correlates and Encoding/Retrieval of Episodic Memory

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR499.26/TT20

Topic: H.07. Long-Term Memory

Support: NIH R01-MH0746492

Title: Does pattern reinstatement at retrieval reflect memory transformation?

Authors: *W. YU[1], A. ZADBOOD[2], A. J. CHANALES[3], L. DAVACHI[2];
[1]Psychology, [2]Columbia Univ., New York, NY; [3]Psychology, NYU, New York, NY

Abstract: Accumulating evidence has suggested that when retrieving a memory, the brain reinstates the activity pattern associated with existing memory representation. However, starting from its initial formation, memory representation in the brain continues to transform following repeated exposures and with offline consolidation. Yet it is unclear whether the dynamic changes in memory representation can be reflected during the reinstatement processes at retrieval. In other words, it remains an open question whether the brain reinstates the most updated representation following the latest transformation, or the activity elicited at very initial encoding. To test this question, we asked 29 participants to study once or thrice presented word-image pairs in an fMRI scanner, which was followed by a post-encoding rest period. Participants were then tested on their memory for the studied pairs immediately following the rest. Using representational similarity analysis, we quantified post-encoding replay during rest, as well as the level of retrieval reinstatement of both the encoded content (i.e., encoding-retrieval similarity) and the replayed content at rest (i.e., rest-retrieval similarity). Hippocampal replay frequency did not differ for once or repeatedly studied information. However, during successful retrieval, neural patterns in the hippocampus were significantly more similar to the rest activity associated with the repeated memories as compared to the once studied memories. In contrast, ventral temporal cortex, retrosplenial cortex and medial prefrontal cortex all significantly prioritized the

replay of thrice presented pairs over the once presented pairs at rest. However, both encoding-retrieval similarity and rest-retrieval similarity in these cortical regions were significantly higher for once studied than repeatedly studied content. Relating level of retrieval reinstatement to behavior, we found that faster response times can be predicted by higher encoding-retrieval similarity but lower rest-retrieval similarity in hippocampus, potentially suggesting that the reinstatement of the initial representation, rather than the replayed content during rest, is more beneficial for retrieval at an immediate memory test.

Disclosures: W. Yu: None. **A. Zadbood:** None. **A.J. Chanales:** None. **L. Davachi:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.01/TT21

Topic: H.08. Learning and Memory

Support: Stanley Fahn Young Investigator Award
Klingenstein-Simon's Foundation Fellowship
Aligning Science Across Parkinson's

Title: Spatiotemporal topography of striatum wide Acetylcholine release during Pavlovian learning and degradation of stimulus outcome associations

Authors: *S. BOUABID, L. ZHANG, M.-A. T. VU, M. HOWE;
Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Striatal cholinergic interneuron ((CINs) and Ach release) in the striatum to conditioned and unconditioned sensory cues and rewards show a multiphasic response that include a short excitation followed by a pause and can be followed by a rebound excitation. These responses are known to change differently with learning, yet little is known about the spatiotemporal dynamic of Ach release across the entire striatum during associative learning and degradation of stimulus-outcome association.Here, we used genetically encoded fluorescent sensors in combination with a novel optical approach to chronically map Ach changes on timescales ranging from 10s of milliseconds to weeks across over 100 striatum locations simultaneously in head-fixed mice during Pavlovian learning and degradation.We established that Ach release to conditioned cues during the acquisition of the predictive value exhibits a topography of region-specific changes in timing, polarity, and amplitudes. Interestingly, the well-characterized dip in Ach release was not present across all striatal regions. Ventral striatum Ach release to conditioned cues exhibited a phasic response that increases with learning. When the stimulus-outcome association was degraded, Ach signals dramatically changed specifically in the anterior medial dorsal striatum (aDMS), Ach dips significantly diminished or disappeared leaving place to a large rebound. Moreover, these changes in aDMS Ach signals aligned well with the strongest dips in DA signals recorded in the same region. This data indicates that Ach

signals are sensitive to outcome degradation and that region selective changes in Ach release occur selectively when cues lose predictive value, possibly promoting flexible degradation of stored information as contingencies change. Next, we determined how excitatory glutamatergic input to CINs shape Ach signals in aDMS during the degradation of stimulus-outcome association. We have found no change in glutamate release onto CINs during the degradation phase. Despite this, the glutamate release increase aligned with the timing of Ach large rebound following the degradation, indicating that the glutamate influence may be "unmasked" to drive the Ach increase.These results establish the striatum-wide acetylcholine release dynamic and suggest a role of Ach increases in aDMS in promoting flexible degradation of stimulus-outcome association.

Disclosures: S. Bouabid: None. **L. Zhang:** None. **M.T. Vu:** None. **M. Howe:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.02/TT22

Topic: H.08. Learning and Memory

Support: NIH Grant UG3MH120094
NIH Grant UF1MH130881
MJFF ASAP-020-519

Title: Transcriptional and anatomical distribution of medium spiny neurons in the primate caudal striatum

Authors: O. R. BRULL[1], G. ABDELHADY[2], J. HE[3], A. R. PFENNING[4], *A. C. BOSTAN[1], W. R. STAUFFER[1,2];
[1]Neurobio., [2]Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [3]Neurobio., Univ. of Pittsburgh, PITTSBURGH, PA; [4]Computat. Biol. Dept., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The striatum is the major input nucleus of the basal ganglia. This expansive nucleus is functionally organized according to cerebral cortical inputs and includes sensorimotor, associative, and limbic territories. Medium spiny neurons (MSNs) represent the majority of striatal neurons. MSNs have been traditionally classified according to their projection targets and their dopamine receptor expression. Using single-nucleus RNA sequencing (snRNA-Seq) we recently revealed that there are at least eight different medium spiny neuron (MSN) subtypes in the rostral regions of the non-human primate (NHP) striatum. The anatomical distribution of MSN subtypes in the rostral striatum differentiated between associative regions of the caudate and putamen in the dorsal striatum, and limbic regions of the ventral striatum, including the Nucleus Accumbens (NAc) and the olfactory tubercule (OT). Here, we aimed to determine the identity and distribution of MSN subtypes in caudal regions of the striatum, including the sensorimotor territory and the caudal ventral putamen. We sampled the head of the caudate, the

NAc, the sensorimotor putamen, and the caudal ventral putamen in three rhesus monkeys and performed snRNA-Seq. We integrated this data with the existing NHP rostral striatum data and identified cell type specific clusters corresponding to major cell classes, including MSNs, interneurons, and glia. The data revealed that MSN subtype clusters in the sensorimotor putamen resembled the MSN subtype clusters from associative regions of the rostral putamen and caudate. MSN subtypes in the caudal ventral putamen, however, clustered more closely with MSNs subtypes located in the NAc. We used fluorescent in situ hybridization (FISH) to confirm the presence of shared key gene markers between the caudal ventral putamen and the NAc. These cell typing results are consistent with previous studies that have shown the NAc and caudal ventral putamen regions share both limbic inputs and protein markers. These results provide new insights into the cell type specific architecture of the primate striatum and provide a roadmap to understanding cell type specific contributions to the wide array of limbic functions and dysfunctions that are associated with the striatum.

Disclosures: O.R. Brull: None. **G. Abdelhady:** None. **J. He:** None. **A.R. Pfenning:** None. **A.C. Bostan:** None. **W.R. Stauffer:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.03/TT23

Topic: H.08. Learning and Memory

Support: Start up funds from University of Missouri-Columbia

Title: Comparative study of the role of dorso-medial striatum (DMS), dorso-lateral striatum (DLS) and nucleus accumbens (NAc) in reinforcement learning and decision making

Authors: *A. BASAK, E. GOODIN, I. OZDEN;
Chem. and Biomed. Engin., Univ. of Missouri, Columbia, Columbia, MO

Abstract: Reinforcement learning (RL) lies at the center of our ability to adapt to a dynamically changing world. It is a process guided by reward and punishment to identify optimal actions and decisions by trial and error. In the brain, the neural circuitry of RL has been associated with the basal ganglia system, particularly the striatum, which receives reward-related information as dopaminergic projections from dopamine centers. Past research has suggested that different parts of the striatum play different functional roles in reward-driven behavior: The dorsomedial striatum (DMS) is involved in learning/selecting appropriate actions based on the expected outcomes. The lateral striatum (LS) is associated with habit formation and translating motor commands into specific actions. And the nucleus accumbens (NAc) is involved in reward expectation and pleasure-seeking. Our lab research focuses on goal-directed action selection, which is mainly associated with the DMS. However, recent studies have shown that other parts of the striatum also play a role in goal-directed behaviors and are modulated by dopaminergic

projections. However, which aspects of the learning process each striatal region specifically contributes to is still under debate. A better understanding of how different parts of the striatum affects basic behaviors might provide insight into their contributions to more complex behaviors. Accordingly, here we report different behavioral consequences of optogenetic stimulation of the direct pathway medium spiny neurons (dMSNs) in different striatal regions in three behavioral paradigms: (1) free behavior under unilateral stimulation of dMSNs; (2) field-preference under bilateral stimulation of the dMSNs; (3) an odor-cued Go-NoGo task under bilateral stimulation of dMSNs during feedback-period. Our results show that unilateral stimulation of the dMSNs of the DMS (n=5) and LS (n=2) led to rotational behavior in mice ($p<0.005$), whereas stimulation of dMSNs in NAc (n=3) did not. In the field-preference test, all mice showed a strong field preference in response to optogenetic stimulus ($p<0.005$). In the Go-NoGo task, we did not see any improvement in learning performance in response to optogenetic stimulation of dMSNs in DMS, however, mice performed the next trial after stimulus faster. These results are consistent with the idea that the DMS and LS associate dopamine input with motor movement, action selection, or motivation rather than with a pure reward signal. On the other hand, in NAc the effect of dopamine input is more consistent with reward. Currently, we are analyzing the data for mice where we stimulated dMSNs of the LS and NAc.

Disclosures: A. Basak: None. **E. Goodin:** None. **I. Ozden:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.04/TT24

Topic: H.08. Learning and Memory

Title: Opposing Memories in the Direct and Indirect Pathways of the Basal Ganglia: Unveiling Preserved Motor Memory in Parkinson's Disease

Authors: *K. WEN[1], Z. SHI[2], P. YU[1,3,4], L. MO[1], V. YANG[1], N. WESTNEAT[1], B. DOIRON[1,3,4], X. ZHUANG[1];
[1]Neurobio., [2]Genetics, Genomics, and Syst. Biol., [3]Statistics, [4]Grossman Ctr. for Quantitative Biol. and Human Behavior, Univ. of Chicago, Chicago, IL

Abstract: Loss of dopaminergic neurons causes motor deterioration in Parkinson's disease patients. We have previously reported that in addition to acute motor impairment, the impaired motor behavior is encoded into long-term memory in an experience-dependent and task-specific manner, a phenomenon we refer to as inhibitory learning, with its associated memory termed inhibitory memory. Surprisingly, we found that normal motor memory acquired prior to inhibitory learning remains preserved in the brain, suggesting the existence of separate engrams for normal motor memory and inhibitory memory. To investigate the neuronal circuits underlying these two memories, we utilized RNA-binding protein YTHDF1, an m6A RNA methylation reader that facilitates protein synthesis and learning/memory processes. Through

conditional knockout of *Ythdf1* in either D1 or D2 receptor-expressing neurons, we found that normal motor memory is stored in the D1 (direct) pathway of the basal ganglia, while inhibitory memory is stored in the D2 (indirect) pathway. Furthermore, our fiber photometry study and computational modeling also support the preservation of normal memory in the D1 pathway after inhibitory learning. These findings hold important implications for novel therapeutic approaches, such as reactivating preserved normal memory in Parkinson's disease and erasing aberrant motor memories in hyperkinetic movement disorders such as chorea or tics.

Disclosures: **K. Wen:** None. **Z. Shi:** None. **P. Yu:** None. **L. Mo:** None. **V. Yang:** None. **N. Westneat:** None. **B. Doiron:** None. **X. Zhuang:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.05/TT25

Topic: H.08. Learning and Memory

Support: JSPS KAKENHI 22J13309
JSPS KAKENHI 22KJ0901
JSPS KAKENHI 18H05525

Title: Learning-dependent neuronal activities in the posterior striatum, lateral geniculate nucleus and visual cortex in the visual discrimination task

Authors: *S. TANIMOTO[1,2], S. FUJISAWA[2,1];
[1]Univ. of Tokyo, Wako / Saitama, Japan; [2]RIKEN Ctr. For Brain Sci., Wako, Japan

Abstract: In everyday life, animals choose options as appropriately and quickly as possible. As they repeatedly experience the same situations, they become able to find important objects more automatically. Recent studies have revealed that the posterior part of the striatum (pStr) is innervated from various sensory areas and is involved in finding familiar objects automatically and quickly. However, how pStr changes its activities and contributes to finding objects quickly through learning remains unknown. In this study, focusing on visual processing, we examined how the representation of stimuli in pStr was shaped, along with its orchestration with the visual thalamus (dorsal lateral geniculate nucleus; dLGN) and cortex (visual cortex; VC). First, we developed a visual discrimination task using a T-shaped apparatus. After several sessions of training, the rats learned to discriminate the images to choose the correct arm, which reflected a success rate of > 80%. Reaction time, defined as the time to take from the start position to either arm of the T-maze, decreased steadily, and the judgement point, where the rats' trajectories were different between left and right chosen trials, got closer to the start position as learning progressed. To investigate the neuronal substrate of this learning process, we recorded the neuronal activities extracellularly using silicon probes from pStr, dLGN and VC simultaneously while the rats performed the task. We found that a sizable amount of neurons in these areas

exhibited judgement-related and/or task-phase-related activities. As the sessions went on, the peak position of firing rates in pStr moved forward to the start position, just before the judgement point. The proportion of these cells decreased through learning, while on the other hand, synchronization between pStr and visual areas got stronger. Our results indicate the involvement of the neuronal activities of the pStr and the orchestration with dLGN and VC in performing the visual discrimination task quickly.

Disclosures: S. Tanimoto: None. **S. Fujisawa:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.06/TT26

Topic: H.08. Learning and Memory

Support: NIH Grant U19 NS113201
NIH Grant R01 NS116753

Title: Diversity of discounting in biological and artificial reinforcement learning

Authors: *P. MASSET[1], P. TANO[2], H. R. KIM[1,3], A. N. MALIK[1,4,5], P. BECH VILASECA[1,6], A. POUGET[2], N. UCHIDA[1];
[1]Harvard Univ., Cambridge, MA; [2]Univ. Hosp. of Geneva, Univ. Hosp. of Geneva, Geneva, Switzerland; [3]Sungkyunkwan Univ., Suwon, Korea, Republic of; [4]Warren Alpert Med. Sch. of Brown Univ., Brown Univ., Providence, RI; [5]Norman Prince Neurosciences Inst., Rhode Island Hosp., Providence, RI; [6]EPFL, Lausanne, Switzerland

Abstract: To thrive in complex environments, animals and artificial agents must learn to act adaptively to maximize fitness and rewards. Reinforcement learning, a type of learning algorithm has been successful at training artificial agents to superhuman performance in an array of complex tasks and that explains dopaminergic neurons' activity as encoding a reward prediction error. In classical reinforcement learning, agents discount future rewards exponentially according to a single time scale, known as the discount factor. This strategy is at odds with the need of agents to make decisions at multiple timescales, and with the empirical observation that humans and animals use non-exponential discounts in many situations. Here, we explore the possibility that the nervous system uses multiple discounting time scales instead. We first show that reinforcement agents learning at multiple timescales possess distinct computational benefits including *(1)* a richer representation of the temporal evolution of rewards, *(2)* an ability to infer reward timing before learning converges and *(3)* better adaptation to alternative discount mechanisms to produce more accurate estimates and behave more efficiently. Next, we report that dopaminergic neurons in mice performing two behavioral tasks encode reward prediction error with a diversity of discount time constant. We recorded the activity of optogenetically identified dopaminergic neurons in mice performing two behavioral tasks: a cued delayed reward

task and navigation in a 1-D virtual reality track. In the cued delayed reward task, each individual neuron's (*n=47* neurons) cue response was well-fit by a distinct exponential discount function. The diversity of discount factors across neurons (*n=111* neurons) provides a vectorized error signal that allows parameter-free decoding of reward timing. In the navigation task, the diversity of ramping activity across dopaminergic neurons can be explained by single neurons with different discount factors experiencing a common value function. Thus, the dopaminergic signal contains distributional information about expected reward timing. Crucially, the measured discount factor of individual neurons recorded in both tasks (*n=40* neurons) is correlated across the two tasks (*r=0.45, p=0.0013*) suggesting that it is a cell-specific property, similar to tuning of visual cortex neurons. Together, our results suggest that the nervous system learns at a multitude of timescales, providing a new paradigm to interpret motivation and timing deficits in disease and opening new avenues for the design of more efficient reinforcement learning algorithms.

Disclosures: **P. Masset:** None. **P. Tano:** None. **H.R. Kim:** None. **A.N. Malik:** None. **P. Bech Vilaseca:** None. **A. Pouget:** None. **N. Uchida:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.07/TT27

Topic: H.08. Learning and Memory

Support: Wellcome Trust
Royal Society
MRC
CIFAR
BBSRC
Gatsby Foundation

Title: Striatal dopamine reflects individual long-term learning trajectories

Authors: ***S. C. LIEBANA GARCIA**[1], A. LAFFERE[1], C. TOSCHI[1], L. SCHILLING[1], J. PODLASKI[1], P. ZATKA-HAAS[1], Y. LI[3], R. BOGACZ[2], A. M. SAXE[4], A. LAK[1];
[1]Dept. of Physiology, Anat. and Genet., [2]MRC Brain Network Dynamics Unit, Univ. of Oxford, Oxford, United Kingdom; [3]Peking Univ., Beijing, China; [4]Gatsby Computat. Neurosci. Unit and Sainsbury Wellcome Ctr., Univ. Col. London, London, United Kingdom

Abstract: Learning from naïve to expert often occurs over long periods of time. This long-term learning entails extensive changes in behavior, supported by changes in underlying neuronal signals. The principles governing behavioral and neuronal dynamics during long-term learning remain unknown. We developed a visually-guided decision task that mice learned over several weeks. The training used a complete psychophysical task from the start, and was kept unchanged

throughout the experiment, allowing us to study learning trajectories without shaping them. Mice adopted sequences of strategies that became more stimulus-dependent over time, showing substantial diversity in the strategies they transitioned through and settled on. These transitions were systematic; the initial strategy of naïve mice predicted their strategy several weeks later. Longitudinal imaging of dopamine release in dorsal striatum demonstrated that dopamine signals reflect each individual's learning trajectories. Stimulus-evoked dopamine signals emerged as animals transitioned to stimulus-dependent strategies, strongly reflecting each individual's strategy in using stimuli to make decisions. Consistently, reward-evoked dopamine signals decreased, mirroring improvements in accuracy throughout learning. A deep neural network model trained on the task with reinforcement learning captured behavioral and dopamine dynamics within and across mice. Analyzing the model revealed saddle points in its loss landscape, where learning trajectories slowed down before strategy transitions, accounting for the animals' trajectories. The model captured dorsal striatum dopamine signals with prediction errors determined by the strategies used by each animal throughout learning. Together, our results demonstrate that long-term learning involves diverse yet systematic transitions through behavioral strategies, and that dopamine signals as well as teaching signals of deep networks exhibit key characteristics to support such learning.

Disclosures: **S.C. Liebana Garcia:** None. **A. Laffere:** None. **C. Toschi:** None. **L. Schilling:** None. **J. Podlaski:** None. **P. Zatka-Haas:** None. **Y. Li:** None. **R. Bogacz:** None. **A.M. Saxe:** None. **A. Lak:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.08/TT28

Topic: H.08. Learning and Memory

Support: R01 NIMH126178

Title: Serotonin modulates the neural representation of action selection in the dorsomedial striatum during goal-directed behavior

Authors: *A. SHARMA[1], K. H. NG[1], S. S. DESROCHERS[1], J. R. MANNING[2], K. NAUTIYAL[1];
[2]Dept. of Brain and Psychological Sci., [1]Dartmouth Col., Hanover, NH

Abstract: The dorsomedial striatum (DMS) is critical for the acquisition and expression of goal directed behavior. Although a large amount of research on learning action-outcome associations and reward processing focuses on the role of dopamine signaling in the striatum, it is clear that serotonin also modulates these behaviors and DMS signaling. Our prior work shows that serotonin modulates reward processing in goal-directed behaviors, specifically through the serotonin 1B receptor (5-HT1BR), however, the underlying neural circuit mechanisms are

unclear. To explore how serotonin could influence the neural encoding of goal-directed actions, we ablated the 5-HT1BR in mice and measured calcium activity in medium spiny neurons during a simple cue-guided operant reward task. We used 1-photon calcium imaging with microendoscopes to record calcium activity in individual neurons in the DMS during the task. First, using trial-based single cell calcium event analysis we found that the majority of trial-responsive neurons showed reductions in the number of calcium events during the cue and reward in control mice. This inhibition was reduced in the mice lacking 5-HT1BR, with fewer cells showing decreased calcium events during the cue and reward periods. To begin to dissociate the neural activity encoding motor behavior from that encoding reward, we analyzed the calcium activity when different motor actions (ie., a poke in the left vs right port) resulted in the same reward outcome. Decoders were able to distinguish between left and right trials using the calcium activity during the cue and reward period. Ongoing work will look for shared patterns in the neural activity encoding left and right trials to determine whether the representations common to these trial types are encoded consistently across trials, sessions, and animals. Additionally, we can examine which aspects of the representations are influenced by our manipulations to serotonin signaling. Overall, this work contributes to our understanding of how the neural activity in the DMS encodes goal-directed behavior, and how it is modulated by serotonin.

Disclosures: A. Sharma: None. **K.H. Ng:** None. **S.S. Desrochers:** None. **J.R. Manning:** None. **K. Nautiyal:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.09/UU1

Topic: H.08. Learning and Memory

Support: NIH Brain Initiative MH120894
Aligning Science Across Parkinson's
Klingenstein-Simons Foundation Fellowship

Title: Multi-fiber photometry reveals functionally distinct spatiotemporal topography of striatum-wide dopamine release

Authors: *M.-A. T. VU[1], M. J. WEN[5], E. H. BROWN[2], Z. ZHANG[3], L. MROZ[6], T. M. OTCHY[3], S. BOUABID[1], D. A. BOAS[4], M. W. HOWE[1];
[1]Psychological & Brain Sci., [2]Grad. Program in Neurosci., [3]Biol., [4]Biomed. Engin., Boston Univ., Boston, MA; [5]Neurobio., Harvard Med. Sch., Boston, MA; [6]Northeastern Univ., Boston, MA

Abstract: Dopamine (DA) release in the striatum is critical for diverse functions, including motivation, reward response, motor control, learning, and memory. Recent studies have provided

evidence that dopamine release to cues, rewards, and movements varies in amplitude and timing across striatal subregions, suggesting that region-specific dopamine signals may support distinct functions in learning and behavior. However, current optical approaches have been limited to measuring dopamine release across only one or two small striatum regions in a given subject, and a complete view of the spatiotemporal topography of rapid dopamine signals and their functional specificity is therefore lacking. To address this, we developed a multi-fiber photometry approach to monitor dopamine release with sub-millimeter spatial resolution and sub-second temporal resolution at over 50 locations simultaneously throughout the striatum in awake, behaving mice expressing the fluorescent dopamine indicator dLight 1.3. Our chronic implants allowed us to record striatal dopamine release over weeks as mice were presented with salient stimuli, ran on a treadmill ball, and were trained in a Pavlovian learning task. Leveraging the simultaneity of our multi-site recordings, we clustered dopamine activity based on short timescale (sub-second) cross-correlations. These clusters showed spatial and functional organization that generalized across mice and behavioral contexts, such that knowing spatial location enabled us to predict functional specialization, and vice versa. Taken together, these initial findings provide the largest scale description of rapid dopamine release topography in the striatum to date and begin to define the spatial territories over which functional dopamine signals may influence distinct aspects of learning and behavior.

Disclosures: **M.T. Vu:** None. **M.J. Wen:** None. **E.H. Brown:** None. **Z. Zhang:** None. **L. Mroz:** None. **T.M. Otchy:** None. **S. Bouabid:** None. **D.A. Boas:** None. **M.W. Howe:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.10/UU2

Topic: H.08. Learning and Memory

Title: Line-1 long non-coding rnas at striatal circuits contribute to the transition from flexible to inflexible behavioral control

Authors: *A. LONGARETTI, D. MANGONI, V. PAGET-BLANC, A. BOENDER, Y. PELLOUX, L. NAVA, M. F. VEGGI, P. LAU, G. TARTAGLIA, S. GUSTINCICH, R. TONINI;
Inst. Italiano di Tecnologia, Genova, Italy

Abstract: The ability to adapt behavior to an ever-changing environment requires flexible control of behavior, which depends on the causal relationship between an action and its outcome (A-O). With repetition, behavior becomes inflexible; actions are no longer sensitive to changes in A-O associations and are primarily elicited by retrospective events. The underlying molecular mechanisms of this transition remain, however, elusive. The epigenetic modification of chromatin offers a mechanistic link between the genome and environmental experience, including the transition from flexible to inflexible behavioral control. In particular, epigenetic

modifications can unsilence the expression of transposable elements, which in turn can act as a key factor for post-transcriptional regulation of gene expression. In this study, we demonstrated that overtraining of an instrumental conditioning task (i.e., nose poke for food reinforcement) is associated with increased RNA levels of the Long Interspersed Nuclear Elements -1 (L1) in the dorsolateral striatum (DLS). Upregulation of L1 RNAs expression occurs in parallel to decreased methylation of L1 promoters and reduced expression of the DNA methyltransferase 3b. Viral-mediated silencing of L1 transcripts in the DLS preserves behavioral flexibility, thus establishing a direct role of L1 upregulation in behavioral control. We are currently investigating mechanisms by which L1 RNAs interfere with bioavailability of key mRNA targets involved in synaptic processes that subserve the updating of behavioral strategy during the presentation of a new A-O contingency. Our findings support the role of (epi)genomic plasticity in instrumental behavior. This study reveals molecular substrates that might be relevant for the numerous neuropsychiatric conditions that are characterized by a loss in behavioral flexibility and striatal circuit dysfunction.

Disclosures: **A. Longaretti:** None. **D. Mangoni:** None. **V. Paget-Blanc:** None. **A. Boender:** None. **Y. Pelloux:** None. **L. Nava:** None. **M.F. Veggi:** None. **P. Lau:** None. **G. Tartaglia:** None. **S. Gustincich:** None. **R. Tonini:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.11/UU3

Topic: H.08. Learning and Memory

Support: 318278-03

Title: Lateral orbitofral cortex controls learning through modulating reward-outcome in striatum via dopamine

Authors: *C. QI[1], C. T. LI[2];
[1]Inst. of Neurosci., ShangHai, China; [2]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai City, China

Abstract: Expectation (prediction) is vital for learning both for animals and human. How expectation guides learning in the brain is poorly understood. To tackle this question, we trained mice to learn an olfactory working memory task with varying reward expectation. Higher reward expectation led to faster learning rate when mice learned the task. With 2-photon calcium imaging technique, we observed that the striatum-projecting neurons in the lateral orbitofrontal cortex (lOFC) could encode the reward expectations. During the expectation period, optogenetically suppressing the activity of the lOFC, or the lOFC-dorsomedial striatum (dmStriatum) pathway, significantly improved the learning rate when the reward expectation was relatively low. Consistently, activating the medial spiny neurons expressing dopamine D2-

receptors (D2-MSNs), but not the D1-MSNs, in the dmStriatum also improved the learning rate. On the contrary, suppressing D2-MSNs activity impaired learning rate. Furthermore, the activity of D2-MSNs could encode both reward expectation and reward outcome. Optogenetically suppressing the activity of lOFC-dmStriatum pathway enhanced the coding ability of D2-MSNs for reward outcome. Besides, suppressing the lOFC-dmStriatum pathway also enhanced encoding of negative prediction error of dopamine activity in the dmStriatum. Consistently, optogenetically suppressing the activity of dopaminergic axons in dmStriatum also improved learning rate. Taken together, the expectation signals in the lOFC modulated coding ability of D2-MSNs for reward outcome via modulating dopamine in the dmStriatum. Our results shed light on the neuronal and the circuitry mechanisms underlying learning modulation by expectations

Disclosures: C. Qi: None. **C.T. Li:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.12/UU4

Topic: H.08. Learning and Memory

Support: NRF-2019R1A2C2005213
NRF-2020M3E5D9079913
NRF-2020M3E5D9079908
Creative-Pioneering Researchers Program through Seoul National University

Title: Tactile value encoding in single neurons of the primate putamen: comparison with visual value encoding

Authors: *J. LEE[1], S. HWANG[2], D. PARK[4], S.-H. LEE[3], H. KIM[1];
[2]SNU, [3]Seoul Natl. Univ., [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [4]Korea Advanced Inst. of Sci. and Technol., KAIST, Daejeon, Korea, Republic of

Abstract: Primates employ their hands and fingers to perceive tactile input and utilize it to optimize decision-making. Understanding the brain mechanism in processing tactile value is crucial for comprehending decision-making across different sensory modalities. The putamen in the striatum is considered an important brain structure that potentially encodes tactile value information due to its anatomical connections with the somatosensory cortex and its plausible role in processing values through inputs from the midbrain dopamine region. Thus, we hypothesize that the tactile value is processed in the putamen to guide decision-making.
To address this, we developed a new task (tactile value task) that allowed macaque monkeys to discriminate between two different tactile stimuli (braille patterns) associated with different rewards (Good-liquid reward vs. Bad-no reward). The association between tactile stimuli and

rewards was reversed in each block. During the task, the same stimulus was presented twice, and we measured monkeys' reaction times when they re-touched the stimulus. After monkeys recognized the associated value, the reaction times became faster for the good tactile stimulus compared to the bad one (386 ± 71 vs. 739 ± 156 ms, respectively). Further validation through two-stimuli-choice trials showed that monkeys successfully selected the good tactile stimuli, achieving a correct rate of 92.72 ± 8.39%.
Next, we recorded putamen neurons during tactile and visual value tasks to investigate whether they exhibit modality-specific preferences or cross-modal value processing. Interestingly, we identified three types of neurons: tactile value-selective, visual value-selective, and both modality value-encoding neurons. Out of 247 value-encoding neurons recorded from two monkeys, 41 neurons (17%) encoded tactile value, 77 neurons (31%) encoded visual value, and 129 neurons (52%) encoded values from both modalities. These results demonstrate that monkeys successfully discriminate and evaluate tactile stimuli, with the putamen serving as a key region for representing tactile values. These three groups of neurons process values from different modalities, possibly allowing primates to recognize shared common values (e.g., good and bad) while also distinguishing their modal origins.

Disclosures: **J. Lee:** None. **S. Hwang:** None. **D. Park:** None. **S. Lee:** None. **H. Kim:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.13/UU5

Topic: H.08. Learning and Memory

Support: Basic Science (NRF-2019R1A2C2005213)
Neurological Disorder (2020M3E5D9079913) Research Programs
Creative-Pioneering Researchers Program

Title: Tactile value representation in the human basal ganglia: Comparison with visual value representation

Authors: *S. HWANG[1], D. PARK[3], S.-H. LEE[2], H. KIM[1];
[2]Seoul Natl. Univ., [1]Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Korea Advanced Inst. of Sci. and Technol., KAIST, Daejeon, Korea, Republic of

Abstract: For primates, including humans, tactile perception through hands and fingers is particularly crucial for collecting value information and making the best decision. It is thus important to understand how the primate brain processes the reward value associated with tactile stimuli. The primate striatum in the basal ganglia is a region of updating value change for cognitive flexibility. However, previous studies have focused on the role of the striatum in visual value processing, which raises a question about its involvement in tactile value processing.To test whether the striatum processes the tactile value information, we conducted a study using

functional magnetic resonance imaging (fMRI) to examine the neural responses of the human striatum during the tactile perception and evaluation of braille patterns (tactile stimuli) associated with the monetary reward. We designed a novel “tactile value discrimination task” where participants were instructed to touch tactile stimuli associated with different monetary rewards (Good: + ₩150 or Bad: - ₩150) and report whether the stimulus was associated with reward or not. In this task, participants had to recognize which tactile stimulus was associated with a monetary reward by trial-and-error with their index finger. We implemented a reversal of the contingency between tactile stimuli and rewards in each block to generate flexible value, and it also minimized potential bias resulting from selective responses to the tactile stimuli. To examine the brain region involved in the tactile and/or visual value processing, we simultaneously presented tactile and visual objects to the participants, and they had to focus on the instructed stimulus for a correct decision. Most participants successfully discriminated the flexible values of objects with both tactile and visual modalities (96 ± 0.59% and 96 ± 0.64% of correct choice, respectively, n = 22). Notably, flexible value of tactile stimuli was primarily decoded from the neural activation pattern in the putamen. We further found that other different areas in the nucleus accumbens and caudate nucleus represented tactile and visual flexible values. These results suggest that tactile and visual flexible values are processed in the different subsets of striatal regions.

Disclosures: **S. Hwang:** None. **D. Park:** None. **S. Lee:** None. **H. Kim:** None.

Poster

PSTR500. Striatal and Corticostriatal Circuits

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR500.14/UU6

Topic: H.08. Learning and Memory

Support: KAKENHI Grant JP21K15210
KAKENHI Grant JP21H00311
KAKENHI Grant JP21K18557
KAKENHI Grant JP22H01105
KAKENHI Grant JP18H02542
KAKENHI Grant JP21H00311
KAKENHI Grant JP22H029440
AMED Grant JP22gm6510012
AMED Grant JP21wm0425010
AMED Grant 21gm1510006
Salt Science Research Foundation Grant 2146
Salt Science Research Foundation Grant 2240
Salt Science Research Foundation Grant 2137
Salt Science Research Foundation Grant 2229

Title: Dual roles for nucleus accumbens core dopamine D1-expressing neurons projecting to the substantia nigra pars reticulata in limbic and motor control

Authors: *S. ATTACHAIPANICH, T. OZAWA, T. MACPHERSON, T. HIKIDA;
Osaka Univ., Osaka, Japan, Japan

Abstract: The nucleus accumbens (NAc) is a critical component of a limbic basal ganglia circuit that is thought to play an important role in decision-making and the processing of rewarding stimuli. As part of this circuit, dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs) of the NAc core are known to send a major projection to the substantia nigra pars reticulata (SNr). Interestingly, the SNr is an important structure in a sensorimotor basal ganglia pathway, highlighting the possibility that SNr-projecting NAc D1-MSNs ($NAc^{D1\text{-}MSN}$-SNr) may be able to influence both limbic and motor functions. Here, we used pathway-specific optogenetic manipulation of the $NAc^{D1\text{-}MSN}$-SNr pathway to investigate its role in reward-related and motor behaviors. In D1-Cre mice, AAV constructs expressing the excitatory opsin channelrhodopsin-2 or the inhibitory opsin archaerhodopsin were infused into the NAc core region and bilateral optic fibers were implanted into the SNr to enable activation or inhibition, respectively, of the $NAc^{D1\text{-}MSN}$-SNr pathway. Activation of the $NAc^{D1\text{-}MSN}$-SNr pathway induced a significant preference for a laser-paired location in a real-time place preference test and augmented an instrumental response for both a laser-paired touch panel and a liquid reward-paired touch panel in two-choice optogenetic self-stimulation tests. Additionally, in an open field arena, bilateral stimulation of the $NAc^{D1\text{-}MSN}$-SNr pathway increased forward locomotion, while unilateral stimulation induced contralateral turning behavior. Interestingly, inhibition of this pathway had no significant effect on either reward-related or locomotor behaviors, suggesting that the $NAc^{D1\text{-}MSN}$-SNr pathway does not bidirectionally control such behaviors. These findings indicate that the NAc D1-MSNs-SNr pathway is able to control both reward-related and motor behaviors and provides evidence of overlap between limbic and motor basal ganglia pathways.

Disclosures: S. Attachaipanich: None. **T. Ozawa:** None. **T. Macpherson:** None. **T. Hikida:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.01/UU7

Topic: H.08. Learning and Memory

Support: NIDA P30 DA048742-01A1
NIH Grant R01-NS104071
NIH Grant F31-NS127417
University of Minnesota's MnDRIVE
McKnight Presidential Fellowship

Title: Hippocampal GABAergic neurons inhibit supramammillary neurons projecting to the dentate gyrus

Authors: *L. GLASSBURN[1], J. WEINER[2], E. KROOK-MAGNUSON[2];
[1]Univ. of Minnesota Grad. Program In Neurosci., Saint Paul, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The supramammillary area (SuM) is a region of the hypothalamus which projects directly to the hippocampal dentate gyrus and CA2, and has been shown to impact hippocampal theta oscillations, spatial and social processing, and memory. In contrast, hippocampal influence over the SuM is largely uncharacterized. Previous work from the Krook-Magnuson lab established that a neuronal nitric oxide synthase (nNOS)-expressing inhibitory neuron population, termed LINCs, project from the hippocampus to the SuM. Using fluorogold retrograde tracing, we find that inhibitory cells from across the hippocampal dorsoventral axis and across hippocampal subregions project to the SuM, of which LINCs is only one subpopulation. Applying immunohistochemistry for somatostatin, we find that many of the hippocampal projection cells are somatostatin-expressing neurons (SOM) (40.4 +/- 8%, n=3 mice), and therefore likely separate from LINCs. By injecting Cre-dependent AAVs into the hippocampus of SOM-Cre and nNOS-Cre mice, we additionally find that hippocampal inhibitory fibers are predominantly from SOM+ neurons, with a smaller contribution from nNOS+ neurons. We performed slice electrophysiology to investigate which SuM cells are directly post-synaptic to hippocampal inhibitory projections. To do so, we injected AAVs into the hippocampus to express axon-targeted ChR2 under the mDlx promoter to target hippocampal GABAergic neurons and retrogradely labeled SuM neurons projecting to the dorsal dentate gyrus (SuM->DG cells). This allowed us to patch fluorescent SuM->DG cells and optogenetically stimulate hippocampal inhibitory fibers in the SuM. We find that hippocampal inhibitory cells synapse directly onto SuM->DG neurons, with apparent preference for retrogradely labeled SuM->DG neurons over neighboring, non-labeled, SuM neurons: 5 of 13 retrogradely labeled SuM->DG cells displayed a postsynaptic response (38%) vs. 3 of 25 unlabeled SuM neurons showed a postsynaptic response (12%). As we therefore hypothesize that this inhibitory connection preferentially modulates SuM signals to the hippocampal dentate gyrus, we hypothesize that hippocampal inhibitory input to the SuM may play a role in modulating spatial processing. Our future experiments will further characterize this previously unexplored inhibitory projection and the role it may play in spatial memory.

Disclosures: L. Glassburn: None. **J. Weiner:** None. **E. Krook-Magnuson:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.02/UU8

Topic: H.08. Learning and Memory

Support: NSERC Discovery 506730
CIHR 507489
NSERC CGS-M
Ontario Graduate Scholarship

Title: Characterization of Mammillary Body Nuclei in Egocentric Spatial Memory

Authors: *K. D. MAR[1], C. SO[2], Y. HOU[2], J. KIM[1,2];
[1]Psychology, [2]Cell & Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The mammillary bodies (MB) have been implicated in processing spatial information however, the role of distinct MB nuclei remains elusive. The organization of pathways between the medial (MM) and lateral (LM) MB nuclei are distinct form one another and are topographically organized which may represent a functional differentiation within the structure. The present study begins to characterize the role of the MB nuclei in egocentric spatial memory through synaptic and optogenetic inhibition of MM or LM circuits. We also sought to characterize egocentric spatial memory deficit in the 5xFAD mouse model of Alzheimer's Disease (AD) that present amyloid-beta burden in the MM and LM at the earliest timepoint. To evaluate egocentric spatial memory, mice were tested on a path integration assay that requires the use of self-motion cues to escape to a previously memorized shelter location in complete darkness. To determine the requirement for the MB nuclei in egocentric spatial memory, we blocked the synaptic vesicle release from either the MM or ML using Cre mediated expression of tetanus toxin light chain in male and female Nts-Cre and Tac2-Cre mice respectively. Silencing the MM impaired performance in the path integration task after 3-weeks, and induced severe deficits in visuospatial movement, balance, posture, and nest making after 4-weeks. To investigate how disease progression of AD impacts egocentric spatial memory, we tested male and female Tg and WT 5xFAD animals at 3- and 5- months of age and characterized the amyloid burden in the MB and connected structures. Transgenic animals were impaired in path integration at 5-months and secondary analysis of sex revealed a more profound deficit in males. Ongoing investigations aim to characterize the requirement for the MB nuclei within subjects using Cre mediated expression of ArchT for optogenetic inhibition.

Disclosures: K.D. Mar: None. **C. So:** None. **Y. Hou:** None. **J. Kim:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.03

Topic: H.08. Learning and Memory

Support: NIH Grant K99AG073507
BBRF Grant 30397
JSPS KIBAN(S) 19H05646

JSPS KIBAN(S) 23H05478
NIH Grant MH118928
NIH/NIA Grant P30AG066512
NIH/NIA Grant P01AG060882
NIH Grant RF1AG072507
NIH Grant R21AG070880
AHA Grant 23PRE1021217
JSPS Grant-in-Aid for Early-Career Scientists 18K14857
JSPS Grant-in-Aid for Young Scientists 16K18373
Grant-in-Aid for JSPS Fellows 16F16386

Title: Hypothalamic Theta Modulation Improves Memory

Authors: *S. CHEN[1,2], L. HE[2,3], S. J. MIDDLETON[2], N. LAM[1], Z. LI[1], Y. XIE[1], J. TANG[1], T. NGUYEN[1], V. BERBANO[1], A. MASURKAR[1], T. M. WISNIEWSKI[1], Z. S. CHEN[1], T. MCHUGH[2];
[1]New York University, Sch. of Med., New York, NY; [2]RIKEN Ctr. for Brain Sci., Wako, Japan; [3]Univ. of California San Francisco, San Francisco, CA

Abstract: Brain rhythms are fundamental features of coordinated neural activity underlying various brain functions and neural mechanisms. In the hippocampus the theta (4-12 Hz) rhythm is crucial for learning and memory, however, uncertainty about the anatomical origin and related circuitry that control theta rhythms remains. In particular, while ascending hypothalamic circuits are known to play a role in theta modulation, how this impacts learning has been understudied. Taking advantage of a transgenic mouse line that allows for specific gene expression in the hypothalamic supramammillary nucleus (SuM), we confirmed the SuM as a modulator of hippocampal theta oscillations. Optogenetic stimulation of the SuM that expresses channelrhodopsins robustly induced hippocampal theta oscillations. Furthermore, the entrained theta rhythm significantly enhances animals' learning of a hippocampal-dependent spatial memory task. To elucidate the physiological mechanism behind SuM theta modulation and associated impact on animals' behavior, we performed in vivo electrophysiological recordings under optogenetic SuM stimulation. We found that SuM-induced theta oscillations globally reshape hippocampal coding, e.g. place cell activity, at both single unit and population levels during different behavioral phases, in particular the synchronization of place cell activities. These results extend our previous findings of the SuM as a hypothalamic hub that routes novelty signals to the hippocampus for memory modulation by highlighting its important physiological role in modulating and synchronizing in vivo hippocampal activities.

Disclosures: **S. Chen:** None. **L. He:** None. **S.J. Middleton:** None. **N. Lam:** None. **Z. Li:** None. **Y. Xie:** None. **J. Tang:** None. **T. Nguyen:** None. **V. Berbano:** None. **A. Masurkar:** None. **T.M. Wisniewski:** None. **Z.S. Chen:** None. **T. McHugh:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.04/UU9

Topic: H.08. Learning and Memory

Support: Hyogo Innovative Challenge, Hyogo Medical University (NHN)
Takeda Science Foundation (NHN)
Grant-in-Aid for Scientific Research (18K06533, 22K07335) of the Japan Society for the Promotion of Science (NHN)

Title: Brain-breath interactions: Memory enhancement and decline induced by central respiratory activity during encoding

Authors: *N. H. NAKAMURA[1], H. FURUE[1], K. KOBAYASHI[2], Y. OKU[1];
[1]Physiol., Hyogo Med. Univ., Nishinomiya, Japan; [2]Viral Vector Develop., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: During offline brain states, such as sleep and memory consolidation, respiration coordinates hippocampal activity. However, the role of breathing during online memory traces remains unclear. Here, we show that respiration is recruited during online memory encoding. Optogenetic manipulation was used to control activation of the primary inspiratory rhythm generator PreBötzinger complex (PreBötC) in transgenic mice. When intermittent PreBötC-induced apnea covered the object exploration time during encoding, novel object detection was impaired. Moreover, the mice did not exhibit freezing behavior during presentation of fear-conditioned stimuli (CS^+) when PreBötC-induced apnea occurred at the exact time of encoding. This apnea did not evoke changes in CA3 cell ensembles of the hippocampus between presentations of CS^+ and conditioned inhibition (CS^-), whereas in normal breathing, CS^+ presentations produced dynamic changes. Notably, different patterns of central respiratory activity (e.g., frequency and phase irregularity) predicted enhanced or failed memory performance. These findings demonstrate that components of central respiratory activity during online encoding strongly contribute to shaping hippocampal ensemble dynamics and memory performance.

Disclosures: **N.H. Nakamura:** None. **H. Furue:** None. **K. Kobayashi:** None. **Y. Oku:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.05/UU10

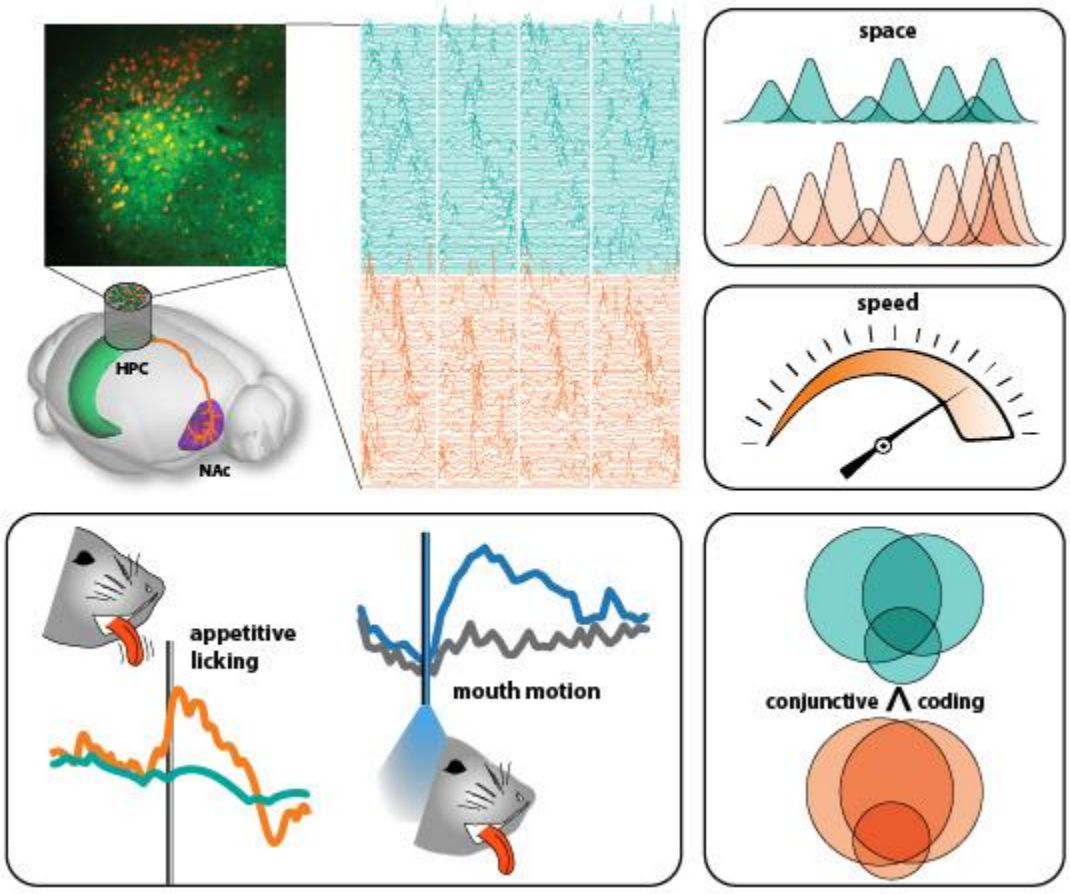
Topic: H.08. Learning and Memory

Support: DFG SFB grant 1089
ERC Consolidator grant Sub-D-Code

Title: A hippocampus-accumbens code guides goal-directed appetitive behavior

Authors: *O. BARNSTEDT, P. MOCELLIN, S. REMY;
Cell. Neurosci., Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: The dorsal hippocampus (dHPC) is a key brain region for the expression of spatial memories, such as navigating towards a learned reward location. The nucleus accumbens (NAc) is a prominent projection target of dHPC and implicated in value-based action selection. To understand how the coding properties of individual NAc-projecting hippocampal neurons ($dHPC^{\rightarrow NAc}$) support spatial reward memory expression, we used *in vivo* dual-color two-photon imaging while mice navigated towards a learned reward zone. In contrast to other dHPC neurons, the $dHPC^{\rightarrow NAc}$ subpopulation contained more place cells, with enriched spatial tuning properties. This subpopulation also showed enhanced coding of non-spatial task-relevant behaviors such as deceleration and appetitive licking, both of which could be elicited by optogenetic activation of dHPC terminals in NAc. A generalized linear model revealed enhanced conjunctive coding in $dHPC^{\rightarrow NAc}$ neurons which improved the identification of the reward zone. We propose that dHPC routes specific reward-related spatial and behavioral state information to guide NAc action selection.



Disclosures: O. Barnstedt: None. **P. Mocellin:** None. **S. Remy:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.06/UU11

Topic: H.08. Learning and Memory

Support: National Research, Development and Innovation Office (NRDI), Hungary K132735
Eötvös Loránd Resarch Network Distinguished Reserach Project grant
NRDI Office of Hungary within the framework of the Artificial Intelligence National Laboratory Program (RRF-2.3.1-21-2022-00004)
NRDI Office of Hungary within the framework of the Translational 943 Neuroscience National Laboratory (RRF2.3.1-21-2022-00011)

Title: Modulation of hippocampal patterns by the raphe-hippocampal glutamatergic connection.

Authors: M. JELITAI[1], A. M. BARTH[1], K. PETRIK[1], T. CHAVES[1], P. BARTHO[2], *V. VARGA[1];
[1]Inst. of Exptl. Med., Budapest, Hungary; [2]Res. Ctr. for Natural Sci., Budapest, Hungary

Abstract: In recent years, one of the centers of the ascending serotonergic system, the median raphe nucleus (MR) has been proved to contain a large glutamatergic neuron group. One of the main targets of these vesicular glutamate transporter type 3 (VGluT3)-expressing median raphe neurons is the dorsal hippocampus, a key node of the brain's episodic memory circuit. We uncovered previously that the raphe-hippocampal glutamatergic connection selectively and highly efficiently activates certain types of hippocampal inhibitory neurons at very short latency. However, the function of this powerful inhibition-targeting modulation is still elusive. In this study, we combined the selective tagging of either VGluT3 MR neurons or their hippocampus-projecting subgroup and high-density silicone probe recording of both MR and dorsal CA1 activity in head-fixed, awake mice. During the recording, the animals had to run on a disc while exposed to both aversive and rewarding stimuli at pre-determined locations. We utilized both excitatory and inhibitory optical tagging by ChR2 and ArchT, respectively. According to our initial results a subset of VGluT3 neurons were triggered by aversive stimuli whereas none of them responded to reward. Additionally, their activation by a 25 Hz pulse train augmented theta and simultaneously induced slow gamma oscillation in the hippocampus. In further analysis we will uncover the modulation of the hippocampal spatial code by the raphe-hippocampal VGluT3 pathway.

Disclosures: **M. Jelitai:** None. **A.M. Barth:** None. **K. Petrik:** None. **T. Chaves:** None. **P. Bartho:** None. **V. Varga:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.07/UU12

Topic: H.08. Learning and Memory

Support: K01 MH097091
R01 MH067924
R01MH10095

Title: Functional Differentiation in The Mesolimbic Hippocampus-VTA-VS Circuit Modulates Exploration-Exploitation Balance in Humans

Authors: *A. OKAN[1], A. DOMBROVSKI[2], M. N. HALLQUIST[3];
[1]Psychology and Neurosci., Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [3]Psychology & Neurosci., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract:
In explore-exploit decision-making, we have previously shown that posterior hippocampus (PH) facilitates exploration and anterior hippocampus (AH) supports converging on the best option (i.e., exploitation). Here, we examined how the Hippocampus (HC) - Ventral Tegmental Area (VTA) - Ventral Striatum (VS) circuit modulates the explore - exploit balance in a spatially structured reinforcement learning task. Given the VTA's role in sending reward prediction error (PE) signals to the hippocampus (HC), helping bind reward value representations into long term memory, we expect that the VTA activity will modulate exploration to find the best option. Additionally, we expect that the VS will modulate choices based only on recent feedback without supporting exploitation due to its role in learning stimulus-outcome associations, but not spatial representations.
70 participants aged 14-30 (M = 21.4, SD = 5.1, 37 female) completed a spatiotemporal reward task where probabilistic rewards varied monotonically with location. Computational model-based voxelwise GLM analyses examined how the HC-VTA-VS circuit was associated with exploration, as defined by trialwise shifts in choices, and exploitation, as defined by convergence on the best option.
When both PH and VTA PE modulation were included as predictors, VTA, but not PH, predicted win-shift choices. VTA also modulated choosing the best option in the next trial following both omissions and rewards, supporting exploitation. VS showed a reward-seeking pattern: VS sensitivity to changes in the number of good options predicted Win-Stay, Lose-Shift responses although it did not predict exploitation.
In sum, the VTA supports both learning different options and maximizing rewards (i.e., exploitation) rather than stochastic exploration. The specificity of the VS in facilitating Win-Stay, Lose-Shift choices but not exploitation suggests that it only holds short-term value representations. These results show that, though the PH does not facilitate exploration above and beyond the effects of VTA, it may contribute to exploration by incorporating local (i.e., trialwise) PEs into cognitive maps while the VTA optimizes the search for maximum reward through goal-directed exploration. Further, VS's contribution to reward learning hinges on environmental demands and may be insufficient for complex spatiotemporal representations, while the AH input is required for exploitation.

Disclosures: A. Okan: None. **A. Dombrovski:** None. **M.N. Hallquist:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.08/UU13

Topic: H.08. Learning and Memory

Support: UNKP-20-3-SE-31 New National Excellence Program of the Ministry of Innovation
UNKP-21-3-SE-9 New National Excellence Program of the Ministry of Innovation
EFOP-3.6.3-VEKOP-16-2017-00009 Semmelweis 250+ Excellence PhD Fellowship

Title: Fear memory recall via hippocampal somatostatin interneurons

Authors: *K. ZICHÓ[1,2], K. E. SOS[1], P. PAPP[1], A. M. BARTH[1], E. MISÁK[1], Á. OROSZ[1], M. I. MAYER[1,2], R. Z. SEBESTÉNY[1], G. NYIRI[1];
[1]Inst. of Exptl. Med., Budapest, Hungary; [2]János Szentágothai Doctoral Sch. of Neurosciences, Semmelweis Univ., Budapest, Hungary

Abstract: Fear-related memory traces are encoded by sparse populations of hippocampal principal neurons that are recruited based on their inhibitory-excitatory balance during memory formation. Later, the re-activation of the same principal neurons can recall the memory. The details of this mechanism are still unclear. Here, we investigated whether disinhibition could play a major role in this process. Using optogenetic behavioral experiments, we found that when fear was associated with the inhibition of mouse hippocampal somatostatin positive interneurons, the re-inhibition of the same interneurons could recall fear memory. Pontine nucleus incertus neurons selectively inhibit hippocampal somatostatin cells. We also found that when fear was associated with the activity of these incertus neurons or fibers, the re-activation of the same incertus neurons or fibers could also recall fear memory. These incertus neurons showed correlated activity with hippocampal principal neurons during memory recall and were strongly innervated by memory-related neocortical centers, from which the inputs could also control hippocampal disinhibition in vivo. Nonselective inhibition of these hippocampal somatostatin or incertus neurons impaired memory recall. Our data suggest a novel disinhibition-based memory mechanism in the hippocampus that is supported by local somatostatin interneurons and their pontine brainstem inputs.

Disclosures: K. Zichó: None. **K.E. Sos:** None. **P. Papp:** None. **A.M. Barth:** None. **E. Misák:** None. **Á. Orosz:** None. **M.I. Mayer:** None. **R.Z. Sebestény:** None. **G. Nyiri:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.09/UU14

Topic: H.08. Learning and Memory

Support: Emory University grant 00115700-URC 2022-23

Title: Hippocampus-amygdala neuronal interactions underlying context-dependent affective memories

Authors: *J. L. KRASNEY[1], N. A. GOSE[2], A. ARUN[2], J. R. MANNS[1];
[1]Dept. of Psychology, [2]Program in Neurosci. and Behavioral Biol., Emory Univ., Atlanta, GA

Abstract: The amygdala and hippocampus support distinct but complementary functions. In particular, the hippocampus serves as a key node for linking objects with a given spatial context, and the amygdala responds to affective or social properties of objects. Previous work has shown that optogenetic stimulation of the basolateral complex of the amygdala (BLA) can accelerate learning nonaffective object-context associations, but it remains uncertain how naturalistic engagement of the BLA via affective stimuli impacts forming or retrieving memories of object-context associations. Accordingly, the present study recorded oscillatory activity in the BLA and ventral hippocampus as female rats formed and retrieved affective and nonaffective object-context associations. Each of the four conditions included two visually unique boxes connected by a tunnel and two objects comprised of unique digging media (e.g., gravel and sand) and unique odorants. Each condition included one nonaffective object scented with a plant odorant (e.g., lavender) and a second object scented with conspecific male urine, conspecific female urine, fox urine, or, as a control, a different plant odorant. Rats learned to retrieve a buried reward in one of the two objects given the current spatial context (i.e., Box 1: A+B-; Box 2: A-B+). Neural analyses focused on local field potentials during the empty context exploration of each trial before objects were presented. The rationale was that neural activity during this period, when no stimuli were present, would reflect memory for the affective object-context associations. Preliminary results suggested that affective and nonaffective object-context associations differentially modulated amygdala-hippocampus interactions during empty context exploration across learning. The results provide insights for the oscillatory interactions between the amygdala and hippocampus that underlie remembering the spatial context in which affective events occur.

Disclosures: **J.L. Krasney:** None. **N.A. Gose:** None. **A. Arun:** None. **J.R. Manns:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.10/UU15

Topic: H.08. Learning and Memory

Support: R00MH118423

Title: Role of hippocampal CA1 catecholamine signaling in movement, novelty, and reward

Authors: *A. L. SOMMER[1], I. PIMENTEL[2], T. N. DONALDSON[3], G. D. NEMER[5], I. CHOI[6], M. KAKANI[4], E. L. NEWMAN[7], S. MCKENZIE[8];
[2]Dept. of Neurosciences, [1]Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM; [3]Dept. Of Psychology, [4]Univ. of New Mexico, Albuquerque, NM; [5]Univ. of Rochester, Rochester, NY; [6]Indiana Univ., Bloomington, IN; [7]Dept. of Psych. and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [8]UNM HSC, Albuquerque, NM

Abstract: Norepinephrine (NE) and dopamine (DA) signaling in the hippocampus are required for normal memory function in part by promoting synaptic plasticity. Microdialysis studies have shown increases in hippocampal DA and NE in response to novelty, however, due to the coarse temporal resolution of microdialysis, it is unknown to what extent DA and NE release in the hippocampus is related to novelty *per se*, or to the movement and arousal changes that accompany exposure to novel stimuli. To better understand the causes of DA and NE release in the hippocampus, we monitored transmitter binding with virally delivered genetically encoded fluorescent indicators GRAB-NE and GRAB-DA as mice engaged in various behavioral tasks. To investigate novelty related signaling, mice were presented with new objects and environments as well as novel object-context pairings and unexpected and predicted rewards. Three strategies were implemented to dissociate catecholamine signaling related to movement versus that related to novelty. First, movement and arousal correlates were captured in the home cage and during running on a familiar track. These correlates were then used to factor out motor-related signaling. Second, mice were trained to run for water on a familiar track and on surprise trials, novel objects were placed beside the track, thus causing mice to stop to explore, rather than initiate movement. Finally mice were trained to run on a running wheel. NE and DA signaling was best explained by transitions between environments and introduction of novel stimuli and only weak motor correlates were observed after regressing out these factors. NE and DA signals were particularly high around the perimeter of the environments and increased around exploratory events such as head sweeps and rearing. We found a strong and reliable DA and NE response to new objects and contexts that decayed as a function of experience. Repeated samplings of the same objects, in which motor output was held constant, were associated with diminishing catecholamine release, supporting our hypothesis that novelty detection contributes to transmitter release. We conclude that apparent movement-related catecholamine signaling in the hippocampus is more closely related to the information gain afforded by those behaviors.

Disclosures: **A.L. Sommer:** None. **I. Pimentel:** None. **T.N. Donaldson:** None. **G.D. Nemer:** None. **I. Choi:** None. **M. Kakani:** None. **E.L. Newman:** None. **S. McKenzie:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.11/UU16

Topic: H.08. Learning and Memory

Support: R01-AG072643-01
McKnight Brain Research Foundation

Title: Determining the age of onset of cognitive impairment in male and female TgF344-AD rats

Authors: *M. ZEMPARE[1], O. GUSWILER[1], A. NETHER[1], B. MALONEY[1], K. BOHNE[1], A. DELGADO[2], M. HUENTELMAN[3], P. WORLEY[2], C. A. BARNES[1];
[1]Univ. of Arizona, Tucson, AZ; [2]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]TGen, Phoenix, AZ

Abstract: Alzheimer's Disease (AD) is characterized by age-dependent cognitive decline and neurodegeneration and is the most common form of dementia in the 65+ aging population in the United States. The pathological hallmarks of AD include the formation and aggregation of amyloid beta plaques and hyperphosphorylated tau proteins leading to failure of critical brain circuit function. Increasing evidence shows that the dorsal hippocampus and medial prefrontal cortex (mPFC) are among the brain regions that are most susceptible to AD pathology. These regions are crucial for learning, memory and spatial navigation and show significant impairment during progression of AD. A novel model of AD was developed by Cohen et al. in 2013 in Fischer 344 rats that express the familial AD human mutant genes: Swedish amyloid cursor protein (APPsw) and presenilin-1 delta E9 (PS1ΔE9). The TgF344-AD model results in a comprehensive set of AD-like phenotypes including: 1) progressive amyloid plaque aggregation and formation, 2) endogenous rather than engineered tauopathy leading to the formation of neurofibrillary tangles (NFTs), 3) cognitive decline and 4) gliosis and neuronal loss. While there has been some characterization of the behavioral status of the TgF344-AD rats, the onset of the behavioral deficit has been roughly determined to be around 9 months in both cross sectional (Cohen et al 2013) and in longitudinal (Berkowitz et al 2018) studies. A more fine-grained month by month analysis of when the behavior begins to change in the TgF344-AD male and female rats has yet to be determined. The purpose of this study was to identify this transition across several behavioral domains including the hippocampus-dependent spatial version of the Morris watermaze task, the medial prefrontal cortex (mPFC)-hippocampus-dependent temporal order recognition (TOR) memory task, and the amygdala-midbrain-dependent elevated zero (EZ) maze task. Six groups (n=65) of male and female TgF344-AD and wildtype (WT) rats at ages 4 months, 5 months, 6 months, 8 months, 9 months, and 10 months of age were tested on the tasks discussed above. Both male and female TgF344-AD rats were comparable in performance to their age-matched WT controls at 4 months, 5 months, 6 months of age on the spatial version of the Morris watermaze, the TOR task and on the EZ maze task. Ongoing testing of male and female TgF344-AD rats at 8 months, 9 months, and 10 months will determine the precise age-of-onset of impairment due to AD pathology across the listed behavioral domains of this study.

Disclosures: M. Zempare: None. **O. Guswiler:** None. **A. Nether:** None. **B. Maloney:** None. **K. Bohne:** None. **A. Delgado:** None. **M. Huentelman:** None. **P. Worley:** None. **C.A. Barnes:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.12/UU17

Topic: H.08. Learning and Memory

Support: R01AG003376
McKnight Brain Research Foundation

Title: Immunohistochemical analysis of Locus Coeruleus neuronal projections and of α1, α2, and β noradrenergic receptors in the hippocampus of cognitively assessed aged and adult rhesus macaques

Authors: *K. MCDERMOTT, I. SINAKEVITCH, C. A. BARNES;
Univ. of Arizona, Tucson, AZ

Abstract: The Locus Coeruleus (LC) is a noradrenaline (NA)-producing brainstem nucleus with wide projections throughout the cortex. NA acts via 3 classes of receptors (α1, α2, β) and this signaling is critical for optimization of cognitive performance. Some histological studies have suggested age-related decreases in NA fiber and varicosity density in the cortex, and autoradiographic studies have shown age- and disease-related decreases in α1 and α2 receptor densities. NA fiber density has not been investigated with density of all 3 NA receptor types or with respect to cognitive performance. We have previously developed a novel protocol for histological analysis of the NA system in rhesus macaques (McDermott et al, Program No. 574.04, 2022 Society for Neuroscience). Here, we utilize coronal brainstem sections from a colony of 30 adult and aged rhesus macaques ranging in age from 8-32 years (24 to 96 human years). All monkeys underwent tests of spatial short-term memory (delayed response), object recognition memory (delayed nonmatching-to-sample), and object discrimination. We used immunofluorescence techniques to identify three NA receptors (α1, α2a, β1) and NA fibers, as well as supporting cell types known to interact with the NA system: vasculature, microglia, and astrocytes. Images from both the dentate gyrus (DG) and CA3 region of the hippocampus from each immunolabeled section were taken at 40X on a high-resolution confocal microscope. NA receptor, NA fiber, glial cell and vascular densities were determined using unbiased stereological techniques. These data were then assessed with respect to the age and cognitive status of the monkeys. Preliminary results from a subset of the 30 animals reveal trends towards higher densities of β1 NA receptors in both DG and CA3 regions of the hippocampus, and higher NA fiber densities in CA3 in the aged macaques relative to the adults. Further data collection will reveal whether these results hold, and if there are age-related alterations in the noradrenergic system that relate to the cognitive impairments observed in the aged macaques.

Disclosures: K. McDermott: None. **I. Sinakevitch:** None. **C.A. Barnes:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.13/UU18

Topic: H.08. Learning and Memory

Support: R01AG003376
McKnight Brain Research Foundation
Arizona Alzheimer's Consortium, Department of Health Services

Title: Detailed examination of the locus coeruleus subnucleus - LC compact - in rhesus macaques

Authors: *I. SINAKEVITCH, K. MCDERMOTT, C. BARNES;
Univ. of Arizona, Tucson, AZ

Abstract: The Locus Coeruleus (LC) is a brainstem nucleus with the largest group of noradrenaline producing neurons. Dysregulation of LC systems contributes to cognitive dysfunctions observed in aging and Alzheimer's disease. We previously reported our results from a study that examined 30 micrometer coronal brainstem sections along the rostral-caudal axis of the LC from a colony of 30 cognitively assessed rhesus macaques ranging in age from 7 to 32 years (human equivalent ~21-96 years). We used AMIRA software to reconstruct the LC from tyrosine hydroxylase (TH)-immunofluorescence and Nissl-stained serial sections aligned with previously collected MRI data. Using this method, we established the 3D structure of the LC nucleus and its subnuclei: LC lateral, LC medial, and LC compact (Sinakevitch et al. Program No. 574.08. 2022 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2022.) Here we present further analysis and description of one of the LC subnuclei: LC compact. The LC compact is the area of the LC with the highest neuronal density. It is located within the LC medial nucleus, which is comprised of both a densely packed region and a more scattered region of TH-positive cell bodies within periaqueductal gray (PAG), which surrounds the 4^{th} ventricle. Analysis from a subset of the macaques (n=8) reveals that the rostro-caudal extent of the whole LC is between 2.10-2.55 mm. LC compact extends rostro-caudally from 1.44-1.95 mm within LC medial and it has TH-positive neurons with similar structure and cell diameters ranging from 29-43 micrometers. In rhesus macaques the LC compact has three subregions: rostral, middle, and caudal. The rostral LC compact begins with a small area of cells with high density near the enlarged mesencephalic nerve (me5) in the PAG. The middle LC compact extends through almost all the LC medial along the dorso-ventral axis, and the caudal LC compact is a small area with the highest density of cells. The volume of the LC compact varied from 0.62-0.92 mm^3 (on each side) and comprises up to 69% of the total TH-positive cells in the LC. The fact that the LC compact closely follows the me5 tract raises the question of whether this structure may interact with the me5 tract. These detailed characterizations of LC compact might be used to further examine the specificity of the impact of age on this LC subnucleus.

Disclosures: I. Sinakevitch: None. **K. McDermott:** None. **C. Barnes:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.14/UU19

Topic: H.08. Learning and Memory

Support: UA Translational Bioimaging Resource (TBIR)
NIH small instrumentation grant (S10 OD02501)
McKnight Brain Research Foundation
Arizona Alzheimer's Consortium, Department of Health Services

Title: Age-related changes in Ex Vivo Female Bonnet Macaque Brains: Insights from Multi-modal MRI Analysis

Authors: *L. DIECKHAUS[1], K. MCDERMOTT[2], A. MURLIKRISHNAN[3], D. T. GRAY[4], C. A. BARNES[2], E. B. HUTCHINSON[1];
[1]Biomed. Engin., [2]Univ. of Arizona, [3]Keep Engaging Youth in Sci. Program, Univ. of Arizona, Tucson, AZ; [4]David Geffen Sch. of Medicine, UCLA, UCLA, Los Angeles, CA

Abstract: Age-related changes in brain morphometry and microstructure play a critical role in both normal and pathological aging. These are often associated with cognitive performance and there is a need to better differentiate normal from disease state alterations. Nonhuman primate brains provide an excellent model to determine lifespan-related brain changes and quantitative MRI offers essential tools for evaluating age-related morphometry and microstructure. Tensor-based morphometry (TBM) can detect local volume changes to identify regions of age-related atrophy while diffusion tensor MRI (DTI) can probe cellular level changes (Ashburner,2000 and Basser, 1994). We examined volumetric changes and structural integrity in the whole brain, hippocampus, and white matter (WM) with TBM and DTI and correlated these with behavioral scores in eight female bonnet macaque brain specimens ranging in age from 10-25 years (human equivalent of 30 to 75 years). We collected high-resolution T2 weighted MRI and DTI (200 and 600 micron isotropic voxels respectively). Adult (n=4; age ranges=10-11 years) and aged (n=4; age ranges= 20-25 years) templates were generated using conventional (Avants, 2004) and diffusion tensor-based registration (Irfannoglu,2016), and comparison of the adult and the aged templates was enabled by warping aged template to adult template. For TBM, LogJ maps were calculated from the non-linear registration between aged and adult templates and showed atrophy in the cortex but not the hippocampus, which was confirmed by ROI analysis (Fig. A&B). Whole brain volume was significantly different between the age groups ($p=0.044$, Cohen's $D= -1.436$) while hippocampal volume was not ($p=0.1$). Fractional anisotropy (FA), derived from DTI, was not different between groups for global WM. Correlation analysis of volume and DTI metrics with previously collected cognitive assessments - Delayed Response (DR), Delayed Non-Match to Sample (DNMS) and Object Discrimination (OD), did not reveal strong relationship between whole brain volume or WM FA, but there was a correlation between hippocampal volume and OD. The results support the idea that hippocampus volume is preserved during healthy aging while the cortex undergoes age-related atrophy, which is relevant for studies of degenerative disease that preferentially affect these structures.

Disclosures: **L. Dieckhaus:** None. **K. McDermott:** None. **A. Murlikrishnan:** None. **D.T. Gray:** None. **C.A. Barnes:** None. **E.B. Hutchinson:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.15/UU20

Topic: H.08. Learning and Memory

Support: McKnight Brain Research Foundation

Title: Possible strain differences of the Fischer 344 rat in a temporal order object recognition task

Authors: *O. GUSWILER, A. NETHER, K. BOHNE, C. BARNES;
Univ. of Arizona, Tucson, AZ

Abstract: Object recognition tasks are commonly used to assess learning and memory processes in rodents. Such tasks are extremely useful as they can be used to investigate the function of targeted brain regions without the need for extensive training protocols. The temporal order memory (TOR) task is a simple and efficient test used to assess recognition memory, specifically, the ability to recall *when* an object or event was committed to memory (Ennaceur & Delacour, Behav. Brain. Res., 1988, 31:47). In this task, animals are allowed to freely explore two different pairs of identical objects across two sample phases, and then, during a test phase one copy of each familiar object is simultaneously presented. Greater exploration of the temporally more remote familiar object over the temporally more recent familiar object has been observed in several rodent species/strains, and it has been shown that lesions to the medial prefrontal cortex significantly disrupt performance on this task in young rats (Mitchell & Laiacona, Behav. Brain Res., 1998, 97:107; Belblidia et al., Behav. Brain Res., 2023, 437:114151; Barker et al., J. Neurosci., 2007, 27:2948; Barker & Warburton, J. Neurosci., 2011, 31:10721). In humans, prefrontal cortex-dependent memory exhibits some of the most dramatic and early changes relative to other brain functions with normative aging (Park et al., Psychology and Aging, 2002, 17:299). Although the TOR task has been utilized in a number of studies of early development, there has been little research on the impact on performance in animals of older ages. The purpose of this study was to investigate whether this task could be used to detect age-related performance changes in a rodent model of healthy aging. We tested male Fischer 344 (F344) rats of three separate age groups, young (4-6mo), adult (8-9mo), and old (23-27mo), using both published protocols, and modified protocols to increase exploratory behaviors. In spite of improvement of overall engagement and exploration with the modified procedures, we were unable to replicate results consistent with what has been reported by others employing the TOR task. A number of reasons for this might be offered, such as strain differences (Ennaceur et al., Behav. Brain Res., 2005, 159:247; van Goethem et al., Behav. Brain Res., 2012, 232:323), and the ages of the animals tested, as ours were clearly mature or old, and most other studies

utilized animals of younger or much younger ages. We report this here to contribute to a growing literature concerning rodent strain related differences in behavioral performance.

Disclosures: **O. Guswiler:** None. **A. Nether:** None. **K. Bohne:** None. **C. Barnes:** None.

Poster

PSTR501. Hippocampal–Subcortical Interactions

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR501.16/UU21

Topic: H.08. Learning and Memory

Support: McKnight Brain Research Foundation
Arizona Alzheimer's Consortium, Department of Health Services

Title: Investigating age-related changes of mPFC neural responses to ventral hippocampus stimulation

Authors: *S. SRIVATHSA, A. VISHWANATH, E. R. CHURCH, S. L. COWEN, C. A. BARNES;
Univ. of Arizona, Tucson, AZ

Abstract: Neural ensembles in the hippocampus (HC) and medial prefrontal cortex (mPFC) play a crucial role in spatial working memory, a process susceptible to decline during aging in mammals. These regions are connected via a monosynaptic, unidirectional projection from the CA1 layer of intermediate (iHC) and ventral (vHC) hippocampus to the mPFC (Jay and Witter, 1991, J. Com. Neurol. 313:574). Damage or inhibition to this connection leads to impairments in spatial working memory tasks. Performance on spatial working memory tasks is known to correlate with increased synchrony of hippocampal theta (8-12 Hz) rhythms to mPFC neural activity. The directionality between hippocampus and mPFC has been mapped out in the phase-locking of mPFC neurons to the hippocampal theta rhythm during working memory tasks; Hhowever, little is understood about how monosynaptic iHC and vHC inputs engage mPFC neural activity along the dorso-ventral axis of the mPFC or how these change with age. To investigate these questions, we delivered a single biphasic electrical pulse (pulse halfwidth: 0.5 ms) of varied intensities (100-600uA) with a 30s interval between pulses to the CA1 layer in iHC and vHC of anesthetized male F344 young (9 months, n = 1) and old (27 months, n = 1) rats. We simultaneously recorded evoked neural activity along the dorsoventral length of the mPFC using Neuropixels probes. Recordings were obtained from neurons spanning 3.84 mm along the mPFC, including the prelimbic and infralimbic regions (areas 24b and 25). As iHC and vHC projections vary across the different layers of the mPFC, we also compare evoked neural responses across different layers of mPFC in response to HC stimulation - by recording first from layer II/III and then from layer V in mPFC. Stimulating both the iHC and vHC, we observed the most robust responses in the infralimbic region of the mPFC across all stimulus intensities. The magnitude of the LFP response, however, was higher with vHC compared to iHC

stimulation at the same stimulus intensity. Furthermore, the slope of the maximum LFP response increased and the response time decreased with increasing intensity of stimulation. While the current study did not have a large enough sample size to compare old versus young animals, it established a foundation for ongoiong experiments that investigate hippocampal input to the mPFC, as well as whether these connections are altered by aging.

Disclosures: S. Srivathsa: None. **A. Vishwanath:** None. **E.R. Church:** None. **S.L. Cowen:** None. **C.A. Barnes:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.01/UU22

Topic: H.08. Learning and Memory

Title: Development of electrophysiological approach for promoting planarian as unique model animal for neurophysiological research

Authors: O. BENITA[1], N. NESHER[2], ***T. SHOMRAT**[2];
[1]Dept of Neurobiology, Hebrew Univ., Jerusalem, Israel; [2]The faculty of Marine Sciences, Ruppin Academic Cen, Michmoret, Israel

Abstract: The remarkable regenerative capacity and rapid regrowth of the planarians, have positioned them as prominent model organisms for research in the fields of regeneration and developmental biology. The aim of the presented work is to promote the planarian as an exceptional research model for neurophysiology as well. The planarian possesses a primal brain which integrates a set of sensory information as the worm navigates through its environment, and is capable of executing relatively complex behaviors. Furthermore, their brain exhibits neuronal features such as multipolar neurons and dendritic spines, reminiscent of those found in vertebrates. They possess most of the qualities of more developed organisms and yet are inexpensive and easy to maintain. Given these advantages, the planarian constitutes a perfect reductionist model, and together with their exceptional regeneration capabilities, allows neurophysiological experiments not possible with any other model animals. In order to facilitate neurophysiological research, we first developed an extracellular multi-unit recording procedure from the planarians brain (*Dugesia*, *japonica* and *sicula*). Once this objective was achieved, we proceeded to search for correlations between different recording sites along the brain "hemispheres" (cephalic ganglia) and specific stimulus modalities and strengths, while applying different types of sensory stimulation, such as light and vibration. Subsequently, we employed recordings from the characterized sensory areas and stimulation procedures to investigate brain neuronal activity during the learning process of habituation to brief exposure to blue light (5-10ms), followed by dishabituation induced by 1s of aversive blue light exposure. We also monitored neuronal activity during classical conditioning processes, where we employed a combination of vibration and mild light exposure, one as conditioned stimulus (CS), and the

other as control stimulus to test the possibility of pseudoconditioning. For the unconditioned aversive stimulus (US), we used 1s exposure to strong blue light or an electric shock (3V/DC). Thus far, we have successfully recorded brain activity that correlates with habituation and short-term dishabituation processes. The classical conditioning experiments have yielded promising preliminary results. Within 3 blocks of ~25 trials there was an increase in the relative neuronal activity response for the CS. In conclusion, the ability to study the well-documented learning behaviors at the neuronal activity level is a significant step toward establishing the planarian as an exceptional model for neurophysiological research.

Disclosures: O. Benita: None. **N. Nesher:** None. **T. Shomrat:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.02/UU23

Topic: H.08. Learning and Memory

Support: DFG Project 376818398

Title: How to improve motor learning in Drosophila

Authors: *R. LYUTOVA-HRISTOVA, S. MARCATO, A. EHWEINER, B. BREMBS; Univ. of Regensburg, Regensburg, Germany

Abstract: Motor learning, skill-learning or habit formation share conceptual similarities, but it is debated how much biology these processes have in common. There is genetic evidence linking motor learning and habit formation in flies, song-learning in birds and language acquisition in humans to an evolutionary conserved operant self-learning process. The FoxP transcription factor family as well the protein kinase C (PKC) family of genes are involved in all of these phenomena. Here we show different biological manipulations of *Drosophila* that all enhance motor learning. Mutations in genes involved in classical conditioning, such as *rutabaga* or *radish*, enhance motor learning (while decreasing or abolishing classical learning).Overexpression of an operant learning gene, atypical PKC (aPKC) enhances motor learning as well as habit formation. Inhibition of a prominent interaction partner of aPKC, *bazooka*, also enhances motor learning. Inhibition of a prominent insect neuropil, the mushroom-bodies (MBs) has been previously reported to enhance habit formation such that premature habits are formed. We show here that this function is mediated via MB output neuron 2 (MBON-02, aka. MBON-β2β'2a). The anatomy of this neuron indicates that non-olfactory MB Kenyon cells of the β2 and β'2-lobes are involved in this enhancement. These neurons receive input via their dendrites in the little-studied lateral (lACA) and dorsal (dACA) accessory calyx regions of the MB. Our preliminary data suggest that thermosensory input from the lACA and visual input from the dACA are both simultaneously necessary for the inhibition of premature habits. However, they are separately sufficient to trigger the inhibition via MBON-02. We will also

show data comparing the motor learning efficacy of *rover* and *sitter* flies, which carry different variants of the protein kinase G (PKG) gene. Given the conserved nature of these learning processes in all bilaterian animals including humans and the role of motor learning in language acquisition, habit formation/addiction and rehabilitation after stroke or spinal cord injury, the diversity of these learning enhancements promises a rich field for the development of medical applications.

Disclosures: R. Lyutova-Hristova: None. **S. Marcato:** None. **A. Ehweiner:** None. **B. Brembs:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.03/UU24

Topic: H.08. Learning and Memory

Support: NIH Grant AG065925

Title: Dopamine neuronal circuit-specific roles of D2-like receptor DD2R in Drosophila olfactory associative learning

Authors: C. QI[1], R. A. COLVIN[2], *D. LEE[2];
[1]Biol., [2]Ohio Univ., Athens, OH

Abstract: Learning and memory is a crucial brain function that helps animals adjust their behaviors to changing environments. In both invertebrates and vertebrates, dopaminergic system plays an important role in learning and memory. Excitatory dopamine D1-like and inhibitory D2-like receptors have distinct roles in mediating various learning tasks. *Drosophila melanogaster* also has a conserved dopaminergic system that mediates associate learning, while its nervous system is much simpler with less redundancy. Previous research has revealed that dopaminergic neurons mediate *Drosophila* olfactory associative learning in both larvae and adults. A D1-like receptor dDA1 in the mushroom body (MB, the learning center in flies) has been proven important for learning. However, the role of D2-like receptor DD2R in fly learning has not been fully investigated. DD2Rs are comprised of pre- and postsynaptic receptors, and known to be involved in larval olfactory learning. In the larval brain, olfactory cues (conditioned stimuli) are transmitted from olfactory receptor neurons to Kenyon cells in the mushroom body via projection neurons, and distinct gustatory information (unconditioned stimuli) is transferred from gustatory receptor neurons to the mushroom body via different dopaminergic neurons (DAN). DANs in DL1 clusters innervating the vertical lobes of MB are necessary for aversive olfactory learning, while those in the pPAM clusters innervating the medial lobes are important for appetitive learning. In this study, we aimed to examine (1) whether DD2Rs are expressed in MB and different DAN clusters, and (2) whether these DD2Rs are involved in *Drosophila* olfactory learning. By using a GFP-tagged DD2R strain, expression patterns of DD2R were explored in

both MB neurons and DANs. In functional assay, knockdown of DD2R with a microRNA strain under 201Y-GAL4 driver impaired both appetitive and aversive learning, showing DD2Rs in MB neurons are necessary for both kinds of larval learning tasks. As to DD2Rs in DANs, aversive learning is completely impaired in larvae with DD2R knockdown in a single DL1 DAN, while the appetitive learning is still intact. Optogenetic activation of the same DAN during training impaired larval aversive learning, indicating DD2R's inhibitory function. In contrast, knockdown of DD2R under a pPAM-specific driver (R58E02-GAL4) completely impaired appetitive learning, suggesting DD2Rs in pPAM clusters have an important role in appetitive learning. In summary, our findings revealed DD2Rs in different brain structures have distinct functions in *Drosophila* larval olfactory learning.

Disclosures: C. Qi: None. **R.A. Colvin:** None. **D. Lee:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.04/UU25

Topic: H.08. Learning and Memory

Support: Invertebrate Neurobiology Research Fund
Duke Univesity Summer Research Fellowship for Third-Year Ph.D. Students and Beyond

Title: Identifying the circuit and molecular mechanisms of non-visual cues-driven spatial learning in Drosophila melanogaster

Authors: *Y. CHEN, R. ALFREDSON, U. STERN, D. MOTEVALLI, S. FOGLEMAN, R. YANG;
Neurobio., Duke Univ., Durham, NC

Abstract: The ability to memorize and navigate to locations of significance is crucial for animals' survival in nature. While much is known about how visual cues enable spatial learning, how animals solve spatial learning tasks without the benefit of visual cues is less well understood. Here we identified specific neural circuits and candidate signaling pathways that support non-visual cues-driven spatial learning (NVSL) in *Drosophila*. Using a high-throughput closed-loop learning platform we developed, we found that flies rely on intact antennae, the associative learning center mushroom bodies (MBs), as well as neurons that signal self-motion (i.e., PFN neurons) for NVSL when the environment is relatively featureless. Further, their learning significantly improves when the environment is enriched with non-visual landmarks. Interestingly, while this improvement still requires antennae and MBs, functional PFN neurons become dispensable. Lastly, transcriptome analysis of flies trained to perform NVSL identified multiple components of the Toll and Imd signaling pathway whose expression in the brain correlates with high NVSL performance. Collectively, our findings suggest that flies can exploit

both environmental and self-motion cues to solve spatial tasks without vision and employ context-specific strategies. We further provide specific circuits - and possibly their transcriptional response - that underlie this form of spatial learning.

Disclosures: **Y. Chen:** None. **R. Alfredson:** None. **U. Stern:** None. **D. Motevalli:** None. **S. Fogleman:** None. **R. Yang:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.05/UU26

Topic: H.08. Learning and Memory

Support: Israel Science Foundation Grant 2396/18
NIH Grant 1R01NS118606-01
National Natural Science Foundation of China Grant 32171011
National Natural Science Foundation of China Grant 62250004

Title: Taste input to feeding command-like neurons is reduced after learning that food is inedible in Aplysia

Authors: M. LEVY[1], G. ZHANG[2], J. JING[2], ***A. SUSSWEIN**[3];
[1]Bar Ilan Univ., Ramat Gan, Israel; [2]Nanjing Univ., Nanjing Univ., Jiangsu, China; [3]Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Repeated failed attempts to swallow food in the mollusc *Aplysia* lead to learning that the food is inedible. After learning, memory is initially characterized by fewer attempts to swallow, followed by a taste-specific cessation of responses to the inedible food. Although the effect of learning on the feeding central pattern generator has been investigated, little is known about how sensory input to the feeding command-like neurons might be affected by learning. Taste of food is conveyed to the CNS via cholinergic inputs to the cerebral ganglion from taste afferents embedded in the lips. Application of a cholinomimetic (carbachol - CCh) to the cerebral ganglion induces repetitive bite-like motor programs. How do animals know that they have tried and failed to swallow a food? Previous data indicated that either an NO donor or histamine paired with lip stimulation induces learning that the food is inedible, indicating that these transmitters are released by failed attempts to swallow, and their release signals the failed attempts when paired with lip stimulation. NO and histamine are the transmitters released by cerebral ganglion sensory neuron C2, which is stimulated by food pressed against the mouth, such as when *Aplysia* try hard to swallow food. We now show that pairing either NO or histamine with application of CCh to the cerebral ganglion caused a decrease of the response to CCh alone 1 hour later. The decrease was not seen with repeated application of CCh alone, or with application of either the NO donor or histamine not paired with CCh. Stimulating neuron C2 produces slow post-synaptic potentials in many of the CBIs (cerebro-buccal interneurons),

command-like neurons that receive input from the lips, and that send axons to the buccal ganglia which organize and effect repetitive feeding behaviors. CCh drives CBI activity, and thereby drives feeding motor programs. Pairing CCh application to the cerebral ganglion with intracellular stimulation of C2 inhibits the subsequent ability of CCh to drive CBI activity. These findings are consistent with a previous suggestion that learning in part arises via a post-synaptic reduction in the response of CBIs to acetylcholine (ACh) released by lip stimulation with food. The reduced response is a result of pairing cholinergic input with NO or histamine, the transmitters released by neuron C2. In summary, activation of command-like neurons by food could be reduced due to pairing of food input and failed attempt to swallowing, demonstrating a role of command-like neurons in learning.

Disclosures: **M. Levy:** None. **G. Zhang:** None. **J. Jing:** None. **A. Susswein:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.06/UU27

Topic: H.08. Learning and Memory

Support: NINDS Grant 15NS118408

Title: Interaction between serotonin and nitric oxide in the formation of long-term sensitization in Aplysia

Authors: *L. RICHARDS, M. WAINWRIGHT, R. MOZZACHIODI;
Life Sci., Texas A&M Corpus Christi, Corpus Christi, TX

Abstract: Learning is a process by which all animals modify their behaviors to ensure increased fitness (Nordell 2021). Long-term sensitization (LTS) in the invertebrate *Aplysia* is an extensively studied example of learning in which defensive responses, such as the tail-siphon withdrawal reflex (TSWR), are heightened for prolonged periods following repeated exposure to noxious stimuli (i.e., aversive training; Kandel 2001). The neurotransmitter serotonin (5-HT) is known to mediate LTS formation in *Aplysia* (Kandel 2001). *In vivo*, 5-HT exposure alone can also induce LTS (Levenson et al. 2000). However, recent findings indicate that the neurotransmitter nitric oxide (NO) is also necessary for LTS formation (Farruggella et al. 2019). Therefore, an experiment was designed to position NO on the 5-HT-mediated biochemical pathway. TSWR durations were initially recorded (pre-tests). Then, animals were injected with either artificial seawater (ASW) as vehicle, or with the NO synthase inhibitor L-NAME. L-NAME concentration was 20 mg/mL, and injections consisted of 1 mL / 200 g of body weight (Farruggella et al. 2019). Animals were subsequently either trained, exposed to 5-HT, or left untrained/untreated. Aversive training consisted of five trials of noxious stimuli spaced 30 min apart. Each trial consisted of 10-s trains of 10 electrical shocks (500-ms duration, 1 Hz, 60-mA intensity). 5-HT exposure consisted of submerging animals in ASW containing 500-µM 5-HT

for 5 min, five times, at 25-min intervals. TSWRs were post-tested 24 h after training/5-HT treatment ceased. Six groups of 15 animals each were utilized: ASW-untrained, ASW-trained, ASW-5-HT, L-NAME-untrained, L-NAME-trained, L-NAME-5-HT. The experimenter performing behavioral tests was kept blind to the training history of the animals to remain unbiased in recording TSWRs. The post-test/pre-test TSWR changes were compared among the six groups using the Kruskal-Wallis H test, followed by the Student-Newman-Keuls *post-hoc* test to isolate the sources of significance (Chatterji et al. 2020). Analysis revealed that LTS occurred in the ASW-trained and ASW-5-HT groups with no statistical difference between them. However, LTS did not occur in either the L-NAME-Trained nor L-NAME-5-HT groups. These results revealed that L-NAME fully blocked both 5-HT-induced LTS and training-induced LTS. The absence of LTS in the L-NAME-5-HT group positions 5-HT upstream of NO signaling. These findings expand our understanding of how different signaling cascades interact and regulate neuronal plasticity, which may have implications for memory formation and related disorders in other organisms, including humans.

Disclosures: L. Richards: None. **M. Wainwright:** None. **R. Mozzachiodi:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.07/UU28

Topic: H.08. Learning and Memory

Support: NINDS Grant 15NS118408
DOE Grant P217A220002

Title: Nitric oxide is required for long-term synaptic facilitation in Aplysia

Authors: A. CHEN, R. MOZZACHIODI, *M. WAINWRIGHT;
Life Sci., Texas A&M Univ. - Corpus Christi, Corpus Christi, TX

Abstract: Two mechanisms are known to sustain long-term memory (LTM): intrinsic plasticity (modulation of neuronal excitability) and synaptic plasticity (facilitation/depression of synaptic responses; Mozzachiodi and Byrne 2010). However, the degree to which each mechanism contributes to LTM is not fully understood. A ubiquitous form of LTM is long-term sensitization (LTS), in which repeated noxious stimuli strengthen defensive responses. In *Aplysia* defensive circuits, LTS is meditated by long-term increased excitability (LTIE) of sensory neurons (SNs) and long-term synaptic facilitation (LTF) of the excitatory postsynaptic potential (EPSP) in the follower motor neurons (MNs; Cleary et al. 1998). Recent studies revealed that the neurotransmitter nitric oxide (NO) is required for LTS but not for LTIE (Farruggella et al. 2019), suggesting that LTF might be NO dependent and the main contributor of LTS. This study aimed to characterize the contribution of NO to LTF by using reduced preparations consisting of the *Aplysia's* isolated nervous system. Ten action potentials in the SN at 10-Hz were initially used to

trigger a summated EPSP in the MN (Phares et al. 2003; pre-test). The recording solution was then exchanged with either vehicle (i.e., L-15 culture medium) or vehicle containing 0.37 mM of L-NAME, which is a selective blocker of NO synthesis (Farruggella et al. 2019). The exchange was followed by the delivery of an *in-vitro* training protocol that induces LTF. This training consisted of four trials of electric shocks to afferent nerves P8 and P9 (10-s, 1 Hz, train of 10 500-ms, 60-Hz, 60-V impulses) spaced 30 minutes apart, which mimics the noxious stimuli that induce LTS *in vivo* (Weisz et al. 2017). The summated EPSP was measured again 24-h (post-test) after training / no training. Four groups of 13 preparations were utilized: trained/vehicle (T-V), untrained/vehicle (UT-V), trained/L-NAME (T-L), and untrained/L-NAME (UT-L). For each preparation, these synaptic parameters were analyzed: EPSP peak amplitude, first EPSP amplitude, last EPSP amplitude, and EPSP area. Percent changes were calculated as [(post-pre)/pre] x100 and compared using the Kruskal-Wallis test followed by the Student Newman-Keuls *post-hoc* test to isolate the sources of significance. For each synaptic parameter examined, statistical analysis revealed that the expected LTF was not observed in T-L preparations compared to T-V preparations. These results indicate that LTF is NO-dependent and suggest that LTF may have a larger contribution than LTIE to LTS expression. These findings provide essential information regarding the roles of intrinsic and synaptic plasticity in the storage of LTM.

Disclosures: **A. Chen:** None. **R. Mozzachiodi:** None. **M. Wainwright:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.08/VV1

Topic: H.08. Learning and Memory

Support: NINDS Grant 15NS118408

Title: Contribution of S-nitrosylation to learning-induced short-term neuronal plasticity in Aplysia

Authors: E. CARRILLO, M. WAINWRIGHT, *R. MOZZACHIODI;
Life Sci., Texas A&M University-Corpus Christi, Corpus Christi, TX

Abstract: In *Aplysia*, *in vivo* training with aversive stimuli (i.e., electrical shocks) causes feeding suppression and decreased excitability of B51, a neuron linked to biting decision-making. A short-term (15 min) decrease of B51 excitability can be induced in a reduced preparation of the *Aplysia* nervous system in which electrical stimulation of afferent nerves serves as *in vitro* training (Weisz et al. 2017). Previous studies revealed that nitric oxide (NO) was necessary for short-term B51 decreased excitability (Farruggella et al. 2019). However, when the downstream targets of NO modulation, guanylyl cyclase (sGC) and protein kinase G (PKG), were pharmacologically blocked, short-term B51 decreased excitability was only partially prevented

(Mozzachiodi et al. 2019), suggesting the contribution of another NO-dependent biochemical cascade. In this study, we examined the role of S-nitrosylation in the expression of short-term B51 decreased excitability by blocking this process with the selective inhibitor TEMPOL (Hsieh et al. 2010). Each preparation was initially incubated for 30 min with either 100 µM TEMPOL or vehicle (artificial seawater; Hsieh et al. 2010). Following incubation, B51 excitability was measured before (pre-test) and 15 min (post-test) after the delivery of trained/untrained protocols. *In vitro* training consisted of a single 10-s, 1-Hz, train of 10 500-ms, 60-Hz, 60-V impulses delivered simultaneously to nerves P8 and P9 (Weisz et al. 2017). Untrained preparations did not receive electrical stimulation. For each excitability measurement, the percent change was calculated as [(post-pre)/(pre) x 100]. Four groups of 15 preparations each were used: untrained/vehicle, trained/vehicle, untrained/TEMPOL, and trained/TEMPOL. Although TEMPOL incubation did not affect B51 excitability before training, a time-dependent drug effect was observed in the untrained/TEMPOL group. Consequently, statistical analysis of B51 excitability changes was performed by comparing the untrained/vehicle group with the trained/vehicle group and the untrained/TEMPOL group with the trained/TEMPOL group using Mann-Whitney tests instead of a multiple comparison across the four groups. Analysis revealed that B51 decreased excitability occurred in the trained/vehicle group but not in the trained/TEMPOL group, indicating that TEMPOL blocked the expression of short-term plasticity in B51. These results indicate a contribution of S-nitrosylation in short-term B51 decreased excitability and suggest that two NO-dependent cascades may sustain this learning-induced plasticity: one mediated by a sGC-PKG pathway and one regulated by a S-nitrosylation process.

Disclosures: **E. Carrillo:** None. **M. Wainwright:** None. **R. Mozzachiodi:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.09/VV2

Topic: H.08. Learning and Memory

Support: NIH Grant NS101356

Title: Combinatorial modulation by operant conditioning of the neural circuit mediating Aplysia feeding behavior.

Authors: *Y. MOMOHARA, C. L. NEVEU, J. H. BYRNE;
Dept. of Neurobio. and Anatomy, McGovern Med. Sch., The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Operant conditioning (OC) is a form of associative learning in which the expression of a behavior is increased by the delivery of a reward contingent with its occurrence. Despite the prevalence of this form of learning, a complete understanding is lacking of how rewards modulate complex neural networks. The neural circuit underlying *Aplysia* feeding is well-

characterized and is modified by OC. Feeding consists of two types of buccal motor patterns (BMPs); ingestion-like BMPs (iBMP) and rejection-like BMPs (rBMP). These can be distinguished by the timing of radula closure activity relative to the two phases of the BMPs, protraction and retraction. In a recent study, Costa et al (2022) used non-negative matrix factorization (NNMF) to analyze the activity of 100s of neurons recorded using voltage-sensitive dyes, and found that OC sped up recruitment of the subpopulation of neurons active during the retraction phase. Here, we use intracellular recording techniques to identify intrinsic and synaptic properties of the retraction neurons that contribute to these effects. We focused on the retraction generator neuron B64, retraction terminator neuron B52, and decision-making neuron B51. The expression of iBMPs was increased in a contingent group compared to a control group (p=0.034, n=11). In the contingent group, the burst threshold of B64 was strongly reduced (p=0.004, n=10) and the number of spikes in B52 elicited by a suprathreshold current injection was decreased (p<0.001, n=11). In B52, the sag potential and input resistance were reduced (sag potential, p<0.003; Input resistance, p=0.027, n=9). These data suggest that OC accelerates initiation of retraction and prolongs its duration, by increasing the excitability of retraction generators and decreasing the excitability of retraction terminating neurons. Furthermore, the inhibitory synaptic connection from B52 to B51 was reduced after OC (p=0.004, n=9). By contrast, there was no statistically significant change in the inhibitory synaptic connection from B52 to B64 (p=0.203, n=7). These changes will be incorporated into a computational model of the feeding CPG (Momohara et al., 2022) to assess their quantitative contribution to the shift observed at the population level observed by NNMF, the increase in retraction duration, and the increase in iBMPs following OC.

Disclosures: **Y. Momohara:** None. **C.L. Neveu:** None. **J.H. Byrne:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.10/VV3

Topic: H.08. Learning and Memory

Support: RF1 NS118606

Title: Examining the neural substrate of egestive repetition priming in Aplysia using voltage-sensitive dye imaging

Authors: *C. NEVEU[1], Y. MOMOHARA[2], E. CROPPER[3], J. BYRNE[4];
[1]UTHealth, Houston, TX; [2]Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX; [3]Icahn Sch. of Med. at Mt. Sinai, New York, NY; [4]McGovern Med. Sch. of UTHSC at Houston, Houston, TX

Abstract: Repeated expression of a behavior increases the performance of that behavior by a form of learning called repetition priming. Although ubiquitous, the mechanism underlying

repetition priming is poorly understood. To better understand repetition priming, we used an egestive repetition priming of the feeding behavior of *Aplysia* (Siniscalchi et al., 2016). *Aplysia* feeding is mediated by motor activity patterns that consist of closure of a tongue-like structure during either the protraction (egestive motor pattern) or the retraction phase (ingestive motor pattern). Feeding behavior is mediated by the buccal ganglia that continue to express fictive motor programs and egestive priming even when isolated from the animal. Previous research indicates that stimulation of the esophageal nerve (EN) that contains buccal ganglia afferents induces motor pattern generation that increases the activity of neurons B8 and B20 that mediate radula closure during protraction (Siniscalchi et al., 2016). We predict that additional neurons are essential for the expression of egestive priming. Therefore, we monitored the activity of 10-100s of neurons by staining the buccal ganglia with voltage-sensitive dye, Di-4-ANNEPS, and imaging before, during and after egestive priming of isolated buccal ganglia. Our results suggest that VSD imaging can reveal new sites of plasticity that mediate repetition priming, which we plan to test by traditional electrophysiological methods. In addition, we have combined VSD recordings with injections of the tracer dye Alexa 647 in identified neurons involved in the selection of egestive (i.e., B52) and ingestive (i.e., B51) motor programs to determine whether the activity of these neurons is modified by egestive priming.

Disclosures: C. Neveu: None. **Y. Momohara:** None. **E. Cropper:** None. **J. Byrne:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.11/VV4

Topic: H.08. Learning and Memory

Support: NIH grant NS101356

Title: In vitro analog of operant conditioning produced persistent changes in population neuronal activity of Aplysia

Authors: *N. O. GONZALEZ[1,2], C. L. NEVEU[1], Y. MOMOHARA[1], J. H. BYRNE[1]; [1]McGovern Med. Sch. of the Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Biosci., Rice Univ., Houston, TX

Abstract: Operant conditioning (OC) is a form of learning in which a specific behavior is reinforced by a reward. The memory for OC has multiple temporal domains, consisting of short-term OC (STOC) lasting a few minutes, to long-term OC (LTOC) persisting 24 h or longer. Analyzing the neural mechanisms of OC has proven challenging due to the complexity of neural circuits in the CNS of vertebrates. Thus, the present study investigated OC using feeding behavior of *Aplysia*. The buccal ganglia control feeding behavior and continue to generate fictive buccal motor programs (BMPs) when isolated from the animal. An *in vitro* analog of OC in which an ingestive BMP is paired with a reward increases the expression of BMPs immediately

(Brembs et al., 2002; Nargeot et al., 1997, 2007) and for at least 24 h after training (Mozzachiodi et al., 2008). A number of neuronal correlates of STOC have been identified (Brembs et al., 2002; Nargeot and Simmers 2011, 2012; Momohara et al., 2021), but other than changes in one neuron, B51 (Mozzachiodi et al., 2008), little is known about correlates of LTOC. To begin to examine the extent to which ST and LT OC share common neuronal correlates, we used voltage-sensitive dye (VSD) imaging. Previously, we used VSD to record the activity of 100s of individual neurons simultaneously immediately after training (STOC) (Costa et al., 2022). During training, monotonic stimulation of the Bn. 2,3 nerve elicited BMPs. The dopaminergic afferent En.2 was stimulated as reinforcement immediately following every ingestive BMP in the contingent group, whereas for a yoked group, En.2 was stimulated with the same timing as the contingent group but without regard to BMP activity. Similar to previous studies, we found that conditioned ganglia produced a greater number of ingestive BMPs than the control ganglia 24 h after training. Importantly, the VSD recordings revealed activation patterns in phase with the BMPs, which can help identify neurons most likely to be modified by OC. The next step will be to use VSD imaging to compare the recruited neural ensembles in LTOC with those previously observed following STOC. The results from this study will elucidate which neurons are specifically involved in OC and how they synergistically interact together to mediate long-term memory.

Disclosures: **N.O. Gonzalez:** None. **C.L. Neveu:** None. **Y. Momohara:** None. **J.H. Byrne:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.12/VV5

Topic: H.08. Learning and Memory

Support: NIH (1R15MH107892-01) to RC-J and IC-J

Title: Transcriptional correlates of long-term sensitization memory in Aplysia: Does long-term memory have a long-term transcriptional cost?

Authors: J. ZARATE TORRES, T. WILSTERMAN, Z. JUAREZ, I. CALIN-JAGEMAN, ***R. CALIN-JAGEMAN**;
Dominican Univ., River Forest, IL

Abstract: The formation of a long-term memory requires transcriptional changes in the nervous system. What happens, though, as the memory is then stored for weeks and months: Do initial transcriptional changes fade or is there an ongoing transcriptional cost for each stored memory? We are addressing this question by tracking transcriptional changes in the nervous system of *Aplysia californica* following long-term sensitization training, a form of pain memory that is conserved across the animal kingdom. *Aplysia* (n = 8 per group) received a 4-day sensitization

protocol, with each day's training consisting of 4 presentations of a painful electrical stimulus to one side of the body. This was sufficient to induce a strong sensitization memory for at least two weeks, expressed as a sharp and persistent increase in reflex duration on the trained side of the body. We are conducting microarray and qPCR to analyze the transcriptional changes occurring 1, 5, and 11 days after training, focusing on the pleural ganglia which contain nociceptive neurons that help store the sensitization memory. Preliminary qPCR results show that training produces strong increases in the expression of several learning-related transcripts, but that these decay within 11 days, much earlier than the behavioral expression of the memory.

Disclosures: J. Zarate Torres: None. **T. Wilsterman:** None. **Z. Juarez:** None. **I. Calin-Jageman:** None. **R. Calin-Jageman:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.13/VV6

Topic: H.08. Learning and Memory

Support: Israel Science foundation (ISF) 2937-21

Title: Activity-dependent LTP induction in the octopus learning and memory network is mediated by a conserved molluscan 5-HT-mediated synaptic plasticity

Authors: I. KATZ[1], T. SHOMRAT[2], N. NESHER[2], F. BIDEL[1], Y. MEIROVITCH[3], *B. HOCHNER[1];
[1]Dept of Neurobiology, Hebrew Univ., Jerusalem, Israel; [2]Fac. of Marine Science, The Ruppin Academic Center,, Michmoret, Israel; [3]Dept. of Mol. and Cell. Biology, Harvard Univ., Cambridge,, MA

Abstract: The octopus' vertical lobe (VL) is involved in memory acquisition, and as typical for analogous brain areas, like the mammalian hippocampus, it possesses an activity-dependent LTP critical for octopus learning. Our past research revealed a unique molecular mechanism that mediates LTP expression and maintenance which involves activity-dependent persistent activation of NO synthase (NOS)(Turchetti-Maia et al 2018). Yet, the mechanism of LTP induction remains unclear. In the current research, we provide indications that the VL LTP induction mechanisms share molecular similarities with the well-characterized 5-HT-induced short-term synaptic facilitation in the defensive reflex of *Aplysia.* Previously we found that the application of 5-HT (100 µM) to VL slice preparations caused short-term synaptic facilitation and in addition, reinforced LTP induction, yet 5-HT alone did not induce LTP (Shomrat et al. 2010). To examine the possible role of 5-HT in the VL LTP, we employed 5-HT receptor antagonists methiothepin, which in *Aplysia* inhibits the adenylyl-cyclase-coupled 5-HT receptors (i.e., cAMP-PKA pathway) and spiperone which blocks PLC-coupled 5-HT receptors (i.e., PKC pathway) (Dumitriu et al. 2006). We found that spiperone (100 µM) attenuated LTP induction

and inhibited 5-HT-induced short-term facilitation, while methiothepin (100 µM) did not affect LTP. This suggests that PKC cascade is involved in activity-dependent LTP induction and 5-HT-induced short-term facilitation. This conclusion is corroborated by the finding that phorbol-ester (PDBu 5µM), a PKC activator, induces activity-independent LTP-like potentiation. Next, we tested the hypothesis that during LTP induction, the strongly activated amacrine interneurons (AMs), which in a recent connectome study were found to innervate serotonergic neuromodulatory processes (Bidel et al. 2023), may lead to the release of 5-HT, which in turn, through extrasynaptic transmission, induces PKC-mediated persistent NOS activation only in the activated AMs. As the AMs are cholinergic, we tested this hypothesis by preventing the AMs from evoking 5-HT release with hexamethonium, an effective ACh-receptor antagonist in the VL. Indeed, hexamethonium (10 mM) attenuated the activity-dependent LTP induction. Moreover, preliminary results showed that in the presence of hexamethonium, LTP induction could be rescued by providing exogenous 5-HT. These results suggest that the mechanism mediating activity-dependent LTP-induction in an advanced cephalopod mollusk has evolved by adapting conserved molluscan serotoninergic-mediated synaptic plasticity mechanisms.

Disclosures: I. Katz: None. **T. Shomrat:** None. **N. Nesher:** None. **F. Bidel:** None. **Y. Meirovitch:** None. **B. Hochner:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.14/VV7

Topic: H.08. Learning and Memory

Support: PAPIIT IN208722

Title: Effect of cycloheximide

Authors: *J. F. VERGARA-OVALLE, H. SANCHEZ-CASTILLO, A. GONZÁLEZ-NAVARRETE;
UNAM, Cd de Mexico, Mexico

Abstract: The Novel Object Recognition task (NOR) is widely used to study vertebrates' memory. It has been proposed as an adequate model for studying memory in different taxonomic groups, allowing similar and comparable results. Although in cephalopods, several research reports could indicate that they recognize objects in their environment, it has not been tested as an experimental paradigm that allows studying different memory phases. In this study we applied the NOR task to octopuses in three different stages of life; posthaching, juveniles and adults. The NOR task consisted of three phases: Habituation, Familiarization and Test. To evaluate the effect of protein synthesis inhibition on the long term recognition memory we treated five octopuses 1h after the familiarization phase with cycloheximide. The animals were anesthesized with 3% ethanol in artificial sea water and administered with cycloheximide (10mg/kg) through the dorsal

aorthic artery.This study shows that two-month-old and older *Octopus maya* subjects can differentiate between a new object and a known one, but one-month-old subjects cannot. Furthermore, we observed that octopuses use vision and tactile exploration of new objects to achieve object recognition, while familiar objects only need to be explored visually. Cycloheximide disrupted long-term recognition memory formation, causing subjects to explore a familiar object as if it were new. To our knowledge, this is the first time showing an invertebrate performing the NOR task similarly to how it is performed in vertebrates. These results establish a guide to studying object recognition memory in octopuses and the ontological development of that memory. PAPIIT IN208722

Disclosures: J.F. Vergara-Ovalle: None. **H. Sanchez-Castillo:** None. **A. González-Navarrete:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.15/VV8

Topic: H.08. Learning and Memory

Support: Glenn Foundation for Medical Research and AFAR Grants for Junior Faculty

Title: Uncovering novel neuropeptide regulators of associative behaviors

Authors: *E. J. LEPTICH[1], R. N. AREY[2];
[1]Neurosci., [2]Ctr. for Precision Envrn. Health, Mol. and Cell. Biol., Baylor Col. of Med., Houston, TX

Abstract: Cognitive decline is a prominent feature of aging across organisms that significantly compromises quality of life in humans. Therefore, it is critical to identify mechanisms that boost learning and memory function. *C. elegans* is a fantastic model for studying this problem, given their short lifespan, invariant cell lineage, and wealth of genetic tools available. Most importantly, *C. elegans'* associative memory behavior is molecularly conserved and declines with age. Our previous research in *C. elegans* shows that gain-of-function mutants in Gαq signaling (*egl-30(gf)*) have enhanced long-term associative memory (LTAM) behavior as young adults, and slowed cognitive aging phenotypes. The enhanced memory ability of young adults requires neuropeptide signaling from a single sensory neuron, the AWC. Growing evidence indicates neuropeptides regulate learning and memory, but their roles in these behaviors are less well-studied than classic neurotransmitters in the context of learning, memory, and cognitive aging. Thus, the identities of memory-promoting neuropeptides and their roles in cognitive aging are unknown. Here, we sought to identify the neuropeptides and their target receptors that boost learning and memory behavior. Using an RNAi-based approach, we screened candidate AWC-expressed neuropeptides for their role in learning and memory behavior in *egl-30(gf)* animals.

We found multiple neuropeptides from different families (neuropeptide-like proteins, FMRFamide-like proteins, and insulin-like peptides) are necessary for enhanced learning and memory behavior. One of the insulin-like peptides we find to be necessary for learning ability, INS-17, is a known antagonist of the *C. elegans* insulin receptor homolog DAF-2. Since *daf-2* mutants are known to have enhanced learning ability with age, our findings suggest that INS-17 may play a role in *daf-2*-dependent learning. Moreover, we have identified evolutionarily conserved target receptors of peptide hits from our screen as novel regulators of learning and memory behavior. In ongoing work, we aim to identify the neuronal sites governing these behaviors. We are also examining if peptide administration using a novel feeding-based approach can slow cognitive aging in the worm. Because many known pathways that slow cognitive aging are shared between species, this research has the potential to uncover novel therapeutic targets for cognitive impairment in higher organisms.

Disclosures: E.J. Leptich: None. **R.N. Arey:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.16/VV9

Topic: H.08. Learning and Memory

Title: Role of CaMKII in signaling competition and learning

Authors: K. NELSON[1], A. H. HUYNH[2], A. B. TOKAR FALATAH[2], *J. ROSE[2];
[1]Biol., [2]Psychology, Western Washington Univ., Bellingham, WA

Abstract: Research has shown that calcium/calmodulin-kinase II (CaMKII) plays a role in neuronal mechanisms associated with learning and memory. In *C. elegans*, the *unc-43* gene is an ortholog of CaMKII with *C. elegans* isoform sequences aligning to mammalian CaMKIIδ or CaMKIIγ. A mutation that affects expression of all UNC-43 isoforms (i.e., *unc-43*(n498)) modulates glutamate receptor expression (specifically GLR-1) in neurons; however, behavioral studies of learning with this strain are limited due to the *unc*oordinated motor phenotype. The *unc-43*(gk452) mutant strain is unique to other *unc-43* mutants as it is superficially wild-type; thus, allowing for behavioral studies of learning and memory. Interestingly, the *unc-43*(gk452) mutation affects the coding region of UNC-43, isoform t, for which the protein sequence is more than 40% identical to CaMKIIγ. Previous studies report that expression of *unc-43*(gk452) regulates learning pathways by activating cAMP-response element binding (CREB) protein. The current study examines the *unc-43(*gk452*)* strain using a relatively novel learning protocol where conditioning is restricted to a brief, discrete time period. Based on signaling competition, this learning assay described previously by our lab employs pairing two stimuli that drive opposing motor responses: blue light ~480 nm elicits forward locomotion while a mild mechanosensory vibration results in backward locomotion. Following multiple pairings, worms typically respond to the presentation of a single stimulus with a prolonged pause motor response. Learning and

memory in *unc-43*(gk452) mutant worms is tested at 10 minutes or 1 hour after one- or three blocks of 5 stimulus pairings. Initial data with this protocol indicates a deficit in learning after one block of training measured at 10 minutes. To examine the effect of *unc-43(gk452)* on GLR-1 glutamate receptor expression, confocal imaging of an *unc-43(gk452)*; GLR-1::GFP cross will be performed. Observing learning and memory, as well as receptor expression in intact animals, we will be able to further describe the role of CaMKIIγ in associative learning and memory.

Disclosures: K. Nelson: None. **A.H. Huynh:** None. **A.B. Tokar Falatah:** None. **J. Rose:** None.

Poster

PSTR502. Learning and Memory: Invertebrate Physiology and Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR502.17/VV10

Topic: H.08. Learning and Memory

Title: A home-constructed behavioral assay demonstrating learning in termites

Authors: Y. E. DING[1], *Z. LI[2];
[1]Boston Latin Sch., Boston, MA; [2]Brigham and Women's Hosp., Boston, MA

Abstract: It is generally believed that termites can't learn and are not "intelligent". Even for termite species that are known for their prowess in collectively building sophisticated mound nests, their demonstrated swarm behavior or self-organization is believed to result from passive and indirect interactions among individuals purely reacting to cues left by others (i.e., stigmergy). This project aimed to test whether termites were capable of learning and developing any form of memory. A Y-shaped test device with one release chamber and two identical test chambers was designed and constructed by 3D printing. A colony of dampwood termites was harvested from the wild and farmed in the basement. Worker termites were randomly selected for experiment. Repellent odors that could mimic the alarm pheromone for termites were first identified. Among all substances tested, a tea tree oil and lemon juice were found to contain repellent odors for the tested termites, as they significantly reduced the time that termites spent in the chamber treated with these substances (N = 10 termites per group). As control, a trail pheromone was found to be attractive, as expected. Subsequently, a second cohort of termites were operant conditioned by punishment using both tea tree oil and lemon juice, and then tested for their ability to remember the path that could lead to the repellant odors. The test device was thoroughly cleaned between trials. It was found that conditioned termites displayed a reduced tendency to choose the path that led to expectant punishment as compared with naïve termites (34.5% odds for the conditioned vs. 50.5% for the naïve, N = 20, $P < 0.01$ with t-test). Thus, it is concluded that dampwood termites are capable of learning and forming "fear memory", indicative of "intelligence" in termites. Follow-up research will examine whether such memory is permanent. Potential neural changes associated with memory formation will also be studied in termites. Data from this study challenge the previous assumption about termites' intelligence.

Disclosures: **Y.E. Ding:** None. **Z. Li:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.01/VV11

Topic: H.09. Spatial Navigation

Support: China Postdoctoral Science Foundation Funded Project (Project No. 212400233)

Title: Long-term calcium imaging of the hippocampal neural activity in freely moving mice

Authors: *Y. LU[1], R. WU[2], X. ZHANG[1], L. CHEN[2];
[1]Beijing Normal Univ., Beijing, China; [2]Inst. of Mol. Med., Peking Univ., Beijing, China

Abstract: Previous studies showed that the hippocampal place cells continuously remapped over repeated exposures to an experimental environment despite the lack of obvious changes in the environmental cues. To better understand the dynamics of such developing neural representation, we used the state-of-the-art miniature two-photon calcium imaging technique (Zong et al., 2021) to track over weeks the hippocampal neural activity while the mice repeatedly explored an open arena. We managed to follow the same group of hippocampal CA1 neurons across 1-4 weeks in seven mice. Our results confirmed that, the constitution of active neural population and their tuning properties continued to deviate from their earliest established patterns for up to a month before we lost sufficient power to register neurons. As we found active neurons dropped in and out over the repeated sessions, we compared the persistent and transient neurons of their contributions to the spatial coding. Results showed that the persistent neurons had higher spatial information and lower spatial decoding error compared to the transient neurons. We further found that, the animal's navigation behavior displayed a distinct transition that characterized by the position sequencing pattern changing from diffusion to superdiffusion regime as they got familiar with the environment (McNamee et al., 2021). We then set out to test whether the neural drifting was driven by changes in the behavioral policy, preliminary evidence supported that the neural ensemble activities reflected behavioral policy.It has always been challenging to track neuronal activities over longer periods and to draw direct comparisons of the same neuronal population. Our results suggested that the hippocampal neural activity were not static representations of space, but rather were continuously evolving ones that updated in response to non-spatial information, such as temporal context, as well as to behavioral policy. Such dynamics of neural representations may play an important role in learning and memory.
1)Zong, W., Wu, R., Chen, S., Wu, J., Wang, H., Zhao, Z., Chen, G., Tu, R., Wu, D., Hu, Y., Xu, Y., Wang, Y., Duan, Z., Wu, H., Zhang, Y., Zhang, J., Wang, A., Chen, L., & Cheng, H. (2021). Miniature two-photon microscopy for enlarged field-of-view, multi-plane and long-term brain imaging. Nature Methods, 18(1), 46-49.2)McNamee, D. C., Stachenfeld, K. L., Botvinick, M.

M., & Gershman, S. J. (2021). Flexible modulation of sequence generation in the entorhinal-hippocampal system. Nature Neuroscience, 24(6), 851-862.

Disclosures: **Y. Lu:** None. **R. Wu:** None. **X. Zhang:** None. **L. Chen:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.02/VV12

Topic: H.09. Spatial Navigation

Support: NIH Grant R01MH112523
NIH Grant R01DA054977

Title: Intentional activation of arbitrary hippocampal place cells in rats

Authors: *Y. YU[1], A. K. UYSAL[1], F. KLOOSTERMAN[2], D. JI[1];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Neuro-Electronics Res. Flanders, Leuven, Belgium

Abstract: Background
Hippocampal place cells fire spikes when an animal is at specific locations of an environment, called place fields. Place cells encode spatial memory and can be selectively activated during memory recall for spatial decisions. However, how these cells are selectively activated to meet intended task demands in the hippocampal neural circuits remains unknown.
Hypothesis
Aiming to establish a rodent model of intentional activation of memory cells, we tested whether rats could be trained to activate arbitrarily chosen place cells in the hippocampus.
Methods
We built a system for online detection of place cell firing activity (spikes), for closed-loop delivery of activity-driven feedback sensory cues (sounds), and for cue-triggered rewards. We trained five freely moving rats in a novel task to activate arbitrarily assigned single hippocampal CA1 place cells for rewards in three environments (Y-maze, rectangle maze, and a confined small space).
Results
In the Y-maze and the rectangle maze, rats were able to trigger the firing of a specific assigned place cell (target cells), by repeatedly running into its place field, which was originally hidden to the animals, like seeking a hidden platform in the Morris water maze. As a result, compared to control running sessions in the absence of sounds or rewards, the target cell's firing rate was increased, and its inter-spike intervals were reduced. As the activity threshold for rewards was gradually increased, the target cell was activated with stronger and stronger intensity to meet the requirement. These changes of activity were specific to the target cell and were not observed in non-target cells that were active during running in the same session. Moreover, in the confined

small space, certain target cells naturally silent or with very low firing rates were activated with much stronger place fields than control sessions in the same small space.

Conclusions

Our findings support our novel task as a rodent model for studying neural circuit mechanisms in intentional memory recall and reveal a remarkable flexibility and selectivity in the activation of hippocampal memory cells.

Disclosures: **Y. Yu:** None. **A.K. Uysal:** None. **F. Kloosterman:** None. **D. Ji:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.03/VV13

Topic: H.09. Spatial Navigation

Title: Post-experience reactivation of hippocampal CA1 and CA3 place cells encoding multiple environments

Authors: *T. YOKOI[1], Y. SHIKANO[2], H. YAGISHITA[1,2], Y. IKEGAYA[2,3], T. SASAKI[1,2]; [1]Tohoku Univ., Sendai, Japan; [2]Univ. of Tokyo, Tokyo, Japan; [3]Inst. for AI and Beyond, Tokyo, Japan

Abstract: The hippocampus plays important roles in learning and memory. In the hippocampus, spatial information is encoded by place cells during an experience and consolidation of spatial memory is supported by reactivation of place cells during rest/sleep periods. It remains to be unknown how multiple spatial experiences that are encoded by a subset of place cell ensembles are reactivated in subsequent rest/sleep periods. To address this issue, we recorded spike patterns of hippocampal CA1 and CA3 place cells in rats that sequentially experienced five different rooms. Our experiments contain seven epochs; pre-rest (30 min), room 1 (Familiar, 10 min), room 2 (Novel, 10 min), room 3 (Familiar, 10 min), room 4 (Novel, 10 min), room 5 (Familiar, 10 min) and post-rest (30 min). We confirmed that CA1 cells showed larger numbers of place fields defined from individual rooms than CA3 cells, suggesting sparser spatial representations by CA3 cells. CA1 place cells kept spatial correlations of firing maps between the Novel rooms, suggesting a common neuronal structure to encode Novel environments. During the post-rest period after experiencing the five rooms, CA1 non-place cells decreased their reactivation rates from the pre-rest to post-rest periods, whereas CA1 and CA3 place cells did not show such changes, suggesting stronger suppression in CA1 non-place cells. Additionally, CA1 cells that showed place fields in room 3, room 4, or room 5 increased their sharp wave ripple-associated reactivation rates, while CA1 cells that showed a place field in room 1 decreased their rates. These results suggest that CA1 neurons that encoded earlier experiences are less reactivated, compared to those with more recent experiences. Such differences across experiences were not observed from CA3 neurons. We are further analyzing synchronous spike patterns to clarify how these neurons that encoded multiple environments are cooperatively reactivated.

Disclosures: **T. Yokoi:** None. **Y. Shikano:** None. **H. Yagishita:** None. **Y. Ikegaya:** None. **T. Sasaki:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.04/VV14

Topic: H.09. Spatial Navigation

Support: NRF Grant 20221R1A2C3005560

Title: Hippocampal encoding of a sequence of rewarded journeys

Authors: ***J. SON**, D. JUNG, S. ROYER;
Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Hippocampal place cells are known to represent discrete positions within both environments and memory-associated spatiotemporal sequences. How environmental cues and spatiotemporal sequence information interact in the hippocampus is unclear. Here we performed silicon probe recordings of hippocampal cells as mice ran head-fixed on a treadmill belt enriched with visual-tactile cues and had to recall a sequence of rewarded journeys that spanned over several belt cycles. A trial consisted of 3 rewarded journeys ending in distinct belt locations and spatially overlapping with one another such that mice could not predict the rewards based only on spatial information but also had to track the sequence of the journeys.
Preliminary analyses indicate that individual CA1 cells encoded fixed positions with respect to either belt layout or rewarded journey sequence, with the mouse performance to predict the rewards being maximal when the cells encode the sequence of rewarded journeys.

Disclosures: **J. Son:** None. **D. Jung:** None. **S. Royer:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.05/VV15

Topic: H.09. Spatial Navigation

Title: Detecting replay in multi-unit spiking data: Bayesian networks

Authors: ***M. YOUSUF**, M. CHERTKOV, J.-M. FELLOUS;
Dept. of Mathematics, Univ. of Arizona, Tucson, AZ

Abstract: “Hippocampal replay” refers to the re-occurrence of population-wide sequences of neural spikes during sleep, similar to sequences observed in a pre-sleep task. The generation of replay during sleep is crucial for memory consolidation and retention and is key to retrieving previous memories during wakefulness. We investigate the generation of replay episodes by leveraging the excitability of neurons as seen in rats during spatial navigation and model the firing of place cells during sleep and pre-sleep tasks. Using the NEURON simulation environment, given well-structured connectivity matrices, we propose an algorithm to extract subgroups of neurons that show repetitive firing patterns at different time intervals. The patterns are viewed as samples generated by Bayesian Networks (BN), which are directed, acyclic graphical models. BN are probabilistic in nature and represent the causal dependency between multiple variables. To observe the causality in neurons during replay, we define neurons as nodes and the causal relationship between excitatory and inhibitory neurons as directed edges in a graphical network to show that the BN framework is suitable for generating replay instances. We further discuss the role of place cells, and the presence of the order of neurons, in the hippocampal replay that provides insights into cognitive functions such as learning and decision-making.

Disclosures: **M. Yousuf:** None. **M. Chertkov:** None. **J. Fellous:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.06/VV16

Topic: H.09. Spatial Navigation

Support: Howard Hughes Medical Institute

Title: The formation of an expanding memory representation in the hippocampus

Authors: *S. P. VAIDYA, R. A. CHITWOOD, J. C. MAGEE;
Howard Hughes Med. Inst. ; Baylor Col. of Med., Houston, TX

Abstract: How episodic memories are stored within brains is poorly understood. While certain memory-retaining neurons have been potentially identified, it is unclear if they retain learned information. Further, there is considerable evidence that neuronal activity is unstable and may require additional mechanisms to support robust memory. To examine these issues, we recorded the activity of a hippocampal CA1 neuronal population for 7 days as mice learned cued reward locations. These data and modelling results suggest that two place cell (PC) pools, distinguished by place field (PF) stability, are formed each day (transient: ~1.5 days; sustained: ~2 weeks). Notably, the proportions of these pools changed across the week as unstable transient PCs were progressively replaced by sustained PCs, markedly enhancing the stability of the total representation. This growing stable representation contained behaviorally relevant information and sustained PCs became active immediately at the start of each session. Finally, the initial

formation of sustained PCs was associated with a higher rate and efficacy of behavioral timescale synaptic plasticity (BTSP) and these PCs showed elevated and more reliable activity. It, therefore, appears that BTSP stabilizes particularly informative PCs, incorporating them into an expanding and readily retrievable representation that displays hallmarks of a long-lasting memory.

Disclosures: S.P. Vaidya: None. **R.A. Chitwood:** None. **J.C. Magee:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.07/VV17

Topic: H.09. Spatial Navigation

Support: Donders Mohrmann fellowship

Title: Coordinated maturation of spatial working memory and hippocampal functional specificity

Authors: *J. BEVANDIC[1,2], F. STELLA[1,2], F. OLAFSDOTTIR[1,2];
[1]Radboud Univ., Nijmegen, Netherlands; [2]Donders Inst., Nijmegen, Netherlands

Abstract: The ability to form precise and stable episodic memories develops gradually. This delayed maturation is thought to reflect the protracted development of the ability to form spatially and temporally precise explicit memories and the ability to retain them over extended periods. Although the neuronal underpinnings of mature episodic memory have been studied extensively over the past decades, we know relatively little about the neuronal processes mediating the maturation of this core cognitive capability.
To close this knowledge gap, we recorded from ensembles (20-50 cells) of CA1 principal neurons (PN) while rat pups (2-4 weeks of age) carried out a working memory (WM) task where they learned to alternate between encoding and retrieval trials on a T-maze. In agreement with past findings, we observed above-chance performance on the task was on average reached by ~3weeks of age. However, individual pups displayed overnight improvements in performance at different ages; suggesting the capability to do this task emerges abruptly. Thus, we sought to investigate if the development of PN function mirrors individual development profiles.
Our results indicate that despite CA1 PNs showing intact spatial encoding at the earliest ages tested, the encoding of task-specific contextual features only emerged as the animals started being able to complete the task accurately. Specifically, as working memory capability abruptly matured, CA1 cells started to display trial-specific activity patterns, remapping between encoding and retrieval phases of the task. Place cells continued to mature during this period (fields became smaller and peak in-field rates higher), but these changes did not account for the emergence of contextual remapping. As memory encoding and retrieval are thought to be supported by differential coupling of CA1 PNs to the HPC theta rhythm – with theta phase

precession thought to support encoding while phase locking may favour retrieval - we investigated how phase locking and precession evolved in development. We found WM maturation was associated with a differential shift in the balance between phase locking and precession during encoding and retrieval trials. For encoding trials, phase locking diminished as WM matured while precession persisted. For retrieval trials, the opposite pattern was observed. In sum, we find WM ontogenesis is associated with the emergence of adult-like contextual remapping and CA1 network mechanisms implicated in core constituent memory processes. Further analyses are underway to understand the nature and specificity of remapping in this developmental period and to assess if reactivation maturity related to WM maturity.

Disclosures: **J. Bevandic:** None. **F. Stella:** None. **F. Olafsdottir:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.08/VV18

Topic: H.09. Spatial Navigation

Support: MOE KUL scholarship

Title: Coordination of neuronal reactivation in the dorsal-ventral hippocampus and the contribution to spatial memory

Authors: *T.-S. SU[1,2], Y.-T. WEI[1,3], V. BONIN[1,3,4,5], F. KLOOSTERMAN[1,2];
[1]Neuro Electronics Res. Flanders (NERF), Heverlee, Belgium; [2]Fac. of Psychology & Educational Sci., [3]Dept. of Biol. & Leuven Brain Inst., KU Leuven, Leuven, Belgium; [4]VIB, Leuven, Belgium; [5]imec, Leuven, Belgium

Abstract: The hippocampus (HPC) is a major part of the brain that is associated with spatial navigation, task learning, and memory consolidation. However, the hippocampus is not a homogeneous structure and significant molecular, anatomical, and functional differences are present along its dorsal-ventral axis. It is unclear how hippocampal neural activity in the dorsal and ventral HPC is coordinated and/or acting independently to support navigation and spatial learning, and how such coordination is expressed during learning.A total of six adults C57BL/6J male mice were chronically implanted with a 4-shank Neuropixels probe that targeted the dorsal and ventral HPC simultaneously. Based on an initial survey of neural signals along the shanks, an animal-specific channelmap was constructed to focus available electrodes on dorsal CA1 (dCA1) and ventral CA1 (vCA1). We successfully recorded from 50-100 cells in both CA1 subregions while mice explored a linear track or performed a spatial alternation task. Preliminary analysis showed that space is coded at different scales along the dorsal-ventral axis of CA1. While both dCA1 cells and vCA1 cells encode location, dCA1 cells have smaller place fields (mean width, dCA1: 26±14 cm, vCA1: 46±24 cm), higher spatial information (46% higher than 0.2 bit/spike, mean spatial information, dCA1: 0.8±0.6 bit/spike, vCA1: 26%, mean=0.5±0.2

bit/spike), and allow for better position decoding (median error, dCA1: 6.7 cm, vCA1: 11.7 cm).When mice pause exploration or consume reward (offline period), we observed spike bursts and associated sharp-wave ripple (SWR) events in both dCA1 and vCA1. SWR events mainly occurred in dCA1 without vCA1 involvement (74% of events). Approximately 20% of events only activated vCA1 and synchronized activation of both subregions occurred in 6% of events. In a fraction of burst events, reactivation of place cell sequences was observed.In conclusion, we have established an experimental approach that uses a 4-shank Neuropixels probe for recording neural signals along the dorsal-ventral axis of the HPC in freely behaving mice. The preliminary results confirm in mice that dorsal and ventral CA1 cells differ in the resolution of their spatial coding and suggest that these subregions function largely independently during offline reactivation.

Disclosures: **T. Su:** None. **Y. Wei:** None. **V. Bonin:** None. **F. Kloosterman:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.09/VV19

Topic: H.09. Spatial Navigation

Title: Mental navigation and telekinesis with a hippocampal map-based brain-machine interface

Authors: *C. LAI, S. TANAKA, T. D. HARRIS, A. K. LEE;
Hhmi/Janelia, Ashburn, VA

Abstract: The hippocampus is critical for recollecting and imagining experiences. This is believed to involve voluntarily drawing from hippocampal memory representations of people, events, and places, including the hippocampus' map-like representations of familiar environments. However, whether the representations in such "cognitive maps" can be volitionally and selectively accessed is unknown. We developed a brain-machine interface (BMI) to test if rats could control their hippocampal activity in a flexible, goal-directed, model-based manner. Our experiments had 3 phases. First, a rat physically ran toward visible goal cues in a 360-degree virtual reality (VR) environment (a 2-dimensional, square open field arena) while its hippocampal activity was recorded. The rat was held in place by a body harness but could turn around unconstrained. Next, we trained a deep net decoder using place field activity from many hippocampal neurons, allowing accurate estimation of the animal's current location from ongoing hippocampal activity. For phase 3, we designed a BMI navigation task (named "Jumper") in which the animal was teleported in VR toward the currently decoded location at each moment. The animal was rewarded when it touched each goal cue. This task assessed whether animals could mentally navigate to arbitrary goal locations in a goal-directed and model-based manner by activating the appropriate hippocampal location representations. Animals demonstrated that they could do so by navigating in efficient paths toward each goal as opposed to randomly sampling locations across the arena. We designed a second BMI task ("Jedi") to test if animals could

control an external object's location using the same world model (i.e. spatial map) from a 3rd-person perspective and hold remote locations in mind for extended periods while remaining stationary. In this task, an object, rather than the animal, was teleported toward the decoded location while the animal remained fixed at the center of the arena. Unlike in the Jumper task, the goal cue remained in place for minutes and the animal continued to get reward as long as the object touched the goal. Animals showed they could move the object to arbitrary, remote goal locations and hold it there for many seconds. The rats' high performance in these tasks showed that they can precisely and flexibly control their own hippocampal activity. This ability to activate and maintain specific non-local neural representations is a fundamental building block of remembering past events and imagining possible future scenarios. Our findings also open up possibilities for high-level neural prosthetics utilizing hippocampal representations.

Disclosures: C. Lai: None. **S. Tanaka:** None. **T.D. Harris:** None. **A.K. Lee:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.10/VV20

Topic: H.09. Spatial Navigation

Support: Wellcome Trust (WT103896AIA)
National Science Foundation (IOS-1844935)
National Institutes of Health (R01MH123466)

Title: Hippocampal replay does not reflect trajectory planning in a spatial planning task

Authors: *E. DUVELLE[1,2], S. MOLAS MEDINA[3,2], E.-Y. HEW[4,2], N. ATESYAKAR[5,2], G. MAKDAH[6,7,2], A. RAWSON[8,2], W. PARRY-JONES[2], R. M. GRIEVES[1,2], M. VAN DER MEER[1], K. J. JEFFERY[9,2];
[1]Dept. of Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Inst. of Behavioural Neurosci., Univ. Col. London, London, United Kingdom; [3]Psychology, Queen Mary Univ. of London, London, United Kingdom; [4]Biomed. Res. Ctr., NIHR Great Ormond Street Hospital, Univ. Col. London, London, United Kingdom; [5]Rutgers Univ., Piscataway, NJ; [6]Ctr. for Interdisciplinary Res. in Biol., Col. de France, CNRS, INSERM, PSL Res. Univ., Paris, France; [7]Assistance Publique-Hopitaux de Paris, Paris, France; [8]Dept. of Clin. Neurosciences, Wellcome Ctr. for Integrative Neuroimaging, Univ. of Oxford, Oxford, United Kingdom; [9]Sch. of Psychology and Neurosci., Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Knowing what our current goal is, and how to reach it, is important both for humans navigating their lives and rats navigating a maze. How does the brain support such planning? In the rodent hippocampus, during pauses (often at reward sites), location-specific 'place cells' reactivate in 'replay' sequences that may reflect the upcoming trajectory and have been proposed to support planning (Pfeiffer & Foster, 2013, Widloski & Foster, 2022). However, this role is

debated, and replay has been suggested to support other processes instead, such as reward-related consolidation (Gillespie et al., 2021).
To tackle this debate, we designed a task that temporally and spatially separates trajectory planning from reward consumption. In a maze with 6 arms radiating from a hexagonal ring, rats had to first discover an unmarked goal location at the end of one of the arms (changed daily), then navigate to it when placed on a randomly allocated start arm. Rats could freely choose between two paths to the goal (usually of unequal length) passing around either side of the hexagonal ring. The necessity to choose a route ensured high planning demand. In a subset of sessions, a transparent barrier was placed on the maze, triggering re-planning events. After a training period, rats were implanted in the hippocampus (dCA1) to record from place cells during planning, running, and reward consumption. We were interested in whether replay sequences related to task parameters such as intended or recent route choice.
Behaviourally, rats demonstrated accurate spatial planning by generally choosing the correct goal, preferring the optimal path, and flexibly using optimal detours when the barrier was present. Surprisingly, we found very few replay sequences at the start of trajectories, likely insufficient to support immediate planning, and those did not preferentially reflect the future path. By contrast, replay events were numerous at the goal, either before or during reward consumption, and represented varied trajectories throughout the maze. Finally, replay rate was also strikingly low during error trials, both at the start and at the unrewarded arms.
In conclusion, it seems that the amount of hippocampal awake replay reflects reward, but not planning demand, while its content generally reflects available routes that are unrelated to immediate behaviour. This suggests that replay does not support trajectory selection, even in a task where rats demonstrate flexible planning. Overall, it is likely that another mechanism, such as hippocampal theta sequences, or prefrontal cortex reactivations, supports the path selection process during flexible navigation.

Disclosures: **E. Duvelle:** None. **S. Molas Medina:** None. **E. Hew:** None. **N. Atesyakar:** None. **G. Makdah:** None. **A. Rawson:** None. **W. Parry-Jones:** None. **R.M. Grieves:** None. **M. van der Meer:** None. **K.J. Jeffery:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.11/VV21

Topic: H.09. Spatial Navigation

Support: University of Connecticut IDEA Grant
University of Connecticut PCLB Grant
University of Connecticut SLAC

Title: Hippocampal place cell firing in a changing aversive-appetitive environment - Comparison along the longitudinal axis

Authors: *R. TROHA[1], S. LEE[2], M. ANAM[1], B. MORTE[1], S. TAVAKOLI[1], E. MARKUS[1];
[1]Univ. of Connecticut, Storrs, CT; [2]Emory Univ., Atlanta, GA

Abstract: The hippocampus has long been known to be important for learning and memory. However, the hippocampus is an elongated structure with functional differences along its longitudinal axis. The dorsal hippocampus seems more involved in spatial memory and navigation, while the ventral regions seem more involved in stress and emotional memory. Less is known regarding the intermediate region and how it processes information compared to the dorsal hippocampus.Strong connectivity between the dorsal and intermediate hippocampus subregions has also been shown, but few studies have examined differences in how these two regions respond to a changing environment. In the current study rats were trained to run back and forth for a food reward on a U-shaped maze. The apex of the runway was connected to a shock generator allowing for a mild current to be activated in this zone. On half the trials, a tone was played signaling the shock zone was active, while on the other half of the trials no tone was played, indicating no current in the zone. Therefore, animals were exposed to a fixed spatial configuration while the emotional situation switched between “safe” and “unsafe” trials. A 64-channel microelectrode array was used to record activity from single units in the dorsal and intermediate hippocampus during this task. Recorded cells in the dorsal and intermediate hippocampus will be compared regarding firing rates and place field characteristics. Furthermore, the response to the change in emotional situation will be quantified and compared between these two regions. This study will further our understanding of how the different regions of the hippocampus work together in processing spatial and emotional information.

Disclosures: R. Troha: None. **S. Lee:** None. **M. Anam:** None. **B. Morte:** None. **S. Tavakoli:** None. **E. Markus:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.12/VV22

Topic: H.09. Spatial Navigation

Support: Grant K99MH129565 (NIMH)
Grant R01MH124047 (NIMH)
Grant R01MH124867 (NIMH)
Grant 1U19NS104590
Grant 1U01NS115530 (NINDS)

Title: Coding and dynamics of neural ensembles along the proximodistal axis of hippocampal CA3 area

Authors: *T. GEILLER[1], E. KONG[1], D. S. PETERKA[2], A. LOSONCZY[1];
[2]Zuckerman Inst., [1]Columbia Univ., New York, NY

Abstract: Recurrent connectivity in the brain is a major anatomical feature underlying higher cortical functions, from sensory perception to learning and memory. In the hippocampus, local excitatory connections between pyramidal cells of the CA3 subregion are thought to support the initial and rapid encoding of contextual representations, providing a basis for memory-guided behaviors.A large body of work has revealed heterogeneities within CA3, organized along a proximodistal gradient, and suggesting that pyramidal neurons in distinct compartments exhibit different patterns of activity, as well as plasticity rules. However, this hypothesis is yet to be examined, as functional imaging approaches have hardly been deployed in the CA3 region of the rodent brain due to unfavorable geometries. The deepest anatomical portions (CA3c) near the dentate gyrus especially lack proper characterization with optical techniques. Therefore, our current knowledge of the sparsity, orthogonality, and stability of the hippocampal code for contexts along the proximodistal axis is still very limited.
To demonstrate the functional heterogeneity of hippocampal CA3 subregions at the cellular-level resolution, we recorded the activity of CA3 pyramidal neurons in the proximal (CA3) and distal (CA3a-b) regions in the same mice, using 2- and 3-photon imaging techniques. Animals were head-fixed, and trained to perform a spatial navigation task in which the reward location was hidden on a treadmill or virtual track. We performed random switches of context where we could monitor immediate network changes. We also tracked the same populations of neurons across multiple days to characterize long-term dynamics. We found prominent differences between the two regions in terms of place cell stability and activity patterns. Our results and ongoing experiments aim at uncovering the functionally structured organization of hippocampal recurrent circuits underlying memory-guided behaviors in awake animals.

Disclosures: **T. Geiller:** None. **E. Kong:** None. **D.S. Peterka:** None. **A. Losonczy:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.13/VV23

Topic: H.09. Spatial Navigation

Support: NIH Grant R01MH100631
NIH Grant R01NS094668
NIH Grant U19NS104590
NIH Grant R01NS067557
NIH Grant R01NS094668
NIH Grant F32MH118716
NIH Grant K99NS127815
Zegar Family Foundation
Foundation Roger De Spoelberch

Title: The in vivo role of distal tuft dendrites in CA1 pyramidal neurons

Authors: *J. K. O'HARE[1], J. WANG[3], M. SHALA[2], F. POLLEUX[1], A. LOSONCZY[1];
[1]Zuckerman Inst., [2]Columbia Univ., New York, NY; [3]Duke Univ., Durham, NC

Abstract: Synaptic plasticity is thought to support behavioral adaptation by updating how information propagates though neuronal circuits, but this process remains enigmatic due to its many levels of complexity. In hippocampal area CA1, pyramidal neurons (CA1PNs) integrate multiple streams of incoming information through three distinct dendritic compartments: basal, radial oblique, and distal tuft. Distal tuft dendrites are believed to play a crucial role in the experience-dependent emergence of spatial feature selectivity: arguably the main computational function of the CA1 circuit. However, their true function has been relegated to speculation due to their experimental inaccessibility in behaving animals. We simultaneously monitored somatic and distal tuft dendritic activity dynamics in individual CA1PNs using single-cell electroporation, two-photon microscopy, and a red-shifted calcium indicator amenable to deep-brain imaging. We find that, despite their ostensibly prominent role in driving neuron-wide plateau potentials to support the emergence of cellular feature selectivity, distal tuft dendrites display a striking degree of autonomous activity. Local distal tuft processing is state-dependent and conveys more spatial information than the cognate CA1PN soma. Finally, using a virtual reality-based “teleporting” paradigm, we provide new insights into the role of distal tuft dendrites in the emergence of spatial feature selectivity in the hippocampus.

Disclosures: J.K. O'Hare: None. **J. Wang:** None. **M. Shala:** None. **F. Polleux:** None. **A. Losonczy:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.14/VV24

Topic: H.09. Spatial Navigation

Support: R01MH124867
R01NS121106
R01MH124047
U01NS115530
U19NS104590
N00014161253
ECCS-2024776
ECCS-1752241
ECCS-1734940
R21 EY029466
R21 EB026180
DP2 EB030992

Title: Orthogonal ensemble emergence for rapid place cell remapping during novel experiences

Authors: *S. TERADA[1], M. RAMEZANI[2], D. KUZUM[3], A. LOSONCZY[4];
[1]Columbia Univ., New York, NY; [2]Univ. of California San Diego, [3]Univ. of California San Diego, La Jolla, CA; [4]Columbia Univ., HHMI Janelia Farm, Westport, CT

Abstract: The circuit balance between stability and plasticity is crucial for memory encoding and storage. While behavioral timescale synaptic plasticity (BTSP) has been considered for a plasticity rule to form the spatial tuning of hippocampal pyramidal cells rapidly, little is known about how this rapid place field formation of single cells is mediated at the circuit level. Using integrated 2-photon imaging and simultaneous extracellular recordings with "E-Cannula", we simultaneously monitored cellular calcium dynamics in large populations of pyramidal cells and local field potentials in hippocampal area CA1 as mice while learning novel spatial cues in order to investigate trial-to-trial transitions of individual place field formations and how they reorganize subsequent network dynamics. We identified that transient spatially tuned sequences of different subpopulations emerged and persisted for a few trials provided that others were absent. In each of these orthogonal ensembles, a certain number of those cells successfully formed stable place fields. The majority lost their spatial tuning after a few trials, which triggered the emergence of the subsequent ensemble of different cells. This cycle of orthogonal ensembles discretely accumulated stable place cells for the spatial map. We further observed the reorganized spatiotemporal patterns of sharp-wave ripples during the subsequent resting period followed by enhanced reactivation of the stable place cells. These results suggest that BTSP is induced in a subset of pyramidal cells while others remain unchanged. When the plasticity selection of those neurons is terminated, the circuit next makes different neurons available.

Disclosures: S. Terada: None. **M. Ramezani:** None. **D. Kuzum:** None. **A. Losonczy:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.15/VV25

Topic: H.09. Spatial Navigation

Support: NIH NINDS R01NS113557

Title: The spatial localization of reward-related changes in hippocampal replay and sharp-wave ripple rate in novel environments requires normal dopamine signaling

Authors: *M. R. KLEINMAN, D. J. FOSTER;
Univ. of California, Berkeley, Berkeley, CA

Abstract: During quiescent states, patterned reactivation of place cells in the hippocampus can represent coherent spatial trajectories at compressed timescales, a phenomenon known as replay. Sharp-wave ripples (SWR) in the local field potential are a signature of the coordinated population bursts comprising replay. Spatially-specific changes in reward value in an environment cause spatially-specific changes in the rate of SWR and replays, as well as changes

in dopamine release from ventral tegmental area (VTA) neurons, but it is unknown whether replay rate is affected by VTA dopamine signaling. To address this question, we used a cre-dependent virus to express the inhibitory DREADD hM4Di in the VTA of TH-cre rats to allow selective and reversible inactivation of VTA dopamine neurons, while recording from dorsal CA1 of the hippocampus using tetrode microdrives. We tested animals on a task where reward volumes at each end of a linear track changed in a blocked design each session: initially equal (0.1 ml, 10-20 laps), then unequal (0.1 ml and 0.4 ml, 10-20 laps), then equal again (0.1 ml, 10-20 laps). Surprisingly, on familiar linear tracks that had been explored several times before, we found VTA inactivation had only modest effects on the selective increase in SWR and replay rate at locations where reward volume increased. In contrast, VTA inactivation in novel environments caused a large disruption in the spatial specificity of SWR and replay changes. SWR rate increased at locations where reward increased but also where reward was unchanged, while replays failed to selectively occur more frequently at the increased reward locations. In a second set of experiments, we tested whether normal SWR and replay recruitment required VTA activity due to novelty per se or alternative features of novel experiences. On familiar tracks, we implemented a new reward schedule, with the "volatile" end of the track having reward volume changing dramatically and pseudorandomly lap-to-lap (range: 0-0.8 ml, mean 0.37 ml), while the "stable" end remained fixed (0.2 ml). With frequent bidirectional changes in reward values, we could also probe whether SWR and replay rate were correlated with value or reward prediction error (RPE). Intriguingly, SWR rate was correlated with RPE, and we found no effect of VTA inactivation in this task in familiar environments. These results reveal VTA dopamine release is required for spatially-specific recruitment of SWR and replay to reward locations when they are not yet well-learned, but is dispensable in familiar environments, where we hypothesize locus coeruleus dopamine release may be sufficient to signal reward changes.

Disclosures: M.R. Kleinman: None. **D.J. Foster:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.16/VV26

Topic: H.09. Spatial Navigation

Support: NIH Grant R01NS113557

Title: Hippocampal replay as veridical representation of spatial experience

Authors: *J. WIDLOSKI[1], M. R. KLEINMAN[2], D. J. FOSTER[3];
[2]Psychology, [3]Univ. of California, Berkeley, [1]Univ. of California, Berkeley, Berkeley, CA

Abstract: Hippocampal cells show precise spatial tuning to the animal's position during behavior. During stopping periods, the hippocampus encodes spatially coherent trajectories through the environment called replays that resemble the animal's behavior. Replay has been

proposed as a form of virtual experience that supplements real experience for the purposes of learning and planning. However, it is unclear how veridical replay is compared to experience, i.e., whether the precise spatial tuning of place cells as a function of animal position is preserved as a function of encoded replay position, independent of the animal's location, let alone any of the higher order spiking statistics within and across cells associated with movement. This is far from obvious, given that replay unfolds at much higher speeds and is likely to involve different mechanisms and circuits. We show that in a 2D environment, spatial tuning in CA1 place cells is preserved as a function of the encoded position during replay (its "replay field") on both coarse- and fine-grained measures, using a leave-one-out analysis that removes the cell's contribution to replay before evaluating its replay field. We further show that replay fields exhibit the same overdispersion or excess temporal variance of spiking that is characteristic of place fields, thought to be due to the top-down modulatory effects of attention and other higher-order cognitive processes, suggesting that firing patterns during replay are influenced by the same extra-positional content as during run. Lastly, we show that during run, this overdispersion is temporally correlated across cells (~1 sec, as predicted by previous models), but during replay is temporally compressed (~1/10 sec), consistent with a model whereby the modulatory extra-positional inputs during run are recapitulated at compressed rate during replay. Thus, according to multiple statistical criteria, replay expresses the same rich dynamics as during run, albeit compressed in time. This correspondence presumably arises through reciprocal connections between hippocampus and cortical and sub-cortical circuits that permit relevant activity patterns in those areas to be retrieved offline as they would online. This could allow for memory consolidation during replay to occur over statistically-matched spiking ensembles during replay as during run.

Disclosures: **J. Widloski:** None. **M.R. Kleinman:** None. **D.J. Foster:** None.

Poster

PSTR503. Place Cells: Sequences, Reactivation, and Remapping

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR503.17/VV27

Topic: H.09. Spatial Navigation

Support: R01NS113557 NIH, NINDS

Title: Reverse replay is temporally segregated from forward replay and uniquely dependent on medial entorhinal cortex

Authors: *C. MALLORY, D. FOSTER;
Helen Wills Neurosci., UC Berkeley, Berkeley, CA

Abstract: During periods of awake immobility, sequences of hippocampal place cells can reactivate in the same or reverse-temporal order as experience (Foster & Wilson, 2006, Diba & Buzsaki, 2007). Forward and reverse sequences appear to be independently regulated, as they

occur at different times relative to behavior, and only reverse replay is sensitive to changes in reward magnitude (Ambrose et al., 2016). Recent work identified a subclass of CA1 neurons ('bimodal cells') that were most active during reverse hippocampal sequences, including replay (Wang et al., 2020). The putative anatomical location of these cells, within deep-CA1, suggests that the upstream medial entorhinal cortex (MEC) may play a particular role in the expression of reverse replay. To explicitly test this hypothesis, we optogenetically inactivated the bilateral MEC and decoded hippocampal replay from up to 150 neurons recorded simultaneously in CA1. First, we found that forward and reverse replays indeed occur at different times relative to the rats' behavior, as previously reported. Strikingly, however, forward replays occurred consistently earlier following the onset of reward-consumption. Thus, the drinking period was marked by two distinct phases: an early phase dominated by forward replay, and a late phase dominated by reverse replay. Second, we show that reverse replay is uniquely dependent on MEC. With MEC input intact, forward and reverse replays were typically accompanied by sharp-wave ripples of varying-but comparable-strength. While the ripple power accompanying forward replays was unaffected by MEC inactivation, that underlying reverse replays was markedly reduced. This effect was strongest during the 'reverse replay phase' of the stopping period, described above, but also observable in reverse replays occurring outside of this time frame. Together these findings point to distinct mechanisms responsible for generating forward and reverse replays, with MEC playing a critical role in the latter.

Disclosures: C. Mallory: None. **D. Foster:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.01/VV28

Topic: H.10. Human Learning and Cognition

Title: Compressing, aligning and transferring knowledge across tasks and domains

Authors: *N. MENGHI[1], S. VIGANÒ[1,2], B. MAESS[1], C. F. DOELLER[1,3,4,5];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Ctr. for Mind/Brain Sciences, Univ. of Trento, Rovereto, Italy; [3]Ctr. for Neural Computation, Kavli Inst. for Systems Neurosci., Trondheim, Norway; [4]Leipzig Univ., Wilhelm Wundt Inst. of Psychology, Leipzig, Germany; [5]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: As we learn, we form models of the world around us. These models have been described as sensory-independent, abstract spaces, where knowledge is represented in a map-like format. During learning, knowledge can be efficiently compressed into lower-dimensional representations, enabling us to generalize and transfer that knowledge across various tasks and domains. Despite decades-long research on learning, the precise dynamics of knowledge interaction and transfer during learning remain elusive. We propose that gaining an understanding of the mechanisms involved in compression is crucial for unravelling the

processes of transfer and generalization. In the present study, we examined task structures to characterize generalization and transfer in a multitask learning experiment. We defined task structure as a one-dimensional manifold that describes the associations between features and outcomes. To this end, we created two experimental conditions (between subjects) in which a spatial and a conceptual task either shared the same underlying structure or not. Participants learned to separately associate spatial or conceptual features with specific outcomes. Subsequently, we evaluated participants' performance using stimuli encountered during training as well as novel stimuli. Using a combination of behavioural and magnetoencephalography (MEG) measures, we characterized the existence of a lower dimensional map-like representation of knowledge, where information can be generalized and transferred within and between tasks. Behaviourally, we found that learning performance differed between the two groups, suggesting that the lower dimensional structure of the task influenced the transfer of knowledge. Neurally, we used representation similarity analysis (RSA) and identified the temporal dynamics of task representations and compression. Our findings reveal that the structure of the task determines how knowledge is represented and transferred. Overall, our results support and expand the cognitive map framework by demonstrating the existence of a general code capable of organizing knowledge derived from diverse experiences.

Disclosures: **N. Menghi:** None. **S. Viganò:** None. **B. Maess:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.02/VV29

Topic: H.10. Human Learning and Cognition

Support: ERC-StG NOAM
ERC-CoG GEOCOG
Max Planck Society
Kavli Foundation
Jebsen Foundation
Helse Midt Norge
Research Council of Norway
ERC-StG 261177
NWO-Vidi 452-12-009

Title: Spontaneous eye movements reflect the representational geometries of conceptual spaces

Authors: *S. VIGANÒ[1,2], R. BAYRAMOVA[1], C. F. DOELLER[1,3,4,5], R. BOTTINI[2];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Ctr. for Mind/Brain Sci. (CIMeC), Univ. of Trento, Rovereto, Italy; [3]Kavli Inst. for Systems Neurosci., Trondheim, Norway; [4]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [5]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Norway

Abstract: Functional neuroimaging studies indicate that the human brain can represent concepts and their relational structure in memory using coding schemes typical of spatial navigation. However, whether we can read out the internal representational geometries of conceptual spaces solely from human behavior remains unclear. Here we report that the relational structure between concepts in memory is reflected in spontaneous eye movements during verbal fluency tasks. In a first experimental condition, we asked participants to randomly generate numbers from 1 to 12, while we monitored their spontaneous gaze behavior with an eye-tracker. Left and right eye movements correlated with numerical differences between mentioned numbers, consistent with the left-right 1-dimensional geometry of the number space (mental number line): the smaller (or larger) the number, the more participants looked to the left (or right) of their visual field before mentioning it. In a second condition, participants randomly mentioned twelve colors, for which they had previously provided pairwise similarity judgments. We used these judgments to reconstruct subject-specific "color wheels" using multidimensional scaling and we observed that Euclidean distances between colors in these reconstructed spaces were correlated with distances covered by bidimensional eye movements during the verbal fluency task: the closer two colors were in the color wheel, the smaller the distance between their corresponding gaze fixation in visual space before they were mentioned, consistent with the 2-dimensional ring-like 2-dimensional geometry of the color space. In a third and last condition, participants randomly generated animal names, for which the underlying representational geometry is complex and multidimensional. We modeled this geometry using a combination of computational linguistics and semantics tools, and we observed that 1-dimensional horizontal eye movements correlated with low-dimensional similarity in word frequency space: the more similar (or different) the frequency values of two mentioned animals, the more participants looked to the left (or right). Taken together, our results suggest that the representational geometries used to internally organize conceptual spaces can be read out from gaze behavior.

Disclosures: S. Viganò: None. **R. Bayramova:** None. **C.F. Doeller:** None. **R. Bottini:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.03/VV30

Topic: H.10. Human Learning and Cognition

Title: Prior Knowledge and Memory Encoding: Investigating the Influence of Congruency and Incongruency on Learning

Authors: *S. ELNAGAR[1], N. MENGHI[1], A. GREVE[2], C. F. DOELLER[1,3,4,5];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]MRC Cognition and Brain Sci. Unit, Univ. of Cambridge, Cambridge, United Kingdom; [3]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and

Technol., Trondheim, Norway; [4]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [5]Dept. of Psychology, Tech. Univ. Dresden,, Dresden, Germany

Abstract: Encoding new memories takes place against the backdrop of a rich library of information acquired through one's life. Several studies show that prior knowledge, such as schemas, strengthens the encoding and accelerates the recall of new memories that are in agreement with it (congruent), while others show the opposite pattern where prediction violation (related to information being incongruent with a schema) facilitates learning. To reconcile the contradictory findings in these two lines of research, a recent framework, the schema- linked interaction between the medial temporal and medial prefrontal regions (SLIMM model), postulates that memory shows a non-linear, U-shaped function with degrees of congruency to prior information. In other words, highly congruent and highly incongruent information with a schema benefit the process of consolidation during learning. However, the SLIMM model remains under scrutiny as not enough evidence have been found to support its hypotheses yet. Furthermore, the neural underpinnings of such learning processes remain unknown. While some models suggest a trade-off between the medial prefrontal cortex (mPFC) and the medial temporal lobe (MTL) structures for congruent and incongruent effects respectively (e.g. SLIMM), other models predict an essential role of MTL structures in encoding information congruent to existing knowledge structures. In this study, we use behavioural methods as well as neuroimaging techniques (fMRI) to understand whether and how the representation of prior knowledge enhance the encoding and retrieval of new events. We developed a novel spatial schema paradigm, which compares three conditions with varying degrees of congruency to previous knowledge and test the three seemingly contradictory behavioural findings in the literature. Our results demonstrate a mnemonic advantage for congruent events, while incongruent events and those lacking a strong prior schema exhibit a disadvantage, suggesting that reaffirming expectations facilitates learning. In the concurrent fMRI study, we will directly compare learning systems in the brain that support learning under certain (congruent) and uncertain (incongruent) conditions and will investigate the formation and update of schema representations with newly acquired information. This study could lead to a better understanding towards a refined neuroscientific model of how brain networks interact to successfully integrate new information with previous knowledge schema.

Disclosures: S. Elnagar: None. **N. Menghi:** None. **A. Greve:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.04/VV31

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
Minerva Fast Track Research Group Grant (Max Planck Society)
Max Planck School of Cognition

Title: Investigating the relationship between connectivity fingerprints and spatial representations in human entorhinal cortex

Authors: *R. M. TENDERRA[1], C. F. DOELLER[1,2,3,4], S. THEVES[1];
[1]Max Planck Inst. For Human Cognitive and Brain Sci., Leipzig, Germany; [2]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [3]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [4]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: The entorhinal cortex (EC) is the key interface between neocortex and hippocampus in which spatial and non-spatial relations between experiences are organized into cognitive maps that support flexible behavior. Computational models suggest that the hippocampal-entorhinal system may specifically support generalization by factorizing the representation of experiences into their structure, by grid- and vector cells in the medial entorhinal cortex (MEC) and their sensory specifics in its lateral counterpart (LEC), allowing their flexible recombination. A functional distinction between MEC and LEC is congruent with their distinct anatomical connectivity with other brain regions, and further corroborated by the specific abundance of grid cells in MEC, but not LEC. Although there is growing evidence for representations of physical and abstract task spaces in the human EC, the functional organization and anatomical distribution of spatial representations remains poorly understood. Here we investigate the regional specificity of spatial representations in EC, assessed with task-based functional magnetic resonance imaging (fMRI), and build on a previously established approach identifying the human homologue of rodent MEC and LEC based on their differential whole-brain functional connectivity profiles. We identify individual connectopic maps reflecting the dominant change in similarity between functional connectivity patterns along the entire EC based on resting state fMRI data. This allows us to probe how these individual connectopic maps correspond to the distribution of spatial representations across the EC. A better understanding of the relationship between representational mechanisms within the EC and its brain-wide connectivity can inform investigations on its wider role in cognition and further contribute to bridging the gap between rodent and human research.

Disclosures: R.M. Tenderra: None. **C.F. Doeller:** None. **S. Theves:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.05/VV32

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: Grid-like coding of an abstract value space for prospective decision making

Authors: *A. NITSCH[1], M. M. GARVERT[1,2,3,4], J. L. S. BELLMUND[1], N. W. SCHUCK[2,3,5], C. F. DOELLER[1,6,7,8];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Max Planck Res. Group NeuroCode, Max Planck Inst. for Human Develop., Berlin, Germany; [3]Max Planck UCL Ctr. for Computat. Psychiatry and Aging Res., Berlin, Germany; [4]Fac. of Human Sciences, Julius-Maximilians-University Wuerzburg, Wuerzburg, Germany; [5]Inst. of Psychology, Univ. Hamburg, Hamburg, Germany; [6]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [7]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [8]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: Everyday decisions require us to predict how valuable different choice options will be in the future. Prior studies have identified a cognitive map in the hippocampal-entorhinal system which encodes relationships between states and enables prediction of future states, but does not inherently convey value during prospective decision making. Here, we investigated whether the entorhinal cortex integrates relational information about changing values by representing an abstract value space. To this end, we combined fMRI with a prospective decision making task which required participants to track and predict changing values of two choice options in a sequence. Such a sequence formed a trajectory through an underlying two-dimensional value space. Our results show that participants successfully integrated and extrapolated changes along the two value dimensions. Participants' choice behavior was explained by a prospective reinforcement learning model and the degree to which they updated values over time correlated with self-reported navigational abilities and preferences. Crucially, while participants traversed the abstract value space, the entorhinal cortex exhibited a grid-like representation, with the phase of the hexadirectional fMRI signal (i.e. the orientation of the estimated grid) being aligned to the most informative axis of the value space. A network of brain regions, including the ventromedial prefrontal cortex (vmPFC) and the hippocampus, tracked the prospective value difference between options and the occipital-temporal cortex represented the more valuable option. These findings suggest that the entorhinal grid system might support the prediction of future values by representing a cognitive map, which might be used to generate lower-dimensional signals of the value difference between options and their identities for choices. Thus, these findings provide novel insight for our understanding of cognitive maps as a mechanism to guide prospective decision making in humans.

Disclosures: A. Nitsch: None. **M.M. Garvert:** None. **J.L.S. Bellmund:** None. **N.W. Schuck:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.06/VV33

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: The role of cognitive maps in prototype-based inference

Authors: *T. A. J. SCHÄFER[1], M. THALMANN[2], E. SCHULZ[2], C. F. DOELLER[1,3,4,5], S. THEVES[1];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Max Planck Inst. for Biol. Cybernetics, Tübingen, Germany; [3]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [4]Wilhelm Wundt Inst. of Psychology, Leipzig, Germany; [5]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: Concepts represent combinations of features shared by similar entities and allow generalization from limited experience to novel situations. It has been proposed that the hippocampal-entorhinal system contributes to concept learning by representing the relations between experiences along relevant feature dimensions. However, how the system provides access to abstracted information remains unclear. In the present fMRI study, we investigated whether this map-like representation of concepts supports the retrieval of category prototypes to guide behavior in a feature inference task. After participants were trained to categorize a set of exemplars based on the combination of two of their features, they were presented with partial stimuli and had to infer the missing feature consistent with a given category label. We found that, congruent with behavioral completion responses, the visual cortex reinstated the pattern of the missing prototypical feature. Specifically, behavioral and neural completion responses were closer to the previously unseen prototype than to the nearest experienced exemplar. Similar to fMRI signatures of pattern completion into experienced event representations, the cortical completion towards the prototypical feature value covaried with hippocampal activity. Furthermore, hippocampal activity during inference reflected sensitivity to the 2D prototype location, alongside a entorhinal grid-like representation of the two-dimensional concept space. Collectively, our results provide novel insight into the neural underpinnings of concept learning and highlight a potential role of cognitive maps in the retrieval of abstracted information during prototype-based inference.

Disclosures: T.A.J. Schäfer: None. **M. Thalmann:** None. **E. Schulz:** None. **C.F. Doeller:** None. **S. Theves:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.07/VV34

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: Geometric determinants of spatial representations in the human hippocampal formation

Authors: *V. REISNER[1], M. KIM[2], T. A. J. SCHÄFER[1], W. DE COTHI[3], C. BARRY[3], C. F. DOELLER[1,4,5,6];
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Insitute of Cognitive Neuroscience, Univ. Col. London, London, United Kingdom; [3]Dept. of Cell and Developmental Biology, Univ. Col. London, London, United Kingdom; [4]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [5]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [6]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: Finding locations in a familiar environment requires accurate representations of the spatial surrounding, so-called 'cognitive maps'. In many animal species, specialized neurons in the hippocampal formation, such as hippocampal place and entorhinal grid cells, provide the neural basis for cognitive maps. Previous research has shown that stretching or compressing environmental boundaries controls the firing pattern of place cells and grid cells in freely moving rodents, as well as homing behavior in humans. This indicates that boundaries defining the geometry of space play an important role in determining the nature of cognitive maps. In this study, we investigated how behavioral changes to environmental deformations are related to those on the neural level as measured with functional magnetic resonance imaging (fMRI) in humans, and how these effects can be explained by computational models. In this two-day study, we first trained participants to learn the location of multiple objects in a virtual arena. During subsequent scanning, we asked them to revisit each location within the arena and actively imagine them outside the arena. Critically, the shape of the arena changed from square to rectangular across days. We observed systematic shifts in spatial memory related to the change in geometry that are best explained by a computational model of boundary vector cells (BVCs) known to respond to the distance and allocentric direction to environmental boundaries. Moreover, our fMRI data suggest that brain representations of locations and distances scale with the current geometry of the environment. Our study extends our understanding of how behaviour and neural representations associated with environmental deformations are interrelated in humans and can be described by a population model of hippocampal cell firing.

Disclosures: V. Reisner: None. **M. Kim:** None. **T.A.J. Schäfer:** None. **W. de Cothi:** None. **C. Barry:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.08/VV35

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: Abstract representation of action plans in the hippocampal-entorhinal system

Authors: *I. BARNAVELI[1], S. VIGANÒ[1,2], D. REZNIK[1], P. HAGGARD[3], C. F. DOELLER[1,4,5,6];
[1]Psychology, Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Ctr. for Mind/Brain Sciences, Univ. of Trento, Rovereto, Italy; [3]Inst. of Cognitive Neuroscience, Univ. Col. London, London, United Kingdom; [4]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [5]Wilhelm Wundt Inst. for Psychology, Leipzig Univ., Leipzig, Germany; [6]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: We have a remarkable ability to form and consider different action plans in order to reach our goals. Effective selection of actions requires weighing them against each other based on how they relate to the world. However, the neural mechanisms underlying these computations are still unknown. Recent evidence suggests that cognitive maps generated in the hippocampal-entorhinal system support the representation of conceptual knowledge by encoding the relational structure of stimuli. Here, we hypothesized that cognitive maps could also organize available action plans in a relational manner according to their expected outcomes, supporting flexible goal-directed behavior. To answer this question, we created a novel behavioral paradigm using immersive virtual reality (VR). Participants (N=46) were trained to execute different actions with their right hand by sequentially changing the position of two virtual joysticks that triggered launching of a ball towards them. They learned to associate different joystick actions with different probabilities to either catch a flying ball (outcome dimension 1) or for the ball to remain visible throughout its flying trajectory (outcome dimension 2). Then, participants compared the action combinations based on the learned action-outcome contingencies. We performed multidimensional scaling on the behavioral data and fitted the subjective judgments to the actual action-outcome space. The results indicate that participants created a mental map of action combinations, corresponding to the distribution of those combinations in the bi-dimensional action-outcome space. To explore the neural mechanisms of this map-like representation, we let participants perform a similar task while recording brain activity with fMRI and applied representation similarity (RSA) and adaptation analyses to the neuroimaging data. Our analyses revealed an integrated and segregated coding of the outcome dimensions in the brain. The distance between relative positions of action combinations in the action-outcome space was represented in the hippocampus, while the general structure of the space elicited a grid-like code in the entorhinal cortex, suggesting a joint representation of both dimensions. Furthermore, activity in anterior intraparietal sulcus selectively scaled with variation in the outcome dimension 'probability of catching', while the signal in visual area V3 was systematically modulated by variation in the outcome dimension 'probability of visibility'. Our findings provide evidence that

cognitive maps could enable efficient selection of actions by encoding multiple relationships between different action plans.

Disclosures: I. Barnaveli: None. **S. Viganò:** None. **D. Reznik:** None. **P. Haggard:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.09/VV36

Topic: H.10. Human Learning and Cognition

Support: Max Planck Society
International Max Planck Research School on Neuroscience of Communication: Function, Structure, and Plasticity (IMPRS NeuroCom)

Title: Integrating knowledge about structure and reward contingencies for generalization and inference

Authors: *F. F. DEILMANN[1], S. THEVES[1], M. M. GARVERT[1,2,3,4], C. F. DOELLER[1,5,6,7]; [1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Max Planck Inst. for Human Develop., Berlin, Germany; [3]Max Planck UCL Ctr. for Computat. Psychiatry and Ageing Res., Berlin, Germany; [4]Fac. of Human Sciences, Julius-Maximilians-University Wuerzburg, Wuerzburg, Germany; [5]Kavli Inst. for Systems Neuroscience, Ctr. for Neural Computation, The Egil and Pauline Braathen and Fred Kavli Ctr. for Cortical Microcircuits, Jebsen Ctr. for Alzheimer's Disease, Norwegian Univ. of Sci. and Technol., Trondheim, Norway; [6]Wilhelm Wundt Inst. of Psychology, Leipzig Univ., Leipzig, Germany; [7]Dept. of Psychology, Tech. Univ. Dresden, Dresden, Germany

Abstract: An adequate internal representation of our environment and its underlying structure is essential for flexible planning and the ability to respond appropriately in novel situations. The hippocampal-entorhinal system is known for abstracting relations among sensory experiences, such as state transition probabilities, within a so-called cognitive map. This form of knowledge representation is assumed to enable fast learning of novel relations and reward generalization, thus facilitating goal-directed behavior as formalized in the reinforcement learning framework. However, in addition to experienced transition probabilities, states may concurrently share other types of relational information, like reward contingencies. Here, we use fMRI to investigate how the neural representation of relational knowledge is influenced by a subsequently learned latent reward structure. We also examine potential neural mechanisms facilitating the utilization of relational knowledge for generalization and inference. Participants initially obtain knowledge about visual object (state) relations based on transition probabilities following a hidden graph structure. Behavioral data suggest that participants successfully acquired structural knowledge and utilized this information to find shortcuts between states of the graph. In a subsequent

decision-making task, each state got associated with fluctuating reward values. Critically, two parts of the graph structure shared the same reward contingencies. Our modeling results indicate that participants could abstract the underlying latent reward structure and effectively generalize over states sharing the same reward contingencies. Furthermore, participants applied their structural knowledge and abstracted reward contingencies to correctly infer reward values of states that they never directly experienced. Representational similarity analysis results reveal hippocampal and vmPFC neural similarity patterns that reflect the underlying structure and reward contingency representations. Finally, multivariate decoding analysis indicates reinstatement of congruent and related states as a plausible neural mechanism supporting generalization of reward contingencies. Overall, our findings demonstrate how an integrated representation of experienced transitions and shared reward contingencies between states enables generalization and inference.

Disclosures: F.F. Deilmann: None. **S. Theves:** None. **M.M. Garvert:** None. **C.F. Doeller:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.10/VV37

Topic: H.10. Human Learning and Cognition

Support: NSF Career Award

Title: Emergent model-based replay in humans as a function of credit assignment

Authors: *C. D. CARRASCO[1], S. A. PARK[2], E. VARGA[1], S. J. LUCK[1], E. D. BOORMAN[1]; [1]Psychology, UC Davis, Davis, CA; [2]Ctr. Natl. de la Recherche Scientifique, Paris, France

Abstract: Sequential patterns of neural reactivation have been shown to be relevant to tracking onto abstract and physical state representations, a phenomenon known as replay. This neurophysiological process is thought to be important for decision making, memory and learning. One way in which learning can take place is through incorporation of explicit knowledge of the structure of an environment. This model can be used to guide reinforcement of relevant representations in the environment that lead to particular outcomes, such as those leading to reward. Whether this type of learning influences the way in which humans replay representations online is still largely unknown.
To test for online model-based replay we had participants perform a localizer and sequence learning task while recording EEG. In the learning task participants were presented with alternating blue and orange bordered images serving as abstract space trajectories leading to an outcome. Participants then reported which of the two trajectory categories they believed contained the relevant sequence of images that lead to reward and were occasionally probed about the exact ordering of the images relevant to reward. The relevant trajectory image

positions and category were changed on a block-by-block basis after a learning criterion was met without explicit feedback. During the localizer, participants viewed stimuli on the screen and a word following; responding if the word matched or mismatched. We generated event related potentials (ERPs) time locked to stimuli and ISI's throughout the tasks. We then trained independent classifiers to identify the neural pattern of activation for each stimulus and used them to identify reactivation of the representations at ISIs following reward. Using these reactivation probabilities, we fit general linear models that identify lag between reactivations of neural representations and relate them to task related transition matrices or 'models', specifying the reactivation of reward target sequences.
Behavioral results, indicate learning in nearly all subjects across search blocks ($N = 20$, $p < 0.05$) and show variations in the rate of learning across participants. Neural results show reverse replay at a 70 ms lag between states following reward, $p < 0.05$. Critically this effect occurs when looking at trials where participants were in an exploit state and rewarded. This indicates that participants had to learn the to apply the model to the environment for replay to facilitate credit assignment to relevant neural states. Future work will include characterization of individual learning differences and their relation to varying differences in replay features.

Disclosures: C.D. Carrasco: None. **S.A. Park:** None. **E. Varga:** None. **S.J. Luck:** None. **E.D. Boorman:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.11/VV38

Topic: H.10. Human Learning and Cognition

Title: Curiosity, Memory, and the Place

Authors: *S. NASIRI, T. BROWN;
Georgia Inst. of Technol., Atlanta, GA

Abstract: An important frontier for spatial navigation research is understanding the influence of curiosity on spatial memory. Architectural science suggests an environment's layout and organization can impact a navigators' affect and motivation to explore, and thus a curiosity may be a key mediator of the association between spatial organization and spatial memory. This experiment investigates the association between spatial organization, curiosity, and memory using immersive virtual environments with head-mounted devices. Informed by architectural design principles, these virtual environments represented simulated homes, with three design strategies: traditional compartmentalized plans, fully open plans, and a mixed semi-open design that retains private space. Based on prior literature, we hypothesized that the open-plan design would generate greater curiosity, encourage exploration, and thus enhance cognitive mapping. Additionally, the open plan was expected to be associated with positive affect and a sense of "flow" and connection. Participants' data, including movement paths, exploration time, and

attentional focus, were collected through Unity programming and eye-tracking technology. Motile neural data using functional near infrared spectroscopy were also recorded to map neural signatures underlying the behavioral data, focusing on the brain's extended dopaminergic reward-processing areas and memory systems related to curiosity and memory signaling. Post-exploration questionnaires gave insight into the subjective experiences of curiosity, affect, and memorability. Preliminary results from participants indicate that it is actually the semi-open plans which generate more distributed curiosity, are more memorable and are perceived as more "livable". Conversely, the compartmentalized plans were both rated more confusing but also less interesting, and found to be less memorable. The study suggests that varying levels of openness evoke different types of curiosity, and affect memorability based on the visual information available for cognitive mapping. Ultimately, this research aims to enhance the design of healthier, more interesting, and memorable environments by understanding human-environment relationships.

Disclosures: S. Nasiri: None. **T. Brown:** None.

Poster

PSTR504. Relational and Spatial Learning

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR504.12/VV39

Topic: H.10. Human Learning and Cognition

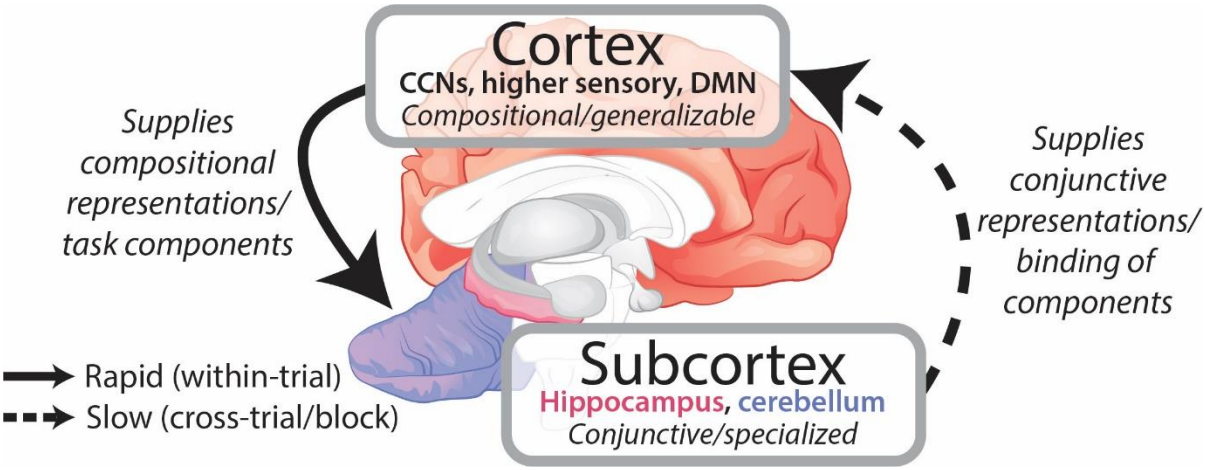
Support: NIH Grant R01 MH109520
NSF Grant 2219323

Title: Neural representation dynamics reveal computational principles of cognitive task learning

Authors: *R. MILL, M. W. COLE;
Rutgers Univ. Newark, Newark, NJ

Abstract: Learning cognitive tasks is a ubiquitous feature of everyday life, yet the neural basis for this essential human skill remains unknown. During learning, neural task representations must be rapidly constructed for novel task performance, then optimized for robust practiced task performance. The present study interrogated changes to neural representational geometry that underpin this transition from novel to practiced task performance. Specifically, we hypothesized that practice involves a shift in the brain from compositional representations (task-general activity patterns that can be flexibly reused across tasks) to conjunctive representations (task-specific activity patterns specialized for the current task). Functional MRI data were recorded with a newly developed paradigm, in which multiple complex tasks were performed from first novel presentation through repeated practice. We developed machine learning-inspired methods to empirically quantify the strength of compositional and conjunctive rule representations as learning progressed over time. The results substantiated the hypothesized dynamic shift from compositional to conjunctive representations with practice, which was associated with reduced

cross-task interference (via pattern separation) and behavioral improvement. Further, we found that conjunctions originated in subcortex (hippocampus and cerebellum) and slowly spread to cortex, extending multiple systems theories of memory to the domain of task learning. The formation of conjunctive representations hence serves as a computational signature of learning, reflecting cortical-subcortical dynamics that optimize task representations in the human brain. Figure legend. Hypothesized cortical-subcortical dynamics underlying the transition from compositional to conjunctive neural representations over task learning.



Disclosures: **R. Mill:** None. **M.W. Cole:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.01/VV40

Topic: H.13. Schizophrenia

Support: NIH (grant no. 5K08GM138981)
RFA (Department of Anesthesia, UCSF)

Title: The Orexin projection to periaqueductal gray mediates Anesthesia Arousal

Authors: ***X. XIANG**, Z. GUAN, W. ZHOU;
Univ. of California San Francisco, San Francisco, CA

Abstract: Anesthesia remains largely mysterious in terms of its underlying mechanisms. The regulation of anesthesia involves intricate neural circuits, including the hypothalamic orexin(Ox) neurons. Ox neurons extensively project throughout the brain and spinal cord, playing crucial roles in regulating arousal, pain perception, and so on. However, the precise interactions between anesthetics and the Ox circuitry remain incompletely understood. Here, we present novel findings regarding the involvement of Ox projections in the periaqueductal gray (PAG) in

anesthesia. Our imaging results demonstrate robust staining of Ox fibers in the PAG. To confirm the presence of Ox terminal innervation of PAG neurons, we employed retrograde adeno-associated virus (AAV) expressing mCherry and regular AAV expressing synaptic vesicle marker tagged with EGFP - synaptophysinEGFP. The results revealed the robust retrograde tracing back to Ox cell bodies and synaptophysin-positive projections into the PAG. To validate the involvement of LHA-PAG Ox circuits in anesthesia arousal regulation, we utilized optogenetics to activate Ox terminals in the PAG, expressing the ChR2-mCherry. We conducted behavioral tests and recorded EEG/EMG during anesthesia induction and emergence. Animals expressing ChR2-mCherry exhibited significantly shorter latency to wake up from light anesthesia compared to the control group expressing only mCherry(7.3 ± 1.92 s vs. 159.3 ± 21.4 s; $P = 0.0007$) and shortened the emergence time after deep isoflurane anesthesia(149.8 ± 22.62 s vs. 231.7 ± 22.67 s; $P = 0.0313$). The noticeable changes in the EEG spectrum upon optogenetic activation, further support the role of PAG as a potential target for Ox neurons in regulating anesthesia arousal. We next utilize the chemogenetics to manipulate the activity of Ox neurons, along with fiber photometry to record local Ox neuropeptide release, presynaptic and postsynaptic calcium dynamics in the PAG. Our results demonstrate the suppression of Ox release during anesthesia, which recovers upon discontinuing isoflurane. The hM3D group exhibited faster emergence from anesthesia, accompanied by a quicker rise and higher levels of Ox signals compared to the hM4D group. Calcium signals from axon-target-GCaMP6f and non-Cre dependent GCaMP6s in the PAG also showed recovery upon anesthesia termination, with hM3D activation augmenting the recovery process neurons. Taken together, this work uncovered the role of Ox-PAG projection in anesthesia arousal and provided valuable insights into the neural circuit mechanisms involved in general anesthesia.

Disclosures: X. Xiang: None. **Z. Guan:** None. **W. Zhou:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.02/VV41

Topic: H.13. Schizophrenia

Title: Chronic exercise alters dorsal hippocampal CA1 synaptic plasticity and improves novel object memory in the sub-chronic phencyclidine rat model for schizophrenia

Authors: *N. SUN[1], M. HARTE[2], J. GIGG[3];
[2]Sch. of Hlth. Sciences, Fac. of Biology, Med. and Heal, [3]Sch. of Biol. Science, Fac. of Biology, Med. and Heal, [1]Univ. of Manchester, Manchester, United Kingdom

Abstract: Introduction: Evidence suggests that chronic exercise improves neurocognition in patients with schizophrenia. In this study, we introduced chronic exercise to the sub-chronic phencyclidine (scPCP) rat model for schizophrenia to investigate its effect on the cognitive impairment associated with schizophrenia. **Methods:** 40 Female Lister Hooded rats were dosed

with either saline or PCP (2 mg/kg, i.p.) twice daily for seven days. Running wheels were provided for aerobic exercise, 1 hour daily for 30 days. Novel object recognition memory was measured after 6 weeks of exercise. Synaptic plasticity in the dorsal hippocampus was then assessed via acute in vivo electrophysiology under urethane anaesthesia (30% w.v.; 1.4g/kg i.p.). The hippocampal CA1 Schaffer-evoked field excitatory postsynaptic potential (fEPSP) was analysed to measure short and long-term synaptic plasticity. Data were analysed with Student t-test and mixed-effect three-way ANOVA. **Results:** Behavioural testing after exercise showed improved novel object recognition memory in the exercised scPCP group. Moreover, Exercise increased long-term potentiation after high-frequency stimulation, prevented depression after low-frequency stimulation in the scPCP group, and improved baseline short-term plasticity in both groups. In conclusion, chronic exercise appears to affect cognitive and hippocampal synaptic deficits in the scPCP rat model.

Disclosures: **N. Sun:** None. **M. Harte:** None. **J. Gigg:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.03/VV42

Topic: H.13. Schizophrenia

Support: AviMed Pharmaceuticlas, LLC
CTSI-AMPD, Bridge to Cures, MCW TAP

Title: The role of the KCNQ (Kv7) potassium channel in regulation of cognitive and negative symptoms of Schizophrenia.

Authors: *L. J. METKO[1], B. FINE-RAQUET[1], T. M. MAXIM[1], M. BERGMANN[1], M. B. GHASEMZADEH[2];
[2]Biomed. Sci., [1]Marquette Univ., Milwaukee, WI

Abstract: Schizophrenia is a chronic mental disorder which affects millions of people worldwide. Changes in neural network activity may contribute to negative and cognitive symptoms of Schizophrenia. While current FDA approved pharmacotherapy treats positive symptoms, they are not effective against the negative and cognitive deficits of schizophrenia. Therefore, there remains a significant unmet medical need for treating critical aspects of disease pathology. Here we have focused on the role of KCNQ (Kv7) voltage-dependent potassium channel in regulation of negative and cognitive symptoms. KCNQ channels function as a subthreshold potassium channel with unique properties of slow activation and non-deactivation, rendering them particularly effective at regulating the membrane potential, generation of action potentials, and neurotransmitter release. These channels augment stimulus-evoked neurotransmitter release in the absence of changes in the basal neurotransmitter levels in the hippocampus and cortex, especially for acetylcholine. It has been suggested that KCNQ channel

properties may enhance signal-to-noise ratio in neural circuits and benefit cognitive processes. Our working hypothesis is that KCNQ channel blockers may ameliorate the negative and cognitive symptoms of schizophrenia through increased neural activity, enhanced information processing, and augmented mutual information sharing amongst neural networks. This hypothesis was examined using the acute systemic phencyclidine (PCP) administration animal model of schizophrenia in rodents using male Sprague-Dawley rats. These studies used acute phencyclidine (PCP, 1.5 mg /kg, sc) administration to induce negative and cognitive deficits and investigated the role of KCNQ channel in regulation of these symptoms. Systemic blockade of the KCNQ channel (XE991 or DMP543) rescued PCP-induced deficits in social interaction, novel object recognition, spatial working memory assessed by delayed alternation task in a T-maze, and prepulse inhibition of startle response. Furthermore, it was demonstrated that KCNQ channel blockade in prefrontal cortex was effective in ameliorating the PCP-induce deficits in spatial working memory while ineffective in nucleus accumbens. In agreement with our hypothesis, pharmacological activation of the KCNQ channel using a channel opener (Retigabine) exacerbated the PCP mediated deficits in social interaction, spatial working memory, and prepulse inhibition of startle response. The results suggest that the KCNQ potassium channel may provide an effective and novel mechanism for ameliorating the negative and cognitive symptoms of schizophrenia

Disclosures: **L.J. Metko:** None. **B. Fine-Raquet:** None. **T.M. Maxim:** None. **M. Bergmann:** None. **M.B. Ghasemzadeh:** 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.; AviMed Pharmaceuticals, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AviMed Pharmaceuticals, LLC.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.04/VV43

Topic: H.13. Schizophrenia

Title: Impaired motor-to-sensory transformation mediates auditory hallucinations

Authors: *F. YANG[1,2], H. ZHU[3], C. ZHANG[2], X. TIAN[3];
[1]Shanghai Mental Hlth. Ctr., Shanghai City, China; [2]Shanghai Mental Hlth. Center, Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [3]New York Univ. Shanghai, Shanghai, China

Abstract: Distinguishing reality from hallucinations requires efficient monitoring of agency. It has been hypothesized that a copy of motor signals, termed efference copy (EC) or corollary discharge (CD), suppresses sensory responses to yield the sense of agency; impairment of the inhibitory function leads to hallucinations. However, how can the sole absence of inhibition yield

positive symptoms of hallucinations? We hypothesize that selective impairments in functionally distinct signals of CD and EC during motor-to-sensory transformation cause the positive symptoms of hallucinations. In an electroencephalography (EEG) experiment with a delayed articulation paradigm in schizophrenic patients with (AVHs) and without auditory verbal hallucinations (non-AVHs), we found that preparing to speak without knowing the contents (general preparation) did not suppress auditory responses in both patient groups, suggesting the absent of inhibitory function of CD. Whereas, preparing to speak a syllable (specific preparation) enhanced the auditory responses to the prepared syllable in non-AVHs, whereas AVHs showed enhancement in responses to unprepared syllables, opposite to the observations in the normal population, suggesting that the enhancement function of EC is not precise in AVHs. A computational model with a virtual lesion of an inhibitory inter-neuron and disproportional sensitization of auditory cortices fitted the empirical data and further quantified the distinct impairments in motor-to-sensory transformation in AVHs. These results suggest that 'broken' CD plus 'noisy' EC causes erroneous monitoring on the imprecise generation of internal auditory representation and yields auditory hallucinations. Specific impairments in functional granularity of motor-to-sensory transformation mediate positivity symptoms of agency abnormality in mental disorders.

Disclosures: **F. Yang:** None. **H. Zhu:** None. **C. Zhang:** None. **X. Tian:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.05/VV44

Topic: H.13. Schizophrenia

Support: NIH T32 MH015330
Abramson Foundation
NIH R21 MH107016
ABCD Charitable Trust

Title: Lower arteriolar cerebral blood volume in recent-onset schizophrenia compared to controls measured by inflow-based vascular-space-occupancy (iVASO) MRI

Authors: A. L. BODNAR[1], A. G. PAEZ[2], K. ULTZ[1], C. A. ROSS[1], J. HUA[2], *R. L. MARGOLIS[1];
[1]Psychiatry, Johns Hopkins Univ., Baltimore, MD; [2]Kirby Ctr., Kennedy Krieger Inst., Baltimore, MD

Abstract: Schizophrenia is a complex and heterogenous disease, prominently associated with metabolic and microvasculature abnormalities. Arterioles may be the most sensitive component of the microvasculature to metabolic disturbances. Based on our preliminary work and other findings, we hypothesize that arteriolar cerebral blood volume (CBVa) is diffusely decreased in

schizophrenia, including in individuals with recent onset of schizophrenia. To explore this hypothesis, differences in CBVa were examined by iVASO (noninvasive blood nulling approach) MRI, extended to 3D whole brain coverage at 7T, between schizophrenia patients (N = 23, duration of illness mean = 1.5 yrs, SD = 1.8 yrs) and healthy controls matched for age and sex (N = 21). CBVa was examined in 98 distinct brain regions. 23/24 subregions within the frontal lobes had significantly reduced CBVa in schizophrenia (ranging from 8.0-41.7%; η^2 = 0.18-0.44). All 10 subregions of parietal lobes had significant decreases in CBVa in schizophrenia (8.1-26.6% change; η^2 = 0.1-0.27) along with 12/14 subregions of the temporal lobes (11.6-41.4% change; η^2 = 0.22-0.35). CBVa in the insular cortices, and cerebellum were also significantly reduced in schizophrenia (8.9-28.7% change; η^2 = 0.19-0.31) along with most occipital regions (7/10 regions; 6.9-23.4% change; η^2 = 0.19-0.32). Similarly, both anterior and posterior cingulate cortices had significantly reduced CBVa in schizophrenia (6/10 regions; 11.3-19.5% change; η^2 = 0.25-0.39). The only cortical region with significant increased CBVa in schizophrenia was the left cuneus (13.4% change; η^2 = 0.19). CBVa was also reduced in subcortical regions, but reduction only reached significance in the pons and thalamus, likely due to a limited number of voxels in most regions. Taken together, these findings replicate and extend previous iVASO results in schizophrenia patients and establish that CBVa is diffusely decreased in almost all areas of the brain, even in patients with recent onset of diagnosable disease. These findings suggest that, rather than being localized to particular circuits, schizophrenia is a disease that affects the whole brain. The relationships between CBVa and longitudinal course of disease (including prodromal stages), treatment response, and treatment resistance, remains to be determined.

Disclosures: **A.L. Bodnar:** None. **A.G. Paez:** None. **K. Ultz:** None. **C.A. Ross:** None. **J. Hua:** None. **R.L. Margolis:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.06/VV45

Topic: H.13. Schizophrenia

Support: Abramson Foundation
ABCD Charitable Trust

Title: A Comparison of Auditory and Visual Event-Related Potentials in Treatment Resistant and Ultra-Treatment Resistant Schizophrenia

Authors: *A. L. BODNAR[1], Y. MO[1], C. MCCAULLEY[1], A. CUERDO[2], L. ZHONG[2], C. BETHANY[2], M. HARPER[2], J. LEONARD[2], C. A. ROSS[3], F. C. NUCIFORA[3], R. L. MARGOLIS[3];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Bayview Med. Ctr., Baltimore, MD; [3]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Schizophrenia is a heterogenous disease that affects ~1% of the population. 30% of patients do not respond to standard antipsychotic medicines (treatment-resistant schizophrenia, TRS), and of these, up to 30% do not respond to clozapine (ultra-treatment-resistant schizophrenia, UTRS). We hypothesize that UTRS patients will have diminished ERPs across multiple sensory modalities, reflecting sensory gating and attentional control deficits. To preliminarily test this hypothesis, ERP responses of UTRS (N = 4) and TRS (N = 8) patients were measured using frequency and spatial auditory oddball and visual Stroop protocols. In the frequency auditory oddball task, P200 (140-180ms) amplitude at central-parietal electrodes was diminished in UTRS compared to TRS ($F = 7.13$, $p = 0.002$, $\eta^2 = 0.42$), and P200 amplitudes were negatively correlated with PANSS scores ($r = -0.554$, $p = 0.031$). In the spatial auditory oddball task, amplitudes at P50 (40-60ms) were non-significantly diminished in UTRS ($p = 0.066$) at right parietal electrodes and were negatively correlated with PANSS scores ($r = -0.756$, $p = 0.002$). In the visual Stroop experiment, P300 (310-500ms) amplitudes were reduced at central-parietal electrodes for both congruent and incongruent trials ($F = 12.96$, $p = 0.006$, $\eta^2 = 0.59$). P300 amplitudes for both congruent ($r = -0.823$, $p = 0.001$) and incongruent ($r = -0.603$, $p = 0.025$) trials were inversely correlated with PANSS scores. Taken together, these preliminary ERP findings suggest that deficits in sensory gating and attentional control may distinguish UTRS from TRS. These results support expansion of this study, including adding patients who respond to standard antipsychotics and healthy controls.

Disclosures: A.L. Bodnar: None. **Y. Mo:** None. **C. McCaulley:** None. **A. Cuerdo:** None. **L. Zhong:** None. **C. Bethany:** None. **M. Harper:** None. **J. Leonard:** None. **C.A. Ross:** None. **F.C. Nucifora:** None. **R.L. Margolis:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.07/VV46

Topic: H.13. Schizophrenia

Support: MH111177

Title: A loss of higher order network features in schizophrenia during learning

Authors: *H. K. SAAD, J. KOPCHICK, V. DIWADKAR, A. Z. CHOWDURY, P. THOMAS, U. RAJAN, D. KHATIB, C. ZAJAC-BENITEZ, L. HADDAD, A. AMIRSADRI, J. A. STANLEY;
Dept. of Psychiatry, Wayne State Univ., Detroit, MI

Abstract: Conventional connectivity analyses typically rely on zero-lag functional connectivity (Silverstein et al., 2016) to quantify 2nd order relationships (between two regions). However, regional effects on the connectome can also be quantified at a higher order (Zhang et al., 2017). Here, it is possible to estimate the similarity in how regions impact the connectome by estimating

the similarity between their connectivity vectors (e.g., for any two regions R_i and R_j, the vectors of their connectivities with all $R_{1\text{-}n}$ in a space of n regions can be estimated). These estimates capture the comparative impacts exerted by pairs of regions on the network. A loss of these higher order features characterizes brain network pathology in neurological conditions (Zhang et al., 2016), but has never been examined in schizophrenia (SCZ), the consummate dysconnection syndrome (Friston et al., 2016). Here from fMRI data acquired using an established learning paradigm (Meram et al., 2023), we quantified higher order feature (HOF) loss in SCZ (n=78, 39 patients) across four experimental conditions (Memory Encoding, Post Encoding Consolidation, Memory Retrieval, Post Retrieval Consolidation). First, in each participant full 2nd order connectivity matrices were estimated for a 90-region bi-lateral network (Tzourio-Mazoyer et al., 2002). Then, HOFs were estimated between all pairs of regions, transforming the 2nd order connectivity matrix into an HOF matrix. Finally, inter-group differences (HC ≠ SCZ) in HOFs were identified (independent samples *t* tests) and statistically thresholded (p_{FDR}<.05). As seen in Figure 1, the relative contributions to HOF loss in schizophrenia are particularly notable in anterior regions of the brain. These include nodes in the frontal, striatal and hippocampal regions (all of which are heavily implicated in learning), but less so nodes in the posterior (sensory) regions. We suggest that the future research attempt to characterize the role of HOF and assess HOF loss in dysconnection syndromes like schizophrenia.

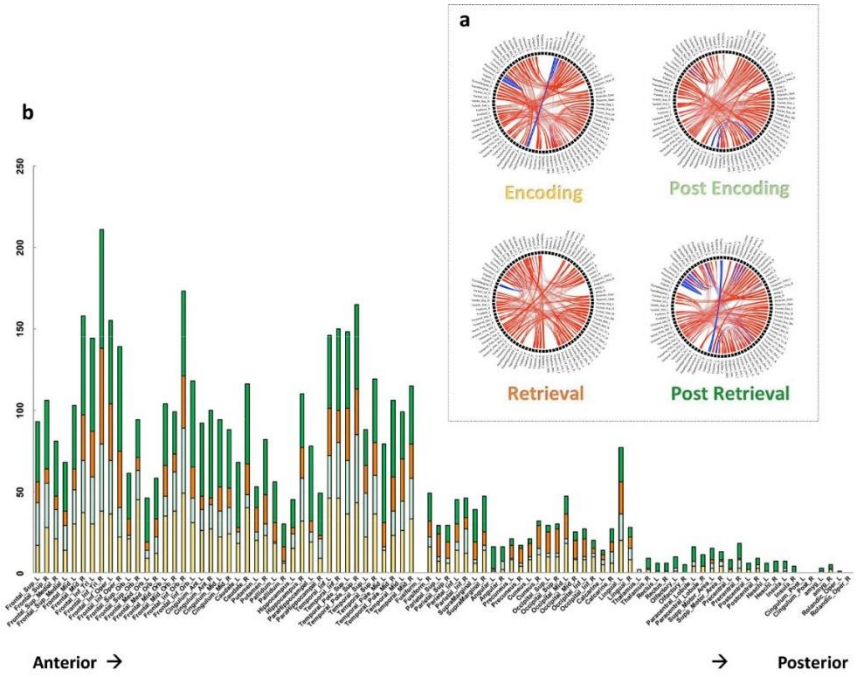


Figure 1a: The chords represent pairs of regions with significant inter-group differences in HOFs (Red: HC > SCZ) in each of the four conditions. **Figure 1b:** From the chord diagrams, the relative contributions of *each node* to HOF loss in SCZ are summarized in the frequency graph. Nodes are organized on an anterior-to-posterior axis. The height of the bar reflects the node's contribution to HOF loss in SCZ. The sub-colors reflect the contributions of the node in each condition (Yellow = Memory Encoding, Teal = Post Encoding, Orange = Memory Retrieval, Dark Green = Post Retrieval).

Disclosures: **H.K. Saad:** None. **J. Kopchick:** None. **V. Diwadkar:** None. **A.Z. Chowdury:** None. **P. Thomas:** None. **U. Rajan:** None. **D. Khatib:** None. **C. Zajac-Benitez:** None. **L. Haddad:** None. **A. Amirsadri:** None. **J.A. Stanley:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.08/VV47

Topic: H.13. Schizophrenia

Support: MRC Grant

Title: Investigating predictive coding mechanisms in the auditory cortex of healthy controls and Schizophrenia patients with 7T fMRI

Authors: *Y. LAZAROVA[1], C. ABBATECOLA[1], L. PETRO[1], J. BALDAQUE[1], K. HAINING[1], P. DHEERENDRA[1], R. KRISHNADAS[1], F. DE MARTINO[2], D. PORTER[1], T. GRENT-'T-JONG[3], P. J. UHLHAAS[3], L. MUCKLI[1];
[1]Univ. of Glasgow, Glasgow, United Kingdom; [2]Maastricht Univ., Maastricht, Netherlands; [3]Charité – Universitätsmedizin Berlin, Berlin, Germany

Abstract: Under the predictive coding account of brain function, our brains build models of the world by making predictions about what should happen next (Clark, 2013). These models are updated by confronting them with prediction errors e.g. from perceiving an unexpected stimulus. An alteration of these mechanisms could be at the origin of the variety of symptoms observed in psychosis (Sterzer et al., 2018). For example, there is evidence for such a difference between Schizophrenia patients and controls in mismatch negativity (MMN) paradigms isolating the response to an unexpectedly different auditory tone in a short sequence of otherwise uniform tones (Erickson et al., 2016). We investigated MMN responses in the auditory cortex of schizophrenia patients and controls using high-field 7T fMRI, to assess differences in cortical laminar-specific microcircuits. We also manipulate participant's attention either towards or away from the auditory stimulation. We conducted 7T fMRI scanning on 32 participants (24 controls and 8 schizophrenia patients) at ICE (Glasgow, Siemens Magnetom Terra). Our trials were composed of six 150 ms tones for a total duration of 1.5s. On 80% of trials, all tones were the same (440 Hz), but in the deviant condition the last tone differed in pitch (620 Hz). To control for attention, we also presented on each trial an unrelated visual stimulation (4 'pacman' shapes which could form an illusory Kanizsa square). Participants performed either an auditory task (responding to trials during which one of the tones is shorter) or a visual task (responding to trials during which the visual stimulation flickers). The task trials were excluded from the analysis to avoid confounds. We acquired a tonotopic mapping scan to identify our region of interest (ROI) in the auditory cortex, by presenting tones of various frequencies in a randomized order. A first deconvolution general linear model (GLM) analysis currently across all cortical layers found that, in all participants, the BOLD response is higher for the deviant trials than the

standard trials. This effect is present bilaterally but is more prominent in the right hemisphere, both with attention directed towards auditory stimulation (controls: $t=3.35$, $p<0.001$, patients: $t=3.33$, $p<0.001$) and away (controls: $t=4.8$, $p<0.001$, patients: $t=3.6$, $p<0.001$). Furthermore, this difference was more pronounced when participants' attention was away from the auditory stimulation. This preliminary data informs how we can understand changes in auditory predictive processing in terms of deviance detection in Schizophrenia, its modulation by attention, and the underlying cortical microcircuitry.

Disclosures: Y. Lazarova: None. **C. Abbatecola:** None. **L. Petro:** None. **J. Baldaque:** None. **K. Haining:** None. **P. Dheerendra:** None. **R. Krishnadas:** None. **F. De Martino:** None. **D. Porter:** None. **T. Grent-'t-Jong:** None. **P.J. Uhlhaas:** None. **L. Muckli:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.09/VV48

Topic: H.13. Schizophrenia

Title: Characterizing interneuron subtypes in the CA1 region of the hippocampus of mice with the 22q11.2 deletion syndrome

Authors: *H. ARAIN[1], E. VAROL[2], S. A. HERRLINGER[1], J. A. GOGOS[3], A. LOSONCZY[3]; [1]Neurosci., [2]Statistics, Columbia Univ., New York, NY; [3]Neurosci., Columbia Univ., New York City, NY

Abstract: Schizophrenia is a mental disorder marked by positive symptoms (i.e. hallucinations), negative symptoms (i.e. apathy), and cognitive deficits including episodic memory deficits. Although present in 1% of the population, the specific neurological effects of the disorder remain poorly understood. Amongst the breadth of symptoms, schizophrenia induced episodic memory deficits are common, but their precise neural correlates are unknown. Given the hippocampus' role in episodic memory, we investigate the hippocampal microcircuitry to explore the reasons for episodic memory deficits in patients with schizophrenia. Prior work in our lab revealed hippocampal place cell deficits in mice with the 22q11.2 deletion syndrome (the largest known genetic risk factor for schizophrenia). The microcircuitry of the hippocampus contains both the excitatory neurons and diverse populations of inhibitory interneurons that suppress and modulate their activity. To better understand how changes in the hippocampal microcircuitry may contribute to place cell instability and possibly manifest as episodic memory deficits, our experiment investigated hippocampal interneuron subtypes to ask if the heterogeneous landscape of interneuron populations are altered. To achieve this, hippocampal sections from wild-type mice (n=6) and Df(16)A+/- mice (n=5) with the 22q11.2 deletion were stained for five interneuron markers and imaged with a confocal microscope. Acquired images were analyzed to determine concentrations of markers within sections through the novel Cell Analysis/Typing Tool (CATT) with the intention of simultaneously troubleshooting development of CATT for

future applications. Analysis of single-marker concentrations between groups and categorized by layer between groups was performed, revealing one statistically significant result - a greater concentration of NPY in the Stratum Radiatum of mice with the deletion - with all other differences being insignificant. These results suggest that the place cell instability apparent in Df(16)A+/- mice may not be attributed to differences in proportions of interneuron subtypes, warranting further investigation into the question.

Disclosures: H. Arain: None. **E. Varol:** None. **S.A. Herrlinger:** None. **J.A. Gogos:** None. **A. Losonczy:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.10/VV49

Topic: H.13. Schizophrenia

Support: NIMH K00MH121382
NIMH R01MH124047
BWF PDEP Award

Title: Comprehensive and simultaneous 3-D imaging of interneuron subtypes in CA1 depicts deficits in interneuron activity resulting in microcircuit disruption in a mouse model for the 22q11.2 deletion syndrome

Authors: *S. A. HERRLINGER[1], B. Y. RAO[2], A. A. TUTTMAN[3], B. VANCURA[4], T. GEILLER[4], A. D. GROSMARK[6], J. A. GOGOS[5], A. LOSONCZY[5];
[1]Zuckerman Inst., [2]Neurobio. and Behavior, Columbia Univ., New York, NY; [3]Columbia Univ., New York City, NY; [4]Columbia Univ., New York, NY; [5]Neurosci., Columbia Univ., New York City, NY; [6]Neurosci., Univ. of Connecticut Hlth. Ctr., Farmington, CT

Abstract: Background: Individuals with the 22q11.2 deletion syndrome (22q11.2DS), one of the strongest genetic risk factors for schizophrenia, demonstrate cognitive impairments, including episodic memory (EM) dysfunction. Our group previously showed that EM is impaired in a mouse model for the 22q11.2DS (*Df(16)A*$^{+/-}$). Place cells, cellular representations of EM, are under strong inhibitory control by heterogeneous subtypes of GABAergic interneurons, which have been implicated in the pathophysiology of schizophrenia. In this study, we examined the contribution of pyramidal cells and hippocampal interneuron subtypes to local microcircuit dysfunction in CA1. **Methods:** 2-photon imaging of CA1 pyramidal neuron population dynamics were performed *in vivo* to characterize plasticity during novel context exposure in a virtual environment in *Df(16)A*$^{+/-}$ and WT mice (n=2 Wt, n=2 Df16). Wild-type and *Df(16)A*$^{+/-}$ mice performed goal-oriented learning, random foraging and reversal learning tasks on a cued belt while undergoing large-scale, unbiased 3D GCaMP-Ca^{2+} imaging of *in vivo* CA1 interneuron dynamics (n=6 Wt, n=5 Df16). Molecular identification of major interneuron subtypes was

performed post-hoc utilizing immunohistochemistry. Interneuron subtype activity was assessed through Pearson cross-correlations with velocity and through peristimulus time histograms around behavioral indicators. **Results:** In *Df(16)A*$^{+/-}$ mice we observe a significant decrease in CA1PC somatic bursting rate during context switch compared with WTs, suggesting that plasticity is suppressed *in vivo* (n=2832 WT, n=1732 *Df(16)A*$^{+/-}$ cells, p<0.001). Interneurons exhibit subtype-specific alterations in activity during locomotion, and a significant overall decrease in place preference (n=8 Wt, n=7 *Df(16)A*$^{+/-}$, F=2.111, p=0.045). **Conclusions:** Results examining CA1 principal neuron dynamics and plasticity collected *in vivo* and *in vitro* suggest that inhibitory circuits are either over-compensating *in vivo* or are intrinsically deficient themselves. We identify subtype-specific alterations in interneuron dynamics, likely contributing to microcircuit dysregulation, ultimately resulting in a less stable microcircuit in CA1 during learning.

Disclosures: S.A. Herrlinger: None. **B.Y. Rao:** None. **A.A. Tuttman:** None. **B. Vancura:** None. **T. Geiller:** None. **A.D. Grosmark:** None. **J.A. Gogos:** None. **A. Losonczy:** None.

Poster

PSTR505. Schizophrenia Relevant Circuits and Systems

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR505.11/VV50

Topic: H.13. Schizophrenia

Support: MR/T033967/1

Title: Schizophrenia, perineuronal nets, and the marmoset hippocampal-prefrontal pathway

Authors: M. GWILT[1], A. R. HODGSON[1], S. F. A. AXELSSON[2], G. J. COCKCROFT[1], J. A. WEST[3], L. B. MCIVER[1], C. MCKENZIE[2], R. N. CARDINAL[4], S. J. SAWIAK[5], ***H. F. CLARKE**[5];
[1]Physiology, Develop. and Neurosci., [2]Psychology, [3]Cambridge Inst. of Therapeut. Immunol. and Infectious Dis., [4]Dept. of Psychiatry, [5]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Schizophrenia is a severe psychiatric disorder associated with three main symptom clusters: positive symptoms that are fairly well treated with antipsychotic drugs, and emotional and cognitive disruptions that are harder to treat, and profoundly impact quality of life . Clinical neuroimaging studies and rodent models of schizophrenia have implicated dysfunction in regions of the PFC (including the dorsolateral prefrontal and orbitofrontal cortices), and glutamatergic overactivity within the anterior hippocampus (aHipp), in the mechanisms underlying schizophrenia symptoms. In addition, disruption of hippocampal parvalbumin-positive inhibitory GABAergic interneurons, and loss of their extracellular matrix support structure, the perineuronal net, induce schizophrenia-like changes in rodent models.
Unfortunately, the causal roles of these changes and their interaction are unknown, and translation from rodent studies is limited. However, while the role of the aHipp has been

considered in the positive symptoms of schizophrenia, the aHipp also projects directly to the PFC, thus aHipp glutamatergic hyperfunction may also induce alterations in PFC function that underlie the cognitive symptoms. Here we demonstrate that enzymatic degradation of the aHipp perineuronal net causes hippocampal overactivity in marmoset monkeys - a species whose PFC is closer to that of humans than rodents. Behavioural changes relevant to schizophrenia were also seen: elevated locomotion and behavioural stereotypy in a novel amphetamine-induced hyperlocomotion paradigm for marmosets, and impaired probabilistic discrimination learning in a task dependent on orbitofrontal-striatal circuitry. These changes were accompanied by increased tonic catecholamine levels in the orbitofrontal cortex. These findings demonstrate a new model for investigating the prefrontal changes that occur in schizophrenia and provide evidence that aberrant hippocampal-PFC communication causes behavioural changes relevant to its symptoms. These studies demonstrate the importance of primate translational neuroscience for the development of a mechanistic understanding of prefrontal cortex function, with a view to developing new treatments for disorders of major personal and societal consequence.

Disclosures: **M. Gwilt:** None. **A.R. Hodgson:** None. **S.F.A. Axelsson:** None. **G.J. Cockcroft:** None. **J.A. West:** None. **L.B. McIver:** None. **C. McKenzie:** None. **R.N. Cardinal:** None. **S.J. Sawiak:** None. **H.F. Clarke:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.01/VV51

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R21DA049635
NIH Grant K01AA029200
NIH Grant R01AA026818
NIH Grant R01AA019526
NIH Grant R01DK110358
NIH Grant 5P30CA042014

Title: Iterative assay for transposase-accessible chromatin by sequencing to isolate functionally relevant neuronal subtypes

Authors: *C. B. MERRILL, I. TITOS, M. A. PABON, A. B. MONTGOMERY, A. R. RODAN, A. ROTHENFLUH;
Univ. of Utah, Salt Lake City, UT

Abstract: The *Drosophila* brain contains tens of thousands of distinct cell types. Numerous transgenic lines reproducibly target specific neuron subsets, yet most still express transgenes in several cell types. Furthermore, most lines were developed without *a priori* knowledge of where the transgenes would be expressed. To aid in the development of cell type-specific tools for

neuronal identification and manipulation, we developed an iterative assay for transposase-accessible chromatin (ATAC) approach. We analyzed open chromatin regions (OCRs) within whole flies and neurons from isolated heads and identified tissue-specific OCRs. Next, we subcloned enriched OCRs from each broad tissue type upstream of Gal4, part of the binary Gal4/UAS system. We show that OCRs that are enriched in neurons, compared to whole bodies, drove transgene expression more highly in the brain than in the fly body. Further, neuron-enriched OCRs drove transgene expression preferentially in subsets of neurons, with each OCR driving transgene expression in a distinct pattern. Then, we used each OCR to drive the expression of a temperature-activated ion channel (TrpA1) and asked whether OCR neuron activation had behavioral relevance. Activating OCR neurons caused distinct sleep phenotypes, including increased nighttime sleep and decreased sleep latency. To home in on the neurons that mediated these sleep phenotypes, we performed a second round of ATAC-seq from the neuron subsets underlying increased nighttime sleep and decreased sleep latency. This analysis revealed additional OCR2s that were enriched within each neuron subset compared to all neurons. To verify that OCR2-expressing neurons were a subset of OCR neurons, we subcloned the OCR2s upstream of flippase, which allowed us to express both OCR and OCR2 transgenes in a combinatorial manner. Our data show that OCR2s further restricted transgene expression within the original neuron subset. Further, each OCR2 drove transgene expression within a distinct neuron pattern. Finally, we analyzed sleep phenotypes after activating OCR2 neurons to determine whether the OCR2 neurons mediated the sleep phenotype we observed upon OCR neuron activation. We observed that activating OCR2 neurons caused sleep phenotypes similar to those observed when activating the parent OCR neurons. Our data show that this iterative ATAC-seq approach allows for continued refinement of transgene expression. We also show that this approach can be used to identify sparse neuronal populations that are relevant for sleep behavior. Furthermore, the iterative ATAC-seq approach we describe is widely applicable to other cell types and to other organisms.

Disclosures: **C.B. Merrill:** None. **I. Titos:** None. **M.A. Pabon:** None. **A.B. Montgomery:** None. **A.R. Rodan:** None. **A. Rothenfluh:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.02/VV52

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant K99EY033457
NIH Grant R00EY028625
NIH Grant R21EY028633
NIH Grant U01MH105960
NIH Grant T32GM007103
Chan-Zuckerberg Initiative CZF-2019-002459
Research to Prevent Blindness and a Klingenstein-Simons Fellowship

Award
Wellcome Trust Investigator Award (210684/Z/18/Z)
ARCS Foundation Scholarship and a Society for Developmental Biology Emerging Models
Children's Glaucoma Foundation Grant
NSF Grant (1827647)

Title: Evolution of neuronal cell classes and types in the vertebrate retina

Authors: *J. HAHN[1], A. MONAVARFESHANI[2], M. QIAO[3], A. KAO[2], Y. KÖLSCH[4], A. KUMAR[1], V. KUNZE[5], A. M. RASYS[6], R. RICHARDSON[7], H. BAIER[4], R. J. LUCAS[7], W. LI[5], M. MEISTER[3], J. T. TRACHTENBERG[8], W. YAN[2], Y. PENG[9], K. SHEKHAR[1], J. SANES[2];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]Dept. of Cell. and Mol. Biol., Harvard Univ., Cambridge, MA; [3]Caltech, Pasadena, CA; [4]Max Planck Inst. for Biol. Intelligence, Stuttgart, Germany; [5]Retinal Neurophysiol. Section, Natl. Eye Inst., Bethesda, MD; [6]Dept. of Cell. Biology,, Univ. of Georgia, Athens, GA; [7]Univ. of Manchester, Manchester, United Kingdom; [8]UCLA, Los Angeles, CA; [9]Dept. of Ophthalmology, UCLA David Geffen Sch. of Med., Los Angeles, CA

Abstract: The neural retina, the portion of the brain that resides in the back of the eye, has a basic structural blueprint that, unlike many other brain regions, is highly conserved among vertebrates. Despite the conservation of this basic plan, species differ profoundly in their visual needs. One might expect that neuronal cell types within the retina evolved to accommodate these varied needs, but this has not been systematically studied. Here, we generated and integrated single-cell transcriptomic atlases of the retina from 17 species: humans, two non-human primates, four rodents, three ungulates, opossum, ferret, tree shrew, a teleost fish, a bird, a reptile and a lamprey. Molecular conservation of the six retinal cell classes (photoreceptors, horizontal cells, bipolar cells, amacrine cells, retinal ganglion cells [RGCs] and Müller glia) is striking, with transcriptomic differences across species correlated with evolutionary distance. Major subclasses are also conserved, whereas variation among types within classes or subclasses is more pronounced. To assess the extent to which retinal cell types are conserved, we performed an integrative analysis, revealing that numerous types are shared across species based on conserved gene expression programs that likely trace back to the common ancestor of jawed vertebrates. The degree of variation among types increases from the outer retina (photoreceptors) to the inner retina (RGCs), suggesting that evolution acts preferentially to shape the retinal output. Finally, we identified mammalian orthologs of midget RGCs, which comprise >80% of RGCs in the human retina, subserve high-acuity vision, and were believed to be primate-specific. Projections of both primate midget RGCs and their orthologous types in mice are overrepresented in the thalamus, which supplies the primary visual cortex. We suggest that midget RGCs are not primate innovations, but descendants of evolutionarily ancient types that increased in number as primates evolved, thereby facilitating high visual acuity and an increased role of the cerebral cortex in primate visual processing.

Disclosures: **J. Hahn:** None. **A. Monavarfeshani:** None. **M. Qiao:** None. **A. Kao:** None. **Y. Kölsch:** None. **A. Kumar:** None. **V. Kunze:** None. **A.M. Rasys:** None. **R. Richardson:** None. **H. Baier:** None. **R.J. Lucas:** None. **W. Li:** None. **M. Meister:** None. **J.T. Trachtenberg:** None. **W. Yan:** None. **Y. Peng:** None. **K. Shekhar:** None. **J. Sanes:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.03/VV53

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Knut and Alice Wallenberg Foundation

Title: A transcription factor atlas of the adult human brain

Authors: *N. MITSIOS[1], W. ZHONG[2], S. BARDE[1], P. OKSVOLD[2], L. FAGERBERG[2], C. ZHANG[2], T. ZHENG[1], K. VON FEILITZEN[2], C. LINDSKOG[3], E. SJOSTEDT[1], Y. LUO[4], E. RENNER[5], M. PALKOVITS[5], T. HOKFELT[1], M. UHLEN[2], J. MULDER[1];
[1]Karolinska Institutet, Solna, Sweden; [2]KTH-Royal Inst. of Technol., Stockholm, Sweden; [3]Uppsala Univ., Uppsala, Sweden; [4]Aarhus Univ., Aarhus, Sweden; [5]Semmelweis Univ., Budapest, Hungary

Abstract: The Human Protein Atlas (HPA; www.proteinatlas.org) is a public online database that provides an integrated overview of protein expression and distribution in all major human tissue types, including brain. There, a comprehensive overview of gene and protein expression in the main anatomical structures of the mouse, pig and human brain is provided, by combining publicly available transcriptomic data and in-house generated RNA sequencing data for 967 samples from 193 microdissected regions and areas of the human brain (Human Brain Tissue Bank, Budapest). These include 10 samples from different basal ganglia, 16 thalamic and 9 hypothalamic nuclei, 9 samples from the hippocampal complex, 5 from the amygdala, over 70 from brainstem (midbrain, pons, medulla) and 5 cerebellar cortical and nuclear samples. In addition, from the cerebral cortex, more than 70 areas, gyri and subregions have also been analyzed. All protein coding genes have now been classified based on regional distribution and co-expression, thus providing lists of genes associated to brain regions, cell types and functions. Transcription factors regulate cell differentiation and specialization. By analyzing the distribution of more than 1800 transcription factors in the above regions of the adult human brain, we have generated a map of their distribution and have identified regionally elevated transcription factors. Moreover, by using integrative approaches, we identify gene regulatory networks that drive brain-regional and cell-type specialization. All presented data are open-access and available in the brain section of version 23 of the Human Protein Atlas.

Disclosures: **N. Mitsios:** None. **W. Zhong:** None. **S. Barde:** None. **P. Oksvold:** None. **L. Fagerberg:** None. **C. Zhang:** None. **T. Zheng:** None. **K. von Feilitzen:** None. **C. Lindskog:** None. **E. Sjostedt:** None. **Y. Luo:** None. **E. Renner:** None. **M. Palkovits:** None. **T. Hokfelt:** None. **M. Uhlen:** None. **J. Mulder:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.04/VV54

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Whole-brain quantification of semaglutide-induced neuronal activation: a study of mRNA and protein signatures in mouse brains using light sheet fluorescence microscopy

Authors: L. LYDOLPH LARSEN[1], S. KANATANI[2], J. LERCKE SKYTTE[1], E. ALEXIOU[1], C. GRAVESEN SALINAS[1], P. UHLÉN[2], U. ROOSTALU[1], *J. HECKSHER-SØRENSEN[1]; [1]Gubra ApS, Hørsholm, Denmark; [2]Dept. of Med. Biochem. and Biophysics, Karolinska Institutet, Stockholm, Sweden

Abstract: Whole-brain mapping combined with light sheet imaging represents a powerful technique for visualization and quantification of targets in intact adult mouse brains, however, technical limitations have complicated 3D spatial RNA imaging. In the present study, we characterized CNS stimulatory effects of semaglutide, currently approved for treatment of diabetes (Ozempic®) and obesity (Wegovy®), by using a novel 3D RNA imaging technique paired with standard 3D protein imaging. Application of this approach allowed 3D mapping of whole-brain c-Fos expression at both mRNA and protein level. To accomplish this, chow-fed male C57BL/6J mice were administered an acute subcutaneous dose of semaglutide (0.04 mg/kg) or vehicle (n=6 per group). Brains were collected 120 min post-dosing and processed for whole-brain c-Fos mRNA or protein detection, respectively. Upon clearing, brains were scanned using light sheet fluorescence microscopy (LSFM) followed by automated 3D quantitative imaging to compare c-Fos mRNA and protein expression patterns in ≥1,100 brain regions. We observed a striking overlap in c-Fos mRNA and protein expression maps, including in brain anatomical hotspots involved in appetite regulation, such as the nucleus of the solitary tract, parabrachial nucleus, and central amygdalar nucleus. Interestingly, the paraventricular hypothalamic nucleus and dorsomedial nucleus of the hypothalamus did not show similar c-Fos responses at the mRNA and protein level. These data shed further light on the dynamic relationship between c-Fos gene expression and protein synthesis, providing valuable insights into the temporal pharmacodynamics of semaglutide in the adult mouse brain.

Disclosures: **L. Lydolph Larsen:** A. Employment/Salary (full or part-time):; Gubra ApS. **S. Kanatani:** None. **J. Lercke Skytte:** A. Employment/Salary (full or part-time):; Gubra ApS. **E. Alexiou:** A. Employment/Salary (full or part-time):; Gubra ApS. **C. Gravesen Salinas:** A. Employment/Salary (full or part-time):; Gubra ApS. **P. Uhlén:** None. **U. Roostalu:** A. Employment/Salary (full or part-time):; Gubra ApS. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Gubra.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.05/VV55

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: 1DP2MH132940

Title: An open-source automated in situ sequencer for high-throughput and cost-effective spatial transcriptomics and barcoded connectomics

Authors: *Z. MALTZER, A. ZHANG, X. CHEN;
Allen Inst., Seattle, WA

Abstract: In situ RNA sequencing allows high-throughput, low-cost, and highly multiplexed detection of gene expression in the context of tissue. In addition to enabling robust and cost-effective spatial transcriptomics, in situ sequencing is the foundation for sequencing-based neuroanatomical techniques (e.g. BARseq) and can potentially be further applied to lineage tracing and multiplexed perturbation experiments. This approach involves performing many rounds of Illumina sequencing-by-synthesis chemistry on tissues and imaging on a microscope after each round, doing the chemistry and imaging manually is labor-intensive and error-prone, and these steps remain a major hurdle in wider dissemination of the technique. To overcome this challenge, we aim to develop an open-source in situ sequencer that can complete the bench chemistry steps for the sequencing reactions and perform imaging without human inputs. This system is designed to include an automated fluidics system for chemical reactions with an integrated heating and cooling element for thermal cycling, an automated microscope for imaging, an integrated software package for data acquisition, handling, and preprocessing, and a cloud-based data processing package. The complete system will lower the cost and increase the throughput of BARseq experiments, allow scalable deployment for large-scale experiments, and can be conveniently set up in any laboratory. In pilot experiments, we show that a prototype fluidics system produced sequencing results that were comparable to those from a manually sequenced experiment. We envision that an automated in situ sequencer will not only enable broad dissemination of in situ sequencing-based high-throughput spatial transcriptomics and neuroanatomical mapping techniques, but also facilitate the development of future techniques based on in situ sequencing.

Disclosures: Z. Maltzer: None. **A. Zhang:** None. **X. Chen:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.06/VV56

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: European Commision Horizon
Medical Research Council

Title: Coppafish 3d: a spatial transcriptomics method compatible with thick fixed tissue sections

Authors: *I. PRANKERD[1], R. TILBURY[1], J. DUFFIELD[1], M. BOURDENX[2], C. MAAT[2], D. NICOLOUTSOPOULOS[1], M. SHINN[1], A. RITOUX[1], P. CAUHY[2], B. ZHOU[1], S. BUGEON[1], Y. ISOGAI[3], K. HARRIS[1];
[1]Univ. Col. London, London, United Kingdom; [2]Dementia Res. Inst., London, United Kingdom; [3]Sainsbury Wellcome Ctr. for Neural Circuits and Behaviour, London, United Kingdom

Abstract: Spatial transcriptomics is a rapidly growing field consisting of methods that detect messenger RNA (mRNA) and allow for the visualization of gene expression in situ. Many methods exist but rarely are they able to detect mRNA expression in 3D fixed tissue sections. We have developed a new method called COmbinatorial Padlock-Probe-Amplified Fluorescence In Situ Hybridisation (coppaFISH) 3D that can detect mRNA with subcellular resolution in sections up to 100um thick. coppaFISH 3D has been successfully used on fresh frozen, PFA-fixed and FFPE mouse brain sections, as well as cultured human cells with thicknesses ranging from 10-100um.
Padlock probes are hybridized to cDNA and undergo ligation and amplification to form rolling circle products (RCPs), each of which correspond to a single mRNA. 3D imaging is performed using a four-camera confocal microscope with automated fluidics, which labels RCPs for each gene with a different sequence of 7 dyes across 7 imaging rounds on our custom microscope and fluidics system. coppaFISH can be combined with multiple rounds of antibody staining and even in vivo imaging (Bugeon et al., Nature, 2022).
The data is analyzed using our open-source python package. First, images are aligned across rounds and color channels via 3D affine transforms, estimated first by Fourier methods and optimized to subpixel level by point cloud alignment. Gene identities are determined using orthogonal matching pursuit, which allows resolution of optically overlapping spots. Cell type identity is determined using pciSeq (Qian et al., Nature Methods, 2000), and results can be viewed interactively in 3D on a web-based viewer.
Abbreviations
PFA = ParaFormAldehyde, FFPE = Formalin Fixed Paraffin Embedded, cDNA = Complementary DNA, pciSeq = Probabilistic Cell typing by In situ SEQuencing

Disclosures: I. Prankerd: None. **R. Tilbury:** None. **J. Duffield:** None. **M. Bourdenx:** None. **C. Maat:** None. **D. Nicoloutsopoulos:** None. **M. Shinn:** None. **A. Ritoux:** None. **P. Cauhy:** None. **B. Zhou:** None. **S. Bugeon:** None. **Y. Isogai:** None. **K. Harris:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.07/VV57

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatial transcriptomics reveals the convergent evolution of mammalian and avian palliumSpatial transcriptomics reveals the convergent evolution of mammalian and avian pallium

Authors: *S. YANG[1], S. LIU[2];
[1]STOmics Americas, San Jose, CA; [2]BGI-Research, Shenzhen, China

Abstract: Amniotes have evolved a complex brain organization, particularly in the telencephalon. However, the identity and evolutionary conservation of the telencephalon at the genoarchitecture scale remains largely unknown. We constructed zebra finch and turtle using the Stereo-seq with single-cell resolution spatial transcriptome telencephalon atlases, to perform comparisons of sauropsids (reptiles and birds) with data from amphibians (axolotls) to mammals (mice and macaques). We identified two major types of gene regulatory models during the evolution of the dorsal ventricular ridge (DVR) in sauropsids: changes in the expression of transcription factors (TF), and changes in the binding motifs of TFs with no difference in expression. We reveal the mechanism by which avian DVR and mammalian neocortex recruit the same effector genes under different transcription factors, indicating their convergent evolution. We also identify a large-scale continuous and graded variation of gene expression along the depth of the DVR, resembling the layers of the neocortex. Overall, our analysis yields insights into the evolutionary relationships of the telencephalon across amniotes.

Disclosures: **S. Yang:** A. Employment/Salary (full or part-time):; STOmics Americas. **S. Liu:** A. Employment/Salary (full or part-time):; BGI.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.08/VV58

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R00EY028625
Glaucoma Research Foundation Catalyst for a Cure 4

Title: Spatial transcriptomic mapping of whole mount retina and the superior colliculus

Authors: *K. NIMKAR[1], N. TSAI[2], M. LUM[2], S. ZHANG[2], P.-Y. LIN[2], X. DUAN[2], K. SHEKHAR[1];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Visual information is transmitted from the retina by the spiking activities of retinal ganglion cells (RGCs), whose axons innervate >50 retinorecipient centers across the brain. In

mice, the main retinorecipient center is the superior colliculus (SC), a midbrain region involved in reflexive eye movements and motor control. Both the retina and SC contain several cell types defined by morphology, function, molecular profile and spatial organization. While advances in transcriptomics, calcium imaging and electron microscopy have classified RGC types and SC neuron types, their spatial organization is still unknown. Here, we used multiplexed error-correcting fluorescent *in situ* hybridization (MERFISH) to produce comprehensive spatial maps of RGC types in the ganglion cell layer (GCL) flat mounts of the mouse retina, and of the neuronal types in SC. MERFISH quantifies ~100-300 genes on tissue sections at 1μm spatial resolution. We constructed optimal gene panels based on prior single-cell (sc) RNA-seq data from the same tissue, and devised machine learning pipelines to segment and annotate the images, align consecutive tissue sections, and impute whole-transcriptome information onto the spatial maps. For the retina, we used a 140 gene panel to spatially map and classify ~27,000 RGCs per retina (on average) on GCL flatmounts. Supervised classification analysis assigned >93% of RGCs to the 45 type identities based on scRNA-seq. Cell type frequencies are highly stable across MERFISH replicates and between MERFISH and scRNA-seq. We recovered previously documented topographic inhomogeneties for well-known RGC types (e.g. αRGCs), and also revealed spatial biases for less well-studied types. In the SC, we used a different 140-marker gene panel to identify 18 inhibitory and 19 excitatory neuronal types that corresponded well to types previously identified by scRNA-seq. Several SC types showed a clear layered spatial arrangement in situ. Taken together, our approach comprehensively maps the spatial distribution of cell types of the retinocollicular pathway without relying on transgenic mice. This will aid future efforts to uncover the cell-type specific wiring diagram between the retina and the SC, and reveal molecular determinants mediating potential synaptic choices.

Disclosures: **K. Nimkar:** None. **N. Tsai:** None. **M. Lum:** None. **S. Zhang:** None. **P. Lin:** None. **X. Duan:** None. **K. Shekhar:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.09/VV59

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Austrian Science Fund (FWF) Grant DK W1232
European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 665385

Title: Visualizing the transcriptional landscape with tissue context

Authors: *N. AGUDELO DUENAS[1], J. LYUDCHIK[1], C. KREUZINGER[1], M. R. TAVAKOLI[1], G. ABAGNALE[2], J. M. MICHALSKA[3], C. SOMMER[1], J. DANZL[1];
[1]Inst. of Sci. and Technol. Austria (ISTA), Klosterneuburg, Austria; [2]St. Anna Children's Cancer Res. Inst. (CCRI), Vienna, Austria; [3]E11 Bio, Alameda, CA

Abstract: Biological systems are intrinsically heterogeneous, from the level of molecular arrangements and interactions to whole tissue organization. To understand the complexity of these systems, it is fundamental to study them in their native context, which requires assessing their intricate structure and function in a spatially informed manner. Over the last decade, there has been a rapid advancement in the field of *spatial omics*, especially at the transcript level measuring gene expression, which has been instrumental in understanding how mRNA distribution and abundance define cell identity and function. This project aims to develop a highly multiplexed and modular methodology for integrated structural and multi-molecular characterization, as a means to visualize the spatial arrangement of the transcriptome with subcellular to tissue context. Given the importance of the compartmentalized organization of mRNAs (*local transcriptome*) in neurons, we apply a 242-gene panel to target neuron-specific transcripts in mouse brain tissue via Multiplexed Error Robust FISH (MERFISH). Importantly, we have adapted our protocols to work with thicker sections and gain 3D MERFISH spatial information. We also combine the transcriptional information with a morphological readout based on labeling the extracellular domain, which provides us with richer contextual information and allows us to locate mRNAs within distinct neuronal compartments at subcellular resolution. We envision that this technology will enable a more accurate characterization of the local transcriptome, to achieve a better understanding of how neurons respond to their functional demands in both health and disease.

Disclosures: N. Agudelo Duenas: None. **J. Lyudchik:** None. **C. Kreuzinger:** None. **M.R. Tavakoli:** None. **G. Abagnale:** None. **J.M. Michalska:** None. **C. Sommer:** None. **J. Danzl:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.10/VV60

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: High-plex single-cell spatial transcriptomic analysis of the mouse brain for massive-scale hypothesis generation

Authors: *C. WILLIAMS[1], A. HECK[2], P. DANAHER[2], Y. CUI[2], M. GRISWOLD[2], A. NAM[2], S. MURPHY[2], Y. LIANG[2], J. REEVES[2], M. RHODES[2], V. DEVGAN[2], J. M. BEECHEM[2]; [1]Res. & Develop., R&D, NanoString(R) Technologies, Seattle, WA; [2]Res. & Develop., NanoString Technologies, Seattle, WA

Abstract: Omics datasets have long been used for hypothesis generation - mass, unbiased discovery of correlations deserving further investigation. Within the brain, neuronal function is heavily dependent on cell-cell interactions, not just on isolated cell states. When applied to the well-researched mouse model, single-cell spatial transcriptomics (SCST) datasets, which record both location and gene expression profiles for potentially millions of cells, would appear to be

particularly promising veins to mine for novel mammalian neurobiology. However, exploratory systems biology analyses are seldom applied in SCST, perhaps because most studies have used panels dedicated to cell-type mapping, with plexity in the low hundreds. Here we demonstrate the utility of a high-dimension systems biology approach to SCST analysis, using the 1,000-plex Mouse Neuroscience Panel with the CosMx™ Spatial Molecular Imager (SMI). This panel covers robust neural and glial cell typing, neurodegeneration, neurodevelopment, and key aspects of cell state and signaling. We generated a dataset of over 150,000 cells from three coronal sections of a healthy, eight-week-old male C57BL/6 mouse, detecting an average of more than 1,200 transcripts per cell across all three samples. Leveraging the 1,000 genes on the panel, we categorized each cell into 50 cell types with distinct expression patterns and anatomical locations comprising excitatory neurons, inhibitory neurons, glia, and vascular populations. Then, we used spatial clustering analysis to partition the tissue into 16 niches with distinct cell-type compositions and calculated the enrichment of > 450 curated gene sets across all the cells in the sample. For cell types with at least 50 cells in four or more niches, we compared pathway activity across niches, testing > 13,000 hypotheses and identifying > 750 pathways with highly concentrated activity (Gini score > 0.3). To better understand the performance of this pathway enrichment, we focused on the amygdala, a bilateral region involved in emotion and memory. As compared to other niches, inhibitory neurons within the amygdala were enriched for expression of the Reactome gene set 'vasopressin-like receptors', and we visualize peptidergic neurons expressing the neuropeptides Avp and Gal specifically in the amygdala. As demonstrated by this close examination of one region in a healthy brain, high-plex single-cell spatial transcriptomics can support massive-scale hypothesis generation and new insights inaccessible to previous technologies. FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Disclosures: **C. Williams:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **P. Danaher:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **Y. Cui:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Griswold:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Nam:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **S. Murphy:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **Y. Liang:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Reeves:** A. Employment/Salary (full or part-time):; NanoString Technologies.

E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Rhodes:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **V. Devgan:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J.M. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.11/VV61

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: A high-plex toolbox for spatial biology: exploring a section of human motor cortex

Authors: *K. F. YOUNG, C. WILLIAMS, A. HECK, M. PATRICK, M. WALTER, R. LIU, J. C. NAVARRO, R. KHAFIZOV, A. WARDHANI, P. DANAHER, S. A. BONNETT, A. ROSENBLOOM, C. KANG, J. REEVES, D. W. RUFF, J. M. BEECHEM;
NanoString Technologies, Seattle, WA

Abstract: The realm of spatial biology is an evolving field full of new innovations that are greatly advantageous for dissecting neurobiological functions and mechanisms, which often depend on spatial relationships between brain cell types. Among these emerging technologies is the CosMx TM Spatial Molecular Imager (SMI), a single-cell spatial biology solution that utilizes the cyclic in situ hybridization chemistry to enable high-plex detection of RNAs and proteins at subcellular resolution in a spatial context. Here, we demonstrate the capability of SMI to detect 6,000 genes covering a variety of biological areas of interest such as protein and RNA metabolism, immune function, development, and disease in a section of the human motor cortex. With more than 4,900 genes particularly relevant to neuroscience and covering over 80 neuronally enriched pathways, the level of detail offered by this high-plex assay allows us to interrogate the tissue microenvironment and explore the spatial context of gene expression, cell states, and ligand-receptor interactions. In the CosMx RNA Assay, the tissue undergoes hybridization with RNA-specific barcoded probes that are detected through several rounds of reporter binding and fluorescence imaging on the CosMx SMI instrument. In addition, morphology markers (histone [nuclei), rRNA [cytoplasm), GFAP [astrocytes)) are utilized by an advanced cell-segmentation algorithm to assign transcripts to single cells, with resolution down to the level of cellular compartments such as the nucleus, cytoplasm, or in segmented cellular projections. The collection of spatially resolved single-cell data enables the annotation of numerous cell types with distinct expression patterns and anatomical locations, including neural

and glial populations, and additional analyses. For example, spatial clustering analysis partitions the tissue into niches based on neighborhood cell type content such that pathway activity and differential gene expression are examined across domains. Moreover, ligand colocalization analyses identify spatially correlated genes such as SST, NPY, and CORT, which helps identify a rare cell type, SST-Chodl neurons. Overall, SMI offers an incredibly versatile toolbox with which we can explore some of the most complicated questions in the field of neuroscience, such as the impact of cellular interactions, cell states, and relative gene expression within a spatial context.

Disclosures: **K.F. Young:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **C. Williams:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **M. Patrick:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **M. Walter:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **R. Liu:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **J.C. Navarro:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **R. Khafizov:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **A. Wardhani:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **P. Danaher:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **S.A. Bonnett:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **C. Kang:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty,

receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **J. Reeves:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **D.W. Ruff:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA. **J.M. Beechem:** A. Employment/Salary (full or part-time):; NanoString® Technologies, Seattle, WA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString® Technologies, Seattle, WA.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.12/VV62

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: In situ transcriptomic and proteomic profiling of the same cells in mouse brain sections with spatial molecular imaging

Authors: *K. VAN RAAY[1], A. ROSENBLOOM[2], B. BIRDITT[1], A. HECK[1], K. YOUNG[3], T. PHAN-EVERSON[2], Z. LEWIS[2], G. ONG[2], S. BONNETT[1], T. RANE[2], N. HANSEN[2], M. VANDENBERG[1], M. KORUKONDA[2], A. WARDHANI[3], P. DANAHER[2], C. BROWN[2], R. KHAFIZOV[2], D. W. RUFF[2], M. HOANG[2], G. GEISS[2], C. STOKES[4], J. BEECHEM[5]; [1]NanoString Technologies, Inc., Seattle, WA; [2]Nanostring Technologies, Inc., Seattle, WA; [3]NanoString Technologies, Seattle, WA; [4]Univ. of Washington / Seattle Children's Hosp., Seattle, WA; [5]Nanostring Technologies, Seattle, WA

Abstract: Spatially resolved, single-cell transcriptomics and proteomics in mouse neural models reveal neurobiological mechanisms relevant to human disease. However, spatial-omic protocols are analyte-specific and fail to capture multi-omic information within the same single cell. Here we develop a proteogenomic workflow on the CosMx™ Spatial Molecular Imager (SMI), a single-cell spatial biology platform that leverages cyclic *in situ* hybridization chemistry to enable high-plex detection of proteins and RNAs at subcellular resolution. We measure 68 proteins (CosMx Mouse Neural Cell Typing and Alzheimer's Pathology Panel) and 1,000 RNA targets (CosMx Mouse Neuroscience Panel) on the same 5 µm thick FFPE section of mouse brain. We test and optimize parameters involved with mouse brain FFPE tissue preparation, including tissue adherence, target retrieval, and permeabilization, to produce high-quality single-cell protein and RNA counts on the same tissue. We add image registration methods to align the protein and RNA images using fiducials and global tissue features. Lastly, we develop new multi-omic algorithms to integrate the protein and RNA data into secondary and tertiary analyses. Our proteogenomic workflow demonstrates significant benefits to cell segmentation

using cell-type specific markers (GFAP, Iba1, NeuN, in addition to a pan soma marker S6 and nuclear stain DAPI) in neural tissue. As a result, the number of transcripts captured within a single cell increased, most notably transcripts distal to cell bodies. This improved the quality of cell typing within the brain, including detection of rare cell types and states. To validate the proteogenomic workflow, we profiled uninfected and infected mouse brain with West Nile Virus (WNV) encephalitis. As expected from MNV encephalitis, we observed persistent neuroinflammation that includes the recruitment and activation of CD8+ T cells and microglia. We captured the major cell types that comprise inflammatory nodules in post-WNV mouse brain (neurons, microglia, and astrocytes) and identified the signaling pathways that underlie persistent microglial-driven neuroinflammation after WNV encephalitis. Taken together, we use the CosMx SMI platform to show, for the first time, that large numbers of mouse neuronal cells can be profiled with both protein and RNA at single-cell resolution in a spatial context. This integrated system maximizes the information content per single cell to enable mechanistic understanding into infectious disease pathology and inflammatory response in the brain.FOR RESEARCH USE ONLY. Not for use in diagnostic procedures

Disclosures: K. van Raay: A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **B. Birditt:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **K. Young:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **T. Phan-Everson:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **Z. Lewis:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **G. Ong:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **S. Bonnett:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **T. Rane:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **N. Hansen:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **M. Vandenberg:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **M. Korukonda:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

NanoString. **A. Wardhani:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **P. Danaher:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **C. Brown:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **R. Khafizov:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **D.W. Ruff:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **M. Hoang:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **G. Geiss:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString. **C. Stokes:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NanoString. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.13/VV63

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Subcellular spatial transcriptomics of astrocytes

Authors: *P. DANAHER[1], A. WARDHANI[1], M. GRISWOLD[1], M. PATRICK[1], C. WILLIAMS[2], A. HECK[3], L. WU[2], A. ROSENBLOOM[4], J. BEECHEM[5];
[1]NanoString Technologies, Seattle, WA; [2]NanoString Technologies, seattle, WA; [3]NanoString Technologies, Inc., Seattle, WA; [4]Nanostring Technologies, Inc, Seattle, WA; [5]Nanostring Technologies, Seattle, WA

Abstract: Spatial transcriptomics depends on successful cell segmentation - the process of defining cell boundaries - to build single cell expression profiles. But many brain cells have thin projections extending far from their nuclei, eluding segmentation by existing algorithms. We have developed techniques for segmenting these projections. Here, we describe the first analysis of spatially resolved gene expression in the projections of astrocytes. Astrocytes have local translation in their peripheral processes; as astrocytes contact synapses, this local translation may allow them to interact differently with different synapses.

We used the CosMx™ 6k Discovery panel to profile a 70 mm^2 area of a FFPE human cerebellum. Expression profiles were derived for 132,779 cells, including 1,624 astrocytes. Astrocyte projections were found in areas enriched with astrocytes, namely white matter, but also extending into the grey matter, where fewer astrocyte cell bodies were found.
We contrasted gene expression between astrocyte projections and astrocyte cell bodies. In projections, we found approximately 2-fold increases in various genes involved in cell motility and differentiation, and greater than 3-fold decreases in genes for water homeostasis, GABA transport, and glucose metabolism.
We clustered projections into 4 groups; these clusters showed remarkably consistent spatial organization. A cluster falling in white matter overexpressed genes involved in regulating water homeostasis, blood pressure, and cytoskeleton organization. A cluster found in grey matter overexpressed genes involved in lipid differentiation and cell signaling. Falling in narrow bands at the white/grey matter interface, one intermediate cluster overexpressed genes involved in stress response, lipid metabolism, and neurotransmission, and another overexpressed glutamate metabolism genes.
Gene clustering analysis found 19 modules of mutually correlated genes. Some modules appeared to correspond to the astrocyte subclusters described above; others were regulated independently of white/grey matter organization. One such module consisted of genes involved in differentiation and adhesion; another had genes involved in hypoxia response and cellular survival.
In summary, we have demonstrated that rich biology can be found in the expression profiles of cellular projections. We have found spatially organized clusters of astrocyte projections. Most diversity in astrocyte projections appears driven by location in white vs. grey matter, but some cellular programs - notably involving differentiation, adhesion, hypoxia and survival - appear to be controlled at a more local scale.

Disclosures: **P. Danaher:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Wardhani:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Griswold:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Patrick:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **C. Williams:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **L. Wu:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.14/VV64

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: German Research Foundation 413668513
SmartAge 859890 H2020-MSCA-ITN2019
FKZ 01EO1002
IZKF AMSP06
IZKF MSP12

Title: Comprehensive optimization of the astrocytic translatome isolation from a single area of mouse brain cortex using the ribotag method

Authors: *V. TRESHIN[1], D. BINOU[1], S. GULL[1], M. HAASE[1], I. INGRISCH[1], A. URBACH[1], J.-C. HENNINGS[2], O. W. WITTE[3], S. SCHMIDT[1];
[1]Dept. of Neurology, Univ. Hosp. Jena, Jena, Germany; [2]Inst. of Human Genetic, Univ. Hosp. Jena, Jena, Germany; [3]Univ. Hosp. Jena, Jena, Germany

Abstract: Astrocytes, initially deemed to be passive supporters of neurons, are now acknowledged as crucial regulators of neuronal network function. However, the underlying molecular mechanisms are still largely unknown. RNA profiling is an invaluable tool in studying cell function. But astrocytes have highly complex morphology with myriads of fine distal processes and a big part of RNA is translocated there and translated locally. Therefore, traditional methods of cell-specific RNA isolation which include tissue dissociation, are not suitable for studying astrocytes since they cause loss of distally localized RNA. A method that addresses this challenge is Ribotag (Sanz et al 2009). It utilizes mice with modified RPL22 gene (RPL22tm1.1Psam strain). After cell-type specific Cre expression it produces an HA-tagged ribosomal protein, providing a way to isolate actively translated mRNA (translatome) from a crude tissue lysate in cell-type specific manner. Though Ribotag has been widely utilized, surprisingly few details are available on it's different aspects. This obstructs the adaptation of the method to analysis of complex cell types as astrocytes, especially, in high-throughput experiments. Here we present an optimization of the original procedure for the isolation of whole astrocytic translatome from a single cortical area with small tissue mass. To achieve this we optimized the method using the Ribotag mice. To induce the astrocyte specific tagging of ribosomes, two ways of Cre delivery were used: AAV.PHP.eB vector based approach with Cre under GFAP104 and gfaABC1D promoters and conventional animal crossing with Aldh1l1-

Cre^{ERT2}(BAC) driver line. The recombination specificity was assessed by immunofluorescent staining. To get a maximum yield of high purity RNA from all compartments of astrocytes, we compared tissue lysis with Dounce homogenizer, Grinding under liquid Nitrogen and their combination. We tested different ratios of the antibody and the lysate material as well as different immunoprecipitation times. On the last step, for elution of immunoprecipitated RNA we compared standard method with silica based microcolumns and our own established approach using Isopropanol coprecipitation with linear polyacrylamide. Performance of the reactions was assessed by RT-qPCR and microcapillary RNA electrophoresis. Our optimized procedure involves induction of recombination by Aldh1l1-Cre driver and lysis of cortex region particles in Dounce homogenizers, 5-hour immunoprecipitation with 0.1% antibody to total protein ratio and our novel RNA elution method. It enables the isolation of astrocytic translatome for large scale deep sequencing.

Disclosures: **V. Treshin:** None. **D. Binou:** None. **S. Gull:** None. **M. Haase:** None. **I. Ingrisch:** None. **A. Urbach:** None. **J. Hennings:** None. **O.W. Witte:** None. **S. Schmidt:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.15/WW1

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant P51OD010425
RF1MH114126-01 from the National Institute of Mental Health to BPL, JTT, and ESL
UG3MH120095-01, -02, -03 from the National Institute of Mental Health to BPL, JTT, ESL, and FKK
UF1MH128339-01 from the National Institute of Mental Health to BT, TB, TLD, BPL, and JTT
RF1MH121274-01 from the National Institute for Mental Health to BT

Title: Enhancer-aavs allow genetic access to diverse populations of astrocytes and oligodendrocytes across species

Authors: J. K. MICH[1], S. SUNIL[5], N. JOHANSEN[6], R. A. MARTINEZ[1], M. LEYTZE[6], B. B. GORE[6], J. MAHONEY[2], Y. KOJIMA[7], S. GIBSON[8], R. CANFIELD[8], N. J. WEED[1], V. OMSTEAD[6], D. MACHEN[9], N. TASKIN[6], E. L. GROCE[6], Y. BISHAW[10], X. OPITZ-ARAYA[6], N. DEE[6], ***J. LIU**[11], T. CASPER[6], N. V. SHAPOVALOVA[6], D. HIRSCHSTEIN[6], H. ZENG[1], T. DAIGLE[1], B. TASIC[3], E. LEIN[9], J. T. TING[4], B. LEVI[1];
[2]Human Cell types, [3]Cell and Circuit Genet., [4]Human Cell Types, [1]Allen Inst. For Brain Sci., Seattle, WA; [5]Allen institute for Neural Dynamics, Seattle, WA; [6]Allen institute for Brain Sci., Seattle, WA; [7]Dept. of Otolaryngology Head and Neck Surgery, [8]Dept. of Physiol. and

Biophysics, Univ. of Washington, Seattle, WA; [9]Human Cell Types, Allen Inst. for Brain Sci., Seattle, WA; [11]Brain Sci., [10]Allen Inst., Seattle, WA

Abstract: Proper brain function requires the assembly and function of diverse populations of neurons and glia. Single cell gene expression studies have mostly focused on characterization of neuronal cell diversity; however, recent studies have revealed substantial diversity of glial cells, particularly astrocytes. To better understand glial cell types and their roles in neurobiology, we built a new suite of adeno-associated viral (AAV)-based genetic tools to enable genetic access to astrocytes and oligodendrocytes. Our oligodendrocyte and astrocyte enhancer-AAVs were highly specific, showed variable expression levels, and our astrocyte enhancer-AAVs showed multiple distinct expression patterns that reflected the spatial distribution of astrocyte subtypes. To provide the best glial-specific functional tools, several enhancer-AAVs were: 1) optimized for higher expression levels, 2) tested in rat and macaque and shown to be functional across species, 3) shown to maintain specific activity in epilepsy where traditional promoters altered expression, and 4) used to drive astrocyte-specific functional transgenes including Cre recombinase, acetylcholine-responsive sensor iAChSnFR and neurotransmitter transporters to further understand their relationship and functionality in glia populations. Together, this collection of glial enhancer-AAVs will enable characterization of diverse glial populations and their roles across species, disease states, and behavioral epochs.

Disclosures: **J.K. Mich:** None. **S. Sunil:** None. **N. Johansen:** None. **R.A. Martinez:** None. **M. Leytze:** None. **B.B. Gore:** None. **J. Mahoney:** None. **Y. Kojima:** None. **S. Gibson:** None. **R. Canfield:** None. **N.J. Weed:** None. **V. Omstead:** None. **D. Machen:** None. **N. Taskin:** None. **E.L. Groce:** None. **Y. Bishaw:** None. **X. Opitz-Araya:** None. **N. Dee:** None. **J. Liu:** None. **T. Casper:** None. **N.V. Shapovalova:** None. **D. Hirschstein:** None. **H. Zeng:** None. **T. Daigle:** None. **B. Tasic:** None. **E. Lein:** None. **J.T. Ting:** None. **B. Levi:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.16/WW2

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIDA U01DA043098
ONR 00014-19-1-2149
The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium
NCI P30CA046592: Cancer Center Shared Resource (Single Cell and Spatial Analysis Shared Resource)

Title: Spatial transcriptomics reveal basal differences in nucleus accumbens gene profiles associated with temperament: studies in a rat model of emotional reactivity

Authors: *M. WASELUS, E. K. HEBDA-BAUER, S. KOONSE, M. DAI, F. MENG, H. AKIL, S. J. WATSON;
Univ. of Michigan, Ann Arbor, MI

Abstract: Differences in emotionality and reactivity to the environment have been modeled using selectively-bred lines of high- and low-responder rats (bHR and bLR, respectively), which exhibit differences in exploratory locomotion, propensity to self-administer drugs of abuse, and anxiety-like behaviors. Anatomical methods (e.g., radioactive *in situ* hybridization) have implicated specific transcripts and brain regions in these behavioral differences, yet classical anatomical tools limit the number of genes examined. Expression profiling (e.g., bulk RNAseq) yielded profound bHR-bLR differences, however more anatomically precise expression profiling approaches are needed to capture regionally defined gene expression differences. Spatial transcriptomic solutions (e.g., Visium-FF, 10X Genomics) combine RNAseq with spatial registration to provide an unbiased global picture of RNA expression with anatomical specificity. Brains from our selective breeding colony (68^{th} generation) were used to examine bHR/bLR differences in basal gene expression. We previously showed significant differences between the lines, including several immediate early genes (IEGs) exhibiting higher expression in the dorsal hippocampus (dHC) of bHRs vs bLRs. In these same rats, we examined the nucleus accumbens (NAc) while delineating differences between the core (NAcC) and shell (NAcS) regions. Differential gene expression was found between the NAcC and NAcS. Both bred lines exhibit higher calcium-binding protein calbindin 1 (Calb1) expression in the NAcC vs NAcS, while neurotensin (Nts) is more abundant in the NAcS vs NAcC. Differences between bHR-bLR were also found. As in the dHC, IEGs Arc and Egr1/zif-268 exhibited higher NAc expression in bHRs vs bLRs, though these differences were restricted to the NAcC, with no IEG differences in the NAcS. In addition, bHRs (vs bLRs) exhibited higher expression of the soluble epoxide hydrolase (Ephx2) in both the NAcC and NAcS, while increased levels of crystallin, alpha B (Cryab) in bHRs were limited to the NAcC. bLR rats expressed patatin-like phospholipase domain containing 1 (Pnpla1) in the NAcC and NAcS, while very little expression was seen in bHRs. Given that many of these genes are associated with inflammatory processes, further investigation of inflammatory pathways and related genes will be examined in both the dHC and NAc. Thus, the unbiased investigation of bHR/bLR brain differences using the Visium platform promises to be a valuable tool for elucidating differences in gene expression and enables subsequent, more refined future anatomical queries aimed at understanding the neurobiology of temperament.

Disclosures: M. Waselus: None. **E.K. Hebda-Bauer:** None. **S. Koonse:** None. **M. Dai:** None. **F. Meng:** None. **H. Akil:** None. **S.J. Watson:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.17/WW3

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Unraveling the transcriptional landscape of Brodmann Area 46 using the ultrahigh 6K discovery panel of CosMx Spatial Molecular Imager (SMI)

Authors: Y. CUI[1], **W. TIAN**[2], S. MURPHY[1], C. WILLIAMS[1], L. WU[1], J. RINK[2], M. BEHRENS[2], J. R. ECKER[2,3], *J. BEECHEM[1];
[1]Nanostring Technologies, Seattle, WA; [2]The Salk Inst. for Biol. Studies, La Jolla, CA; [3]Howard Hughes Med. Inst., La Jolla, CA

Abstract: Understanding the intricate molecular architecture of the human brain is essential for unraveling its complex cognitive functions. In this study, we employed the cutting-edge CosMx™ 6K Discovery panel to comprehensively map gene expression in the prefrontal cortical region known as Brodmann Area 46 (BA46). This high-plex approach enabled us to investigate the molecular signatures and cellular diversity underlying the cognitive processes associated with this key brain region. We have successfully profiled the expression of approximately 6,000 genes in about 400,000 cells within fresh-frozen human brain tissues, which yielded ~1,200 transcripts from ~800 unique genes per cell. By establishing a transcriptional atlas of BA46, we are gaining novel insights into the cell types, gene networks, and functional pathways that define the region's unique characteristics. Our results revealed distinct cell populations in BA46, including pyramidal neurons and various subtypes of inhibitory interneurons, aligning with previous knowledge of the prefrontal cortex cellular composition. Furthermore, we identified specific expression patterns linked to synaptic transmission, plasticity, and neurotransmitter receptors, highlighting the molecular machinery that defines the cell states in this brain region. Through integrative analyses, we are testing 160,000+ ligand pairs for *in situ* correlations with the aim to unveil co-expression networks that would shed light on the functional organization of BA46. We plan to identify clusters of genes displaying coordinated expression in shared biological processes. These results should provide new insights into key signaling pathways and molecular cascades that may be ultimately involved in executive function, working memory, cognitive control, and language processing. This comprehensive transcriptomic map of BA46 serves as a valuable resource for future studies aiming to decipher the molecular mechanisms underlying higher-order cognitive processes. The intricate interplay between cell types, gene networks, and functional pathways revealed in this study will provide a foundation for understanding the complex architecture of the dorsolateral prefrontal cortex. In conclusion, our research showcases CosMx SMI's capacity to unravel the transcriptional landscape of BA46 with subcellular resolution, deepening our understanding of the molecular composition in this important brain region. These findings pave the way for future investigations into the role of specific genes, cell types, and pathways in cognitive disorders and guide the development of targeted therapeutic interventions.

Disclosures: **Y. Cui:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **W. Tian:** None. **S. Murphy:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **C. Williams:** A. Employment/Salary (full or part-time):; Nanostring Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies. **L. Wu:** A. Employment/Salary (full or part-time):;

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

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.18/WW4

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIMH IRP

Title: Investigating the impact of high-risk copy number variants on the human brain transcriptome at a single cell level

Authors: *G. DUGAN[1], N. AKULA[2], S. MARENCO[3], P. AULUCK[3], A. RAZNAHAN[2], S. LIU[2], A. R. DECASIEN[2], Q. XU[3], N. FENG[3], B. KOLACHANA[3], X. JIANG[2], M. GREGORY[4], K. F. BERMAN[4], F. MCMAHON[2], A. SCHULMANN[2];
[1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, VA; [2]NIH, Natl. Inst. of Mental Hlth. (NIMH), Human Genet. Br., Bethesda, MD; [3]NIH, Natl. Inst. of Mental Hlth. (NIMH), Human Brain Collection Core (HBCC), Bethesda, MD; [4]NIH, Natl. Inst. of Mental Hlth. (NIMH), Clin. and Translational Neurosci. Br., Bethesda, MD

Abstract: Copy number variants (CNVs) are chromosome segments that undergo duplications or deletions. Some large and recurring CNVs have been strongly associated with increased risk for neuropsychiatric disorders. However, little is known about their impact on the human brain transcriptome. We aim to enhance understanding of high-risk CNV effects at the single-cell level in the human brain. We collected single-nucleus RNA-sequencing data from the dorsolateral prefrontal and anterior cingulate cortex of 13 carriers of neuropsychiatric CNVs (22q11.2, 16p11.2, 1q21.1, 7q11.23 del, and 15q11.2), along with 24 non-carriers matched for age, race, sex, and psychiatric diagnosis. Data were processed using CellRanger, filtered and quality controlled using Seurat, and annotated for cell type with Azimuth. Differential gene expression analyses were performed across nine cell types using Dreamlet, and functional enrichments were conducted using Zenith. As expected, the expression of genes within CNV regions corresponded to the number of copies present in those regions. Individuals with deletions exhibited more differentially expressed genes (DEGs; FDR < 0.1) than those with duplications. Deletion carriers showed functional enrichments associated with cellular stress and energy metabolism. Topic modeling of enriched terms across CNVs reiterated these findings. Notably, individuals with the 22q11.2 deletion and 7q11.23 deletion showed variations in the number of DEGs across cell

types. In the case of the 22q11.2 deletion, astrocytes had more than five times the number of DEGs compared to other cell types. These genes were enriched in terms related to glycolysis, cellular stress response (specifically hypoxia), and circadian regulation of gene expression across various cell types. The 7q11.23 deletion exhibited nearly ten times the number of DEGs in excitatory neurons compared to other cell types. These genes were enriched in terms associated with oxidative phosphorylation and mitochondrial function, particularly showing higher numbers of DEGs in excitatory neurons. This is the first transcriptomic analysis of high-risk CNVs in the human brain at single-cell resolution. Deletions have a stronger impact on gene expression than duplications, aligning with their greater phenotypic effects. Specific CNVs have differential effects on cell types, highlighting the importance of cellular resolution. Deletion carriers show upregulated genes associated with cellular stress responses across major cell types, suggesting neurobiological changes due to CNVs. Future research will further explore convergent and divergent effects of distinct CNVs.

Disclosures: G. Dugan: None. **N. Akula:** None. **S. Marenco:** None. **P. Auluck:** None. **A. Raznahan:** None. **S. Liu:** None. **A.R. DeCasien:** None. **Q. Xu:** None. **N. Feng:** None. **B. Kolachana:** None. **X. Jiang:** None. **M. Gregory:** None. **K.F. Berman:** None. **F. McMahon:** None. **A. Schulmann:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.19/WW5

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatially resolved, single-cell transcriptomic imaging with protein biomarkers in human Alzheimer's Disease brain samples

Authors: *B. WANG, Y. SUN, R. CHEN, C. CHEN, S. KAUSHAL, L. COLBERT, J. HE; Vizgen, Cambridge, MA

Abstract: Elucidating the underlying molecular alterations in Alzheimer's Disease (AD) pathology is essential for understanding disease mechanisms and developing effective therapeutic interventions. In recent years, spatial imaging techniques such as Multiplexed Error-Robust Fluorescence in situ Hybridization (MERFISH) have emerged as powerful tools for comprehensive molecular and cellular profiling at single-cell resolution. In this study we used the Vizgen® MERSCOPE® Platform, a single-cell, spatially resolved transcriptomic imaging platform utilizing MERFISH technology, to study the spatial distribution of biomarkers and cellular heterogeneity of the human AD brain by simultaneously imaging 6 protein biomarkers and hundreds of RNA species together. Specifically, we employed a representative 6-plex neuronal protein panel comprising cell type markers (GFAP, Iba-1, MBP), a vascular protein (CD31), and neuropathological markers (mOC23, AT8) in conjunction with a customized 244-plex gene panel to explore the intricate molecular and cellular signatures within affected brain

regions. We demonstrated that MERSCOPE protein co-detection did not significantly impact the detection of RNA transcripts in tissue, and that simultaneous protein staining revealed distinct morphological and pathological features of the disease. Additionally, in situ profiling of hundreds of RNAs on the same tissue section at single-cell resolution enabled us to map and catalog distinct cell types. By simultaneously examining markers for neurons, astrocytes, microglia, and other relevant cell populations, we can elucidate the cellular composition and potential alterations in cellular states associated with AD progression. The proposed utilization of spatial multiomics profiling of both protein and RNA with MERSCOPE offers a comprehensive approach to investigate protein expression patterns, cellular heterogeneity, and cell-cell interactions in AD pathology, highlighting the power of spatial multiomics in elucidating contributing factors to complex diseases such as AD. This information will contribute to a deeper understanding of the cellular and molecular mechanisms involved in the disease, potentially identifying novel targets for therapeutic intervention.

Disclosures: **B. Wang:** A. Employment/Salary (full or part-time):; Vizgen. **Y. Sun:** A. Employment/Salary (full or part-time):; Vizgen. **R. Chen:** A. Employment/Salary (full or part-time):; Vizgen. **C. Chen:** A. Employment/Salary (full or part-time):; Vizgen. **S. Kaushal:** A. Employment/Salary (full or part-time):; Vizgen. **L. Colbert:** A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.20/WW6

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Efficient whole transcriptome library preparation with whole transcriptome amplification (WTA) for low-input RNA without ribosomal RNA depletion

Authors: *K. SAITO[1], J. KOSHY[5], J. LIU[2], Y. V. JALIKOP[6], C. LANDERS[3], R. WIESE[4]; [1]Mol. Assays R&D, Life Sci. Biology, Sci. and Lab. Solutions, MilliporeSigma, Temecula, CA; [2]Bioinformatics, [3]Mol. Prep & PCR, Life Sci. Biology, Sci. and Lab. Solutions, [4]Singleplex & Special Protein Platforms R&D, Life Sci. Biology, Sci. and Lab. Solutions, MilliporeSigma, Saint Louis, MO; [5]Bioinformatics, [6]Mol. Prep & PCR, Life Sci. Biology, Sci. and Lab. Solutions, Merck, Bangalore, India

Abstract: RNA-Seq has revolutionized gene expression analysis, offering two popular methods: whole transcriptome sequencing and mRNA-seq. Whole transcriptome sequencing provides a comprehensive view, including non-coding RNA, and can process degraded RNA, including RNA from FFPE tissue. However, the high abundance of ribosomal RNA (rRNA) poses a challenge, requiring time-consuming rRNA depletion during library preparation. To overcome this limitation, we developed an efficient WTA-based method for Illumina platform. WTA eliminates fragmentation, end repair, and intermediate cleanup steps, saving time and resources.

In this study, we compared WTA workflow (WTAW) to an adapter-ligation-based workflow (Ligation W), with and without rRNA depletion, analyzing ribosomal RNA, coding genes, and non-coding genes. Ligation W, without rRNA depletion, suffered from 90% of reads aligning to rRNA, limiting the analysis to 10% of reads. WTAW, utilizing proprietary semi-degenerate primers that preferentially amplify non-ribosomal RNA, achieved a substantial improvement, with 38% rRNA alignment, leaving 62% of reads for transcriptome analysis. With rRNA depletion, WTAW further reduced rRNA alignment to 3%. Without rRNA depletion, Ligation W identified 10,000 genes, while WTAW detected 23,000 genes. Notably, WTAW excelled in identifying non-coding RNA genes, revealing 7,400 genes compared to Ligation W's 500-600 genes.In summary, our WTA-based workflow enables efficient gene expression analysis. It outperforms ligation-based workflow, identifying more coding and non-coding genes from low-input RNA (less than 5 ng). By eliminating the need for time-consuming rRNA depletion, the WTA streamlines the workflow and provides valuable insights into the transcriptome.

Disclosures: K. Saito: None. **J. Koshy:** None. **J. Liu:** None. **Y.V. Jalikop:** None. **C. Landers:** None. **R. Wiese:** None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.21/WW7

Topic: I.02. Systems Biology and Bioinformatics

Support: Wings for Life - Spinal Cord Research Foundation
Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Functional Genomic Approaches to Identify Transcriptional Regulators of Neural Repair and Inform Therapeutic Development

Authors: *Y. CHENG[1], F. TIAN[3], Y. YIN[3], A. ZHANG[1], C. J. WOOLF[3], M. V. SOFRONIEW[2], L. I. BENOWITZ[3], Z. HE[3], D. H. GESCHWIND[1];
[1]Dept. of Neurology, Psychiatry, and Human Genetics, David Geffen Sch. of Med., [2]Dept. of Neurobiology, David Geffen Sch. of Med., UCLA, Los Angeles, CA; [3]F.M. Kirby Neurobio. Center, Boston Children's Hospital, and Dept. of Neurol., Harvard Med. Sch., Boston, MA

Abstract: The inability of adultneurons to efficiently self-repair is a major impediment to improving outcomefrom traumatic brain damage, spinal cord injury, and other neurodegenerativedisease. Understanding how neurons detect and respond to such insults couldhold the key to developing novel protective and regenerative strategies. Aspecific cell state ultimately represents the readout of specific geneexpression programs expressed at the time. When we investigated thetranscriptional state of injured RGC (Cheng et al., 2022, Nat Commun 13:4418;Tian and Cheng* et al., 2022, Neuron 110:2607; Cheah and Cheng* et al., JNeurosci. 2023. * co-first), we found that they are unable to maintain geneprograms needed for survival

and axon regeneration, while sustaining geneprograms for cell death. Thus, the central vision of our research is to rewirethe transcriptional state of the damaged neurons, so as to improve theirability to survive and regenerate. Leveraging functional genomics techniques toprofile transcriptome and epigenome of nerve-damaged neurons, we identified keyregulators of their growth and survival state, including transcription factors,environmental signals, and repurposed drugs. We validated our computationalpredictions across multiple disease-relevant models including spinal cordinjury, optic nerve injury, and glaucoma, demonstrating improved neuronalsurvival and/or axon regeneration upon interventions of these modulators.Together, our findings demonstrate the power of a systems biology approachinvolving integrative genomics and bioinformatics to prioritize hypothesesrelevant and inform potential therapeutic strategies relevant toneuroprotection and neural repair.

Disclosures: Y. Cheng: None.

Poster

PSTR506. Genomic and Transcriptomic Techniques

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR506.22/WW8

Topic: I.02. Systems Biology and Bioinformatics

Support: Vizgen

Title: Unveiling Brain Complexity with the MERSCOPE Platform: Insights into Healthy and Diseased States

Authors: *R. CHEN, Y. CAI, D. PATEL, S. KAUSHAL, L. COLBERT, J. HE;
Vizgen, Cambridge, MA

Abstract: The neuronal system consists of various molecularly unique cell types arranged into different anatomical structures. Over the past decade, single-cell sequencing has made considerable strides in elucidating the cell heterogeneity of the nervous system. However, most sequencing-based methodologies require cell dissociation, thus posing challenges to directly linking the molecular characteristics of analyzed cells to their anatomical and functional properties. Recently, the advent of spatially resolved genomic technologies enables the probing of molecular profiles, such as gene and protein expression, with subcellular precision while maintaining intact tissue context. Among these, Multiplexed Error-Robust Fluorescence in situ Hybridization (MERFISH) stands at the forefront of spatial transcriptomics, enabling comprehensive mapping of cellular composition and spatial organization within many brain structures.In this study, we showcase the capabilities of the Vizgen® MERSCOPE® Platform—an end-to-end solution for MERFISH technology—in deciphering the molecular and cellular features of both mouse and human brain tissue under physiological and pathological conditions. We leveraged the vast gene repertoire of a 960-plex gene panel to achieve unprecedented resolution in neuronal and non-neuronal cell types across disparate mouse brain regions,

enabling the molecular and cellular features underpinning various anatomical structures to be uncovered. Moreover, the integration of molecular and spatial information derived from the same tissue facilitated an in-depth understanding of cell-type-specific signaling and regulatory mechanisms. Furthermore, to understand the pathogenesis of Alzheimer's Disease (AD) we conducted a multiomics imaging assay on MERSCOPE, concurrently measuring gene expression and protein staining in human brain samples. This enabled us to identify cell-type-specific molecular and cellular adaptations, highlighting a spatially dependent response associated with the disease.In summary, the application of the MERSCOPE Platform not only allows us to understand the spatial organization and interaction of different cell types in the brain, but also sheds light on the fundamental processes of neurobiology and the complex alterations occurring in neurodegenerative diseases such as AD.

Disclosures: **R. Chen:** A. Employment/Salary (full or part-time):; Vizgen. **Y. Cai:** A. Employment/Salary (full or part-time):; Vizgen. **D. Patel:** A. Employment/Salary (full or part-time):; Vizgen. **S. Kaushal:** A. Employment/Salary (full or part-time):; Vizgen. **L. Colbert:** A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.01/WW9

Topic: I.04. Physiological Methods

Support: Peking-Tsinghua Center for Life Sciences and the State Key Laboratory of Membrane Biology at Peking University School of Life Sciences

Title: Revealing the in vivo dynamics and molecular mechanisms of neuropeptide release in vivo using a new GRAB sensor

Authors: *X. XIA, Y. LI;
Peking Univ., Beijing, China

Abstract: Neuropeptides are a class of neuronal chemicals that are involved in many physiological and pathological processes, and they often are colocalized with small molecule neurotransmitters of neuronal signaling in almost all animals. The resulting co-transmission, which provides a more stable and flexible system that helps animals survive in fast-changing environments. However, the precise spatial and temporal dynamics and molecular mechanisms of neuropeptides and small molecule neurotransmitters release from the same neurons remain poorly understood. In this study, we describe a new genetically encoded G-protein-coupled-receptor-activation-based (GRAB) short neuropeptide F (sNPF) sensor called $GRAB_{sNPF1.0}$. $GRAB_{sNPF1.0}$ enables the detection of sNPF release *in vivo* with suitable sensitivity, specificity, and spatiotemporal resolution. Using this sensor, we systematically characterized the spatial and

temporal dynamics of sNPF release with Acetylcholine (ACh) release by combining the well-characterized ACh3.0 sensor from Kenyon cells (KCs). sNPF shows broader release in the neuronal system than ACh, and sNPF is slower than ACh in both rise and decay time constants, what's more, sNPF release pattern is opposite with ACh release. Finally, we found that loss of function of syt7 and sytα suppressed neuropeptide release but not impair small molecule neurotransmitter release. Thus, $GRAB_{sNPF1.0}$ provides insight into the different release dynamics and molecular mechanisms of neuropeptide release from small molecule neurotransmitter release.

Disclosures: X. Xia: None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.02/WW10

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative (grant nos. 1U01NS113358)
NIH BRAIN Initiative (grant nos. 1U01NS120824)

Title: Improved dual-color GRAB sensors for monitoring dopaminergic activity in vivo

Authors: *Y. ZHUO[1,2], B. LUO[1,2], X. YI[1], H. DONG[1], J. WAN[1], R. CAI[1], J. T. WILLIAMS[3], T. QIAN[1], M. G. CAMPBELL[4], H. WANG[1], X. MIAO[5], B. LI[6], Y. WEI[1], G. LI[1], Y. ZHENG[1], M. W. UCHIDA[4], Y. LI[1,2,7,8,9];
[1]Peking Univ., Beijing, China; [2]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [3]Oregon Hlth. Sci. Univ., Oregon Hlth. Sci. Univ., Portland, OR; [4]Harvard Univ., Harvard Univ., Cambridge, MA; [5]Capital Med. Univ., Beijing, China; [6]Chinese PLA Gen. Hosp., Beijing, China; [7]Chinese Inst. for Brain Res., Beijing, China; [8]Inst. of Mol. Physiol., Shenzhen Bay Lab., Shenzhen, China; [9]Peking-Tsinghua Ctr. for Life Sci., Beijing, China

Abstract: Dopamine (DA) is a crucial monoamine neurotransmitter involved in many physiological and pathological processes through a complex network of dopaminergic projections, and the ability to directly monitor DA dynamics is essential for understanding its physiological functions. Despite the widespread use of genetically-encoded dopamine fluorescent sensors in vivo, the detection of DA is often limited to highly innervated regions. Consequently, cortical DA detection has received comparatively little attention due to the undetectable of current generations of sensors. To address this gap, we have developed a series of green and red fluorescent G-protein-coupled receptor (GPCR) activation-based DA ($GRAB_{DA}$) sensors employing different DA receptor subtypes. These sensors display highly improved sensitivity, selectivity, and signal-to-noise properties, with subsecond response kinetics and the ability to detect a broad range of DA concentrations. Using these sensors, we have measured optogenetically-evoked and behaviorally-relevant DA release in mice, while

concurrently monitoring neurochemical signaling in the nucleus accumbens, amygdala, and cortex. Furthermore, with these sensors, we have identified spatially-resolved heterogeneous cortical DA release during various behavioral tasks. Thus, these new DA sensors provide an extended toolbox for multifaceted *in vivo* DA imaging under a variety of complex behavior contexts, which can in turn promote the understanding of diverse aspects of dopamine biology.

Disclosures: **Y. Zhuo:** None. **B. Luo:** None. **X. Yi:** None. **H. Dong:** None. **J. Wan:** None. **R. Cai:** None. **J.T. Williams:** None. **T. Qian:** None. **M.G. Campbell:** None. **H. Wang:** None. **X. Miao:** None. **B. Li:** None. **Y. Wei:** None. **G. Li:** None. **Y. Zheng:** None. **M.W. Uchida:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.03/WW11

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative 1U01NS113358
NIH BRAIN Initiative 1U01NS120824

Title: A chemigenetic far-red dopamine sensor for multiplex imaging in vivo

Authors: ***Y. ZHENG**[1], R. CAI[1], J. ZHANG[1], H. DONG[1], B. LUO[1], J. GRIMM[2], K. JOHNSSON[3], E. R. SCHREITER[2], L. D. LAVIS[2], Z. CHEN[1], Y. LI[1];
[1]Peking university, Beijing, China; [2]Howard Hughes Med. Institute, Janelia Farm Res. Campus, Ashburn, VA; [3]Max Planck Inst. for Med. Res., Heidelberg, Germany

Abstract: Dopamine (DA) is a critical monoamine neurotransmitter that regulates various physiological functions, including motor control and reward. Abnormal DA signaling is also associated with many brain disorders. Moreover, many of these conditions involve with the release and regulation of several other neurotransmitters. To gain a better understanding of their dynamics and functional roles in both health and disease, there is an urgent need to expand the spectral of neurochemical sensors for simultaneous multi-color imaging of several neurochemicals. To meet this challenge, we developed a far-red DA sensor based on the G protein-coupled receptor activation (GRAB) strategy, named GRAB-HaloDA (HaloDA1.0 in short), which takes advantage of the circularly permutated self-labeling protein HaloTag and bright far-red fluorescent dyes conjugated with HaloTag ligand (HTL). In cultured cell lines and primary cultured neurons, HaloDA1.0 labeled with a far-red dye, exhibits over 1000% fluorescent response to DA, with subsecond kinetics, good membrane trafficking and molecular selectivity. In acute brain slices, HaloDA1.0 can resolve both spontaneous and evoked endogenous DA release in nucleus accumbens. Using fiber-photometry recording, we successfully detected optogenetically and reward-elicited DA release in freely moving mice using HaloDA1.0. More importantly, the far-red DA sensors enable three-color imaging with

other green and red sensors in mice during pavlovian conditioning task. Overall, HaloDA1.0 represents a power tool for monitoring DA dynamics in vitro and in vivo, facilitating study of the regulation between neurotransmitters.

Disclosures: **Y. Zheng:** None. **R. Cai:** None. **J. Zhang:** None. **H. Dong:** None. **B. Luo:** None. **J. Grimm:** None. **K. Johnsson:** None. **E.R. Schreiter:** None. **L.D. Lavis:** None. **Z. Chen:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.04/WW12

Topic: I.04. Physiological Methods

Support: National Basic Research Program of China 2021YFF0502904
NIH Grant 1U01NS120824
NSF Grant 31925017

Title: Monitoring norepinephrine release in vivo using next-generation $GRAB_{NE}$ sensors

Authors: ***J. FENG**[1], H. DONG[1], J. E. LISCHINSKY[2], J. ZHOU[3], F. DENG[1], H. WANG[1], H. XIE[4], G. CUI[3], D. LIN[2], Y. LI[1];
[1]Peking Univ., Beijing, China; [2]Sci. Building- Neurosci. Inst., New York Univ. SOM, New York, NY; [3]NIEHS, RTP, NC; [4]Dept. of Automation, Tsinghua Univ., Beijing, China

Abstract: Norepinephrine (NE) is an essential biogenic monoamine neurotransmitter, yet researches using prototype NE sensors were limited by their low sensitivities. Here, we developed next-generation versions of GPCR activation-based NE sensors ($GRAB_{NE2m}$ and $GRAB_{NE2h}$) with a superior response, high sensitivity and selectivity to NE both *in vitro* and *in vivo*. Notably, these sensors can detect NE release triggered by either optogenetic or behavioral stimuli in freely moving mice, producing robust signals in the locus coeruleus and hypothalamus. With the development of a novel transgenic mouse line, we recorded both NE release and calcium dynamics with dual-color fiber photometry throughout the sleep-wake cycle; moreover, dual-color mesoscopic imaging revealed cell type–specific spatiotemporal dynamics of NE and calcium during sensory processing and locomotion. Thus, these new $GRAB_{NE}$ sensors are valuable tools for monitoring the precise spatiotemporal release of NE *in vivo*, providing new insights into the physiological and pathophysiological roles of NE.

Disclosures: **J. Feng:** None. **H. Dong:** None. **J.E. Lischinsky:** None. **J. Zhou:** None. **F. Deng:** None. **H. Wang:** None. **H. Xie:** None. **G. Cui:** None. **D. Lin:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.05/WW13

Topic: I.04. Physiological Methods

Title: A toolbox of genetically encoded GRAB sensors for multiplex imaging of purinergic transmission

Authors: *B. LI[1,2], A. D. UMPIERRE[3], Z. WU[2], L. WANG[2], S. PAN[2], L.-J. WU[4], Y. LI[2,5,6]; [1]Ctr. for Life Sci., Peking Univ., Beijing, China; [2]PKU-IDG/McGovern Inst. for Brain Res., Beijing, China; [3]Neurol. Dept, [4]Neurol., Mayo Clin., Rochester, MN; [5]Peking-Tsinghua Ctr. for Life Sci., Beijing, China; [6]State Key Lab. of Membrane Biology, Peking Univ. Sch. of Life Sci., Beijing, China

Abstract: Purinergic transmitters, such as extracellular ATP, ADP, adenosine and UDP, play essential roles in both peripheral and central nervous systems. With a range of endogenous purinergic transmitters interacting with over ten native receptors, and the possibility of complex conversions among certain neurochemicals, it becomes crucial to develop tools that can monitor them concurrently with high molecular specificity and high spatial-temporal resolution. To achieve this, we developed and optimized a series of GPCR-Activation-Based (GRAB) sensors, which are capable of detecting various purinergic transmitters including ATP, ADP, adenosine and UDP. These sensors show good plasma membrane localizations, high sensitivity, and importantly, high selectivity in distinguishing them from other structurally similar neurochemicals. Novel UDP sensors allowed us to observe increased UDP release e following epileptogenic *in vivo*. Furthermore, the development and optimization of red-shifted purinergic GRAB sensors made it possible to achieve dual-color imaging of different neurochemicals. Specifically, by combining adenosine and ATP sensor with separated fluorescent spectrums, we were able to simultaneously record their dynamics in culture neurons. Taken together, this expanded purinergic sensor toolbox enabled monitoring of purinergic transmission *in vitro* and *in vivo*, unlocking new avenues for comprehending the dynamic and regulation of the purinergic system.

Disclosures: B. Li: None. **A.D. Umpierre:** None. **Z. Wu:** None. **L. Wang:** None. **S. Pan:** None. **L. Wu:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.06/WW14

Topic: I.04. Physiological Methods

Support: Beijing Municipal Science & Technology Commission Z181100001318002
Beijing Municipal Science & Technology Commission Z181100001518004
National Natural Science Foundation of China 31925017
Shenzhen-Hong Kong Institute of Brain Science NYKFKT2019013
NIH grant NS103558
NIH grant NS99457
NIH grant R01NS104944
NIH grant R01MH101214
STI2030-Major Projects 2022ZD0208300

Title: Development and application of genetically encoded sensors for endocannabinoids

Authors: *R. CAI[1], A. DONG[1], S. CAI[1], K. HE[1], B. DUDOK[2], J. FARRELL[3], W. GUAN[4], L. WANG[1], B. LI[4], I. SOLTESZ[3], C. SONG[1], Y. LI[1];
[1]Peking Unversity, Beijing, China; [2]Stanford Univ., [3]Stanford Univ., Stanford, CA; [4]Cold Spring Harbor Lab., Cold Spring Harbor Lab., Cold Spg Hbr, NY

Abstract: Endocannabinoids (eCBs) are crucial retrograde neuromodulators involved in diverse biological processes. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are prominent eCBs, but their in vivo dynamics remain poorly understood due to limited probes with adequate spatiotemporal resolution. Here, we developed genetically encoded eCB sensors by inserting cpEGFP into the ICL3 of the human CB1 receptor. The first-generation sensor, $GRAB_{eCB2.0}$, displayed proper cell membrane trafficking and a robust fluorescence change at physiological eCB concentrations. Using this sensor, we monitored eCB dynamics in several biological conditions in vitro and in vivo. Furthermore, we developed $GRAB_{AEA1.2}$ and $GRAB_{2\text{-}AG1.2}$ by structure-guided protein engineering based on $GRAB_{eCB2.0}$. These sensors showed specific responses to AEA and 2-AG respectively in HEK293T cells and cultured neurons. They were able to reliably detect endogenous 2-AG and AEA signals in the DLS region of acute brain slices following electrical stimulation. Moreover, we improved the performances of these eCB sensors by point mutation screening to optimize signal amplitude and apparent affinity. We developed the next generation eCB sensors, including $GRAB_{eCB3.0}$, $GRAB_{AEA1.5}$ and $GRAB_{2\text{-}AG1.5}$, which showed improved performance and maintained the pharmacological properties. In summary, $GRAB_{eCB}$ sensors are robust probes for measuring the dynamics of eCBs under both physiological and pathophysiological conditions, providing more details about endocannabinoid mediated neuromodulation.

Disclosures: **R. Cai:** None. **A. Dong:** None. **S. Cai:** None. **K. He:** None. **B. Dudok:** None. **J. Farrell:** None. **W. Guan:** None. **L. Wang:** None. **B. Li:** None. **I. Soltesz:** None. **C. Song:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.07/WW15

Topic: I.04. Physiological Methods

Title: Development and optimization of genetically encoded sensors for oxytocin and arginine vasopressin detection

Authors: *L. GENG, H. WANG, B. LUO, T. QIAN, Y. LI;
Peking Univ., Beijing, China

Abstract: Oxytocin (OT) and Arginine vasopressin (AVP) are two neuropeptides that play important roles in both the peripheral system and the central nervous system. While OT is associated with the contraction of uterine smooth muscle and milk secretion in the peripheral, AVP is essential for regulating the osmolarity homeostasis. In the central nervous system, OT is involved in pair-bonding and AVP is important in stress behaviors. To understand their important functions, it is critical to be able to monitor OT and AVP dynamics *in vivo* with high selectivity, sensitivity and good spatiotemporal resolution. To achieve this goal, we have developed genetically encoded sensors for the detection of OT and AVP respectively based on the principle of GRAB (G protein-coupled receptor activation–based) sensors, $GRAB_{OT1.0}$ and $GRAB_{AVP0.2}$. Further optimization of the sensors' cpEGFP, GPCR backbone and linker peptide resulted in OT and AVP sensors with higher signal-to-noise ratio (SNR). Moreover, the new OT sensor shows higher selectivity to OT vs AVP. The sensors can detect relevant peptides with fiber photometry recording upon hypertonic stimulation in freely moving mice with good temporal resolution, potentially contributing to a better understanding of OT/AVP functions in both physiological and pathophysiological conditions.

Disclosures: **L. Geng:** None. **H. Wang:** None. **B. Luo:** None. **T. Qian:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.08/WW16

Topic: I.04. Physiological Methods

Support: NIH BRAIN Initiative 1U01NS120824
NIH BRAIN Initiative 1U01NS113358

Title: Next-generation genetically encoded sensors for neuropeptides with high selectivity and sensitivity

Authors: *S. FU, Y. YAN, H. WANG, H. DONG, S. XIE, J. WAN, Y. ZHAO, B. LUO, L. WANG, Y. LI;
Peking Univ., Beijing, China

Abstract: Neuropeptides play a crucial role in regulating various physiological processes such as energy balance, sleep-wake cycles, stress, and social behaviors. To understand the functions of neuropeptides, it is essential to have tools that can monitor their dynamics *in vivo* with high specificity, sensitivity, and spatiotemporal resolution. Here we developed a series of GRAB (G protein-coupled receptor activation-based) sensors for detecting somatostatin (SST), substance P (SP), orexin (OX), neuropeptide Y (NPY), neurotensin (NTS), glucagon (GCG), vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP). These fluorescent sensors use the corresponding GPCRs as the sensing module with a circular-permutated GFP as the optical reporter. This design detects the binding of specific neuropeptides at nanomolar concentration with a robust increase in fluorescence. We used these GRAB neuropeptide sensors to measure the dynamics of endogenous neuropeptide release with good selectivity and spatiotemporal resolution *ex vivo* and *in vivo*. Furthermore, the neuropeptide sensors are able to reveal endogenous peptide release. To further improve the signal-to-noise ratio and dynamic range of the sensors, we developed next-generation neuropeptide GRAB sensors with significant improvements in both response and affinity. These new sensors establish a robust toolkit for studying the function and regulation of neuropeptides.

Disclosures: S. Fu: None. **Y. Yan:** None. **H. Wang:** None. **H. Dong:** None. **S. Xie:** None. **J. Wan:** None. **Y. Zhao:** None. **B. Luo:** None. **L. Wang:** None. **Y. Li:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.09/WW17

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH RF1MH130391
NIH U01NS128537
NIH R01GM139850

Title: Application of Optogenetic Microwell Array Screening System (Opto-MASS) to identify an ultra-sensitive genetically encoded fluorescent opioid sensor

Authors: *L. TORP[1], S. WAIT[2], S. SHARMA[1], M. RAPPLEYE[1], A. BERNDT[1];
[1]Bioengineering, [2]Mol. Engin. and Sci., Univ. of Washington, Seattle, WA

Abstract: Engineering fluorescent protein indicators for endogenously and exogenously released opioids can help elucidate new mechanisms for opioid signaling and eventually lead to a better understanding of addiction and pain mechanisms in the brain. The time and resources required for mammalian-based in vitro screening and the large mutational space occupied by mutagenesis libraries designed to increase sensor specificity and selectively greatly impede the development of robust opioid sensors. The Optogenetic Microwell Array Screening System (Opto-MASS) was developed to address these challenges by presenting a high-throughput screening platform

capable of determining the functional characteristics of thousands of variants in a single day. This is achieved by physically separating unique variants into individual components on microwell arrays and selecting top-performing variants in real time. Previously Opto-MASS has successfully identified an improved opioid sensor among 23,000 linker variants, designated μMASS^{2A}. μMASS^{2A} demonstrated increased fluorescence response to 500nM (~4.6 fold) and saturating (~3.8 fold) concentrations of the synthetic endogenous opioid [D-Ala2 , N-MePhe4 , Gly-ol]- enkephalin (DAMGO) compared to the mu-opioid receptor (MOR) sensor mLight. μMASS^{2A} was also well expressed and showed increased fluorescence response to endogenous and exogenous opioids in primary rat cortical neurons. Building on the success of Opto-MASS in identifying improved opioid sensors, several efforts have been introduced to engineer a more sensitive opioid sensor. Next generation μMASS libraries were developed by introducing novel mutations in the intracellular region of μMASS^{2A}. Mutations within this region may confer additional sensitivity by decreasing coupling to downstream G-protein machinery. A new linker library was also developed to further saturate either side of the cpGFP insertion site, generating a library with 10^4 times the variation of the original libraries screened on the Opto-MASS platform. Finally, coupling this increase in variation to pre-selection using flow cytometry narrowed down the number of variants screened on the platform to only top performers, and improved the system as a whole. Taken together, Opto-MASS is a versatile high-throughput screening platform, capable of engineering sensitive opioid sensors for robust imaging in order to gain a deeper understanding of how opioids contribute to neuromodulation.

Disclosures: L. Torp: None. **S. Wait:** None. **M. Rappleye:** None. **A. Berndt:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.10/Web Only

Topic: I.04. Physiological Methods

Support: ERC StG 891959
ERC-H2020-ICT 101016787
SNSF 310030_196455
DFG 178316478-B8
NIH BRAIN Initiative U19 NS107464

Title: A new family of multicolor genetically-encoded indicators for fast, sensitive and selective in vivo imaging of norepinephrine

Authors: Z. KAGIAMPAKI[1], V. ROHNER[1], C. KISS[1], S. CURRELI[3], A. DIETER[4], M. WILHELM[1], M. HARADA[1], S. N. DUSS[5], J. DERNIC[1], M. A. BHAT[1], X. ZHOU[1], L. RAVOTTO[1], T. ZIEBARTH[6], L. M. WASIELEWSKI[6], L. SOENMEZ[6], D. BENKE[1], B. WEBER[1], J. BOHACEK[5], A. REINER[6], S. J. WIEGERT[4], T. FELLIN[3], ***T. PATRIARCHI**[2]; [2]Inst. of Pharmacol. and Toxicology, [1]Univ. of Zurich, Zurich, Switzerland; [3]Italian Inst. of

Technol., Genova, Italy; [4]Univ. of Heidelberg, Mannheim, Germany; [5]ETH Zurich, Zurich, Switzerland; [6]Ruhr Univ. Bochum, Bochum, Germany

Abstract: Norepinephrine (NE) is a pivotal neuromodulator that regulates several aspects of brain function, including alertness, wakefulness, learning and memory, among others. Genetically-encoded tools that enable the detection of NE at high-resolution in behaving animals have transformed the field and started to advance our understanding of the NE system. However, currently-available indicators suffer from low dynamic range, limited NE-selectivity, slow kinetics, limited compatibility with pharmacological compounds and are exclusively green. Here we present a new family of multicolor genetically-encoded NE indicators, named nLightG (green) and nLightR (red), that overcome these limitations. We thoroughly benchmarked these indicators and show that they exhibit enhanced sensitivity, ligand selectivity, ON and OFF kinetics, and a unique pharmacological profile compared to state-of-the-art $GRAB_{NE}$ indicators. Through in vitro, ex vivo, and in vivo experiments, we demonstrate that these indicators sensitively detect the release of endogenous norepinephrine (NE). To assess the performance of the nLightG indicator in vivo, we employed multi-site fiber photometry recordings, allowing us to simultaneously monitor optogenetically induced NE release in both the mouse locus coeruleus and hippocampus. Furthermore, utilizing two-photon imaging techniques with nLightG, we observed transient NE signals in the dorsal CA1 area of the hippocampus during locomotion and reward-related activities. The introduction of nLightG and nLightR represents an important addition to the existing suite of indicators and provides researchers with the necessary tools for comprehensive investigations into the intricate workings of the norepinephrine system.

Disclosures: Z. Kagiampaki: None. **V. Rohner:** None. **C. Kiss:** None. **S. Curreli:** None. **A. Dieter:** None. **M. Wilhelm:** None. **M. Harada:** None. **S.N. Duss:** None. **J. Dernic:** None. **M.A. Bhat:** None. **X. Zhou:** None. **L. Ravotto:** None. **T. Ziebarth:** None. **L.M. Wasielewski:** None. **L. Soenmez:** None. **D. Benke:** None. **B. Weber:** None. **J. Bohacek:** None. **A. Reiner:** None. **S.J. Wiegert:** None. **T. Fellin:** None. **T. Patriarchi:** Other; T.P. is a co-inventor on a patent application related to the technology described in this project..

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.11/WW18

Topic: I.04. Physiological Methods

Title: Genetically encoded sensor for epinephrine and norepinephrine detection

Authors: *A. PUTANSU;
Chem., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: G-protein coupled receptors' (GPCRs) role is to initiate signal transduction through response to external stimuli, placing them at a critical junction for regulation of many cell

physiological processes such as muscle contraction and cognition. Dysregulation of GPCRs and their downstream signaling is often associated with pathology, and approximately 35% of FDA approved drugs target GPCRs. Currently there is a lack of tools for researchers to study endogenous GPCR ligand localization across the brain at cellular resolution due to either limitations in spatial resolution, size of the imaging area, or GPCR selectivity. To address this need, I propose expanding a genetically encoded integrator sensor motif, **S**ingle-chain **P**rotein-based **O**piod **T**ransmission **I**ndicator **T**ool (SPOTIT), to detect other GPCR ligands. This new tool motif will be called SPOTall. I will focus on designing and optimizing SPOTall for mapping neurotransmitter catecholamines epinephrine and norepinephrine. The Beta-2 Adrenergic Receptor (B2AR)-SPOTall was designed in the Wang lab by Kayla Kroning. I will describe the effort to enhance the brightness of B2AR-SPOTall by screening linker lengths and improve endogenous ligand sensitivity using directed evolution methods. The improved B2AR-SPOTall will have more robust applications in animal models.

Disclosures: A. Putansu: None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.12/WW19

Topic: I.04. Physiological Methods

Support: The University of Michigan

Title: Designing sensors for α-melanocyte-stimulating hormone

Authors: *R. SINGER[1], J. SHEN[1], L. VAZQUEZ-RIVERA[1], W. WANG[2,3];
[2]Dept. of Chem., [3]Life Sci. Inst., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: The melanocortin peptide derived from pro-opiomelanocortin (POMC) hormone α-melanocyte-stimulating hormone (α-MSH) regulates a variety of biological processes including, melanin production, hypothalamic-pituitary-adrenal axis, exocrine gland function, and energy homeostasis. Each distinct biological function is a result of receptor-subtype specific interactions within the melanocortin signaling system. Furthermore, additional signaling partners, coreceptors, and alternate endogenous ligands for melanocortin receptors add additional complexity to the phenotypic responses to α-MSH stimulation. One such receptor for α-MSH, melanocortin-4 (MC4R), helped show the role of the central melanocortin system in energy homeostasis, nutrient uptake, feeding, and body weight. In fact, 2-5% of early onset syndromic obesity cases have been shown to be caused by mutations to the MC4R receptor, making MC4R mutations the most common cause for this condition. However, further study of α-MSH microcircuitry is required to understand the complex environment of POMC neurons. Optogenetic and chemogenetic methods have been employed to study the role of AgRP (an antagonist of MC4R) in POMC neurons. However, this has yet to be done with α-MSH. In this

poster, I will show preliminary designs for α-MSH sensors by designing binders for the hormone.

Disclosures: R. Singer: None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.13/WW20

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Brain Canada
NSERC

Title: Engineering a genetically encoded fluorescent D-serine sensor

Authors: *R. DALANGIN[1], L. PAQUET[1,2], A. SCHOHL[5], D. FOUBERT[5], A. BARBEAU[1], A. G. GODIN[1,3], E. S. RUTHAZER[5], M.-E. PAQUET[1,4];
[1]CERVO Brain Res. Ctr., Quebec, QC, Canada; [2]Fac. of Sci. and Engin., [3]Dept. of Psychiatry and Neurosci., [4]Dept. of Microbiology, Biochem. and Bioinformatics, Univ. Laval, Quebec, QC, Canada; [5]Montreal Neurolog. Institute-Hospital, McGill Univ., Montreal, QC, Canada

Abstract: The last two decades have seen a growing interest in the role of D-amino acids within the nervous system. In particular, D-serine is now recognized as a key neuromodulator in its role as a more potent co-agonist than glycine for N-methyl-D-aspartate receptors (NMDARs), which are widely recognized as the key receptor responsible for synaptic plasticity. Accordingly, aberrations in D-serine signalling have been consistently associated with several pathological conditions, including schizophrenia, Alzheimer's disease and epilepsy. However, despite our understanding of D-serine's role in the nervous system, the molecular mechanisms that govern its dynamics remain unclear, with recent works challenging its role as a gliotransmitter. Thus, a more thorough understanding of D-serine dynamics is necessary to properly understand its role in both healthy and disease states. To address these gaps in knowledge, tools with the requisite spatiotemporal resolution, such as genetically encoded fluorescent protein-based indicators, are necessary to monitor D-serine dynamics. To date, the only genetically encoded indicator for D-serine is a FRET-based indicator, called DserFS, based on a bacterial periplasmic binding protein (PBP). PBPs are ideal scaffolds for sensor engineering because they are orthogonal to neurons, offer large changes in fluorescence in response to ligand binding and can be targeted to arbitrary cellular compartments. However, DserFS shows a limited dynamic range relative to single fluorescent protein-based indicators and requires exogenous addition for imaging in brain slices. Here we present our work on engineering a genetically encoded single fluorescent protein-based indicator for D-serine from DserFS. Indeed, preliminary results indicate that our D-serine sensor shows large fluorescence changes with micromolar affinities and good membrane localization.

We anticipate that our new D-serine indicator will open new avenues for investigating D-serine dynamics within the nervous system.

Disclosures: **R. Dalangin:** None. **L. Paquet:** None. **A. Schohl:** None. **D. Foubert:** None. **A. Barbeau:** None. **A.G. Godin:** None. **E.S. Ruthazer:** None. **M. Paquet:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.14/WW21

Topic: I.04. Physiological Methods

Title: Development and Validation of Stable In Vivo Glutamate Sensor MEA with Nano-Platinum

Authors: *B. WONG[1,2], E. ROBBINS[1], M. PWINT[1], B. WU[1], S. SALAVATIAN[2], A. MAHAJAN[1,2], X. CUI[1];
[1]Bioengineering, [2]Anesthesiol. and Perioperative Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Glutamate (GLU) is a major neurotransmitter in the nervous system and an important biomarker to track excitatory activity, especially in traumatic brain injury and ischemia research. Enzymatic biosensors are the current state of the art for detecting GLU *in vivo* at a sub-second time resolution. GLU sensors typically are made by crosslinking GLU oxidase (GluOx) onto the electrode. However, chronic GLU sensing has not been possible due to the instability of such sensors *in vivo*. *In vivo,* enzyme degradation due to H_2O_2 most directly impacts the loss of GLU sensitivity. Here, we demonstrate long-term stability of GLU sensors optimized with nano-platinum (nanoPt). *In vitro,* platinum wires with electrochemically deposited nanoPt prior to enzyme coating were incubated in groups in PBS, 10mM GLU (high), 100μM GLU (low), 10mM H_2O_2 (high), or 100μM H_2O_2 (low) for up to 3 weeks checking sensitivity regularly compared to smooth wires without nanoPt. These conditions investigate failure due to enzyme degradation that will be encountered *in vivo*: H_2O_2 both present and generated from the enzymatic GLU reaction. GLU sensors with nanoPt maintained sensitivity up to 3 weeks compared to smooth sensors, which failed after 1 week. After 3 weeks, the GLU sensors with nanoPt had no loss of sensitivity while incubated in PBS, high and low GLU, and low H_2O_2. Sensitivity was only significantly reduced while incubated in the high concentration of H_2O_2 (Initial: 0.246 ± 0.017 nA/μM; Post H_2O_2 incubation: 0.0304 ± 0.006 nA/μM; $p<0.0001$). This indicates that only a concentration of H_2O_2 much higher than what is found *in vivo* is able to significantly degrade the enzyme. Thus, our nanoPt GLU sensors can maintain sensitivity while exposed to expected *in vivo* GLU and H_2O_2 concentrations for long-term studies in the nervous system. Currently, our methodology has been translated to microelectrode arrays for detecting GLU *in vivo* in pig spinal cord and rat striatum, where sensitivity was maintained for 7 days without degradation contrast to smooth sensors. Thus, long-term studies involving the detection of GLU can be optimized with nanoPt. The rough surface of nanoPt increases electrode surface

area and creates additional anchor points for the GluOx solution to tether. Introducing a nanoPt deposition step prior to coating the enzyme increases the longevity of the GLU sensor. For the future, we are exploring platinum nanoparticles loaded with GluOx to electrochemically deposit rather than manually drop-casting the enzyme onto our electrodes. This methodology can be applied to other enzymatic sensors dependent on the oxidation of H_2O_2, such as those for lactate and acetylcholine.

Disclosures: **B. Wong:** None. **E. Robbins:** None. **M. Pwint:** None. **B. Wu:** None. **S. Salavatian:** None. **A. Mahajan:** None. **X. Cui:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.15/WW22

Topic: I.04. Physiological Methods

Support: NIH Grant RO1NS123424-10
NIH Grant T32 GM008804

Title: Simultaneous monitoring of phasic and tonic dopamine release: a platform for long-term studies in awake and behaving animals

Authors: *N. C. WEINTRAUB, M. L. HEIEN;
Chem. and Biochem., Univ. of Arizona, Tucson, AZ

Abstract: Dopamine release in the striatum is integral to motor control, reward-guided learning, and decision making. Long-term dysfunction in dopamine signaling is associated with a range of neurological disorders, such as Parkinson's disease, depression, and addiction. Dopamine neurotransmission has two signaling modalities that occur over different timescales, making concurrent monitoring technically challenging. Phasic signaling is a rapid burst fire, occurring on the order of milliseconds to seconds, in response to salient stimuli, while tonic signaling is a continuous, steady-state release establishing the baseline level of dopamine activity and which changes over the course of minutes to hours. Another challenge for long-term electrochemical measurements is a loss of sensitivity due to biofouling, which is the term given to describe the cascade of immune responses that occur after electrodes are implanted in the brain, which degrades voltammetric performance obscuring dopamine detection. This work presents a new platform to simultaneously measure phasic and tonic dopamine release while mitigating the effects of biofouling for long-term studies in freely moving animals. Fast-scan cyclic voltammetry (FSCV) is an electrochemical technique which is used to monitor phasic dopamine release through oxidation and reduction of dopamine at a carbon-fiber microelectrode surface. Fast-scan controlled adsorption voltammetry (FSCAV) is a complimentary technique which allows for quantification of tonic (i.e. basal) levels of extracellular dopamine using the same experimental design. To perform simultaneous FSCV-FSCAV, we developed a single board

potentiostat capable of outputting multiple waveforms, new custom-built inhouse software, and a novel multichannel headstage based on a three-electrode design using a common Ag/AgCl reference, platinum counter, and multiple carbon fiber working electrodes. The three-electrode design mitigates changes in the electrode signal due to biofouling occurring at the electrode surface, preserving dopamine sensitivity over long term recording. This platform lays the foundation for future research into the complex dynamics of dopamine release and how both modalities of dopamine signaling correlate with behavioral and physiological responses, all while reducing the time and number of animal subjects required for future studies, contributing to more ethical and efficient research practices.

Disclosures: **N.C. Weintraub:** None. **M.L. Heien:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.16/WW23

Topic: I.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research JP20J14411
Grant-in-Aid for Scientific Research JP16J06838
Grant-in-Aid for Scientific Research JP19K23820
Grant-in-Aid for Scientific Research JP20K16118
Grant-in-Aid for Scientific Research JP17K08529
Grant-in-Aid for Scientific Research JP20H00575
Grant-in-Aid for Scientific Research JP20H04121
Grant-in-Aid for Scientific Research JP20H04765
Grant-in-Aid for Scientific Research JP20H04836
Grant-in-Aid for Scientific Research JP16K01922
Grant-in-Aid for Scientific Research JP18H04832
Kowa Life Science Foundation
Uehara Memorial Foundation
Research Foundation for Opto-Science and Technology
Yamaguchi Educational and Scholarship Foundation

Title: Intracellular cGMP dynamics during incretin secretion revealed by red fluorescent protein-based cGMP sensor

Authors: *Y. OSUGA[1], M. TAKIZAWA[1], R. ISHIDA[1], M. MITA[1], K. HARADA[1], T. KITAGUCHI[2], T. TSUBOI[1];
[1]Tokyo Univ., Tokyo, Japan; [2]Tokyo Inst. of Technol., Kanagawa, Japan

Abstract: Glucagon-like peptide-1 (GLP-1) is secreted by enteroendocrine cells in the small intestine when they sense nutrients in the intestinal lumen. Although intracellular Ca^{2+} and

cAMP are both involved in GLP-1 secretion, the relationship between GLP-1 secretion and cGMP remains unclear.
In this study, we developed a cGMP sensor called Red cGull employing a red fluorescent protein, mApple. Red cGull consists of two split segments of mApple with the cGMP-binding domain PDE5α inserted between them. Upon treatment with cGMP, Red cGull showed a maximal 6.7-fold increase in fluorescence intensity, and the response was shown in a dose dependent manner.
Using live-cell imaging analysis, we were able to simultaneously detect intracellular cGMP and Ca^{2+} dynamics in Red cGull-expressing HeLa cells. Furthermore, we co-expressed Red cGull with photoactivatable soluble guanylate cyclase in the cells. After exposure of the cells to blue light, the fluorescence intensity of Red cGull increased, indicating that it can be used in conjunction with an optogenetic tool.
The GLP-1 secretagogue L-arginine was administered to Red cGull expressing GLUTag cells, a mouse small intestinal enteroendocrine cell line. We found that L-arginine treatment increased intracellular cGMP levels. This increase in cGMP levels was inhibited by NOS inhibitor, suggesting that increased intracellular cGMP levels are involved in GLP-1 secretion.
We propose that Red cGull will facilitate the understanding of enteroendocrine cell signaling not only in relation to cGMP, but also in relation to other signaling molecules that regulate GLP-1 secretion. In addition, Red cGull will be applied to other excitable cells such as neurons, smooth muscle and endocrine cells.

Disclosures: **Y. Osuga:** None. **M. Takizawa:** None. **R. Ishida:** None. **M. Mita:** None. **K. Harada:** None. **T. Kitaguchi:** None. **T. Tsuboi:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.17

Topic: I.04. Physiological Methods

Support: NIH grant UF1NS107705
NIH grant U01NS128665
NiH grant U01NS103517
NIH U01NS090565
DARPA N6600117C4012
DARPA N6600119C4020

Title: Engineering of genetically encoded voltage indicators for two-photon imaging

Authors: *J. PLATISA[1,2], P. F. O'BRIEN[1], R. F. O'BRIEN[1], X. YE[3], A. AHRENS[3], C. LIU[3], A. CHEN[3], L. TIAN[3], J. L. CHEN[3], V. A. PIERIBONE[2];
[1]The John B Pierce Lab., New Haven, CT; [2]Yale Univ., New Haven, CT; [3]Boston Univ., Boston, MA

Abstract: Understanding information flow and processing across neural networks is tied up with understanding the electrical events of the neuronal plasma membrane. Two-photon voltage imaging with genetically encoded indicators (2p GEVIs) is an emerging approach to non-invasively record neuronal electrical transients with cellular resolution and from deeper brain structures. We recently developed a set of novel tools, including a positive-going VSD-based voltage indicator with improved spike detection (SpikeyGi2), a kilohertz two-photon microscope ('SMURF'), and a self-supervised denoising algorithm (DeepVID) for inferring fluorescence from shot-noise-limited signals. This multi-component system allows for sustainable (>1 hour), high-speed (~1kHz), and deep-tissue imaging of large neuronal populations (>100 neurons) in awake-behaving mice. However, reliable detection of subthreshold events requires 2p GEVIs with higher sensitivity and photostability. The opsin-based GEVIs show sub-millisecond kinetics, high sensitivity, and better optical properties than VSD GEVIs. Unfortunately, reported voltage sensitivity under multiphoton excitation for this class of GEVIs has been limited to none. Here, we combined high-throughput protein engineering and a 2p-based screening system to investigate the voltage sensitivity of the novel best-in-class opsin-GEVIs for multiphoton imaging.

Disclosures: **J. Platisa:** None. **P.F. O'Brien:** None. **R.F. O'Brien:** None. **X. Ye:** None. **A. Ahrens:** None. **C. Liu:** None. **A. Chen:** None. **L. Tian:** None. **J.L. Chen:** None. **V.A. Pieribone:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.18/WW24

Topic: I.04. Physiological Methods

Support: NSF Grant 1707359
NIH R01 1R01EB027145
NIH U01 U01NS113294
NIH U01 NS118288
NSF Grant 1935265
Welch Foundation Grant Q-2016-20190330
Klingenstein-Simons Fellowship Award in Neuroscience
F.S.-P. is supported by the McNair Medical Institute

Title: Enhancing green negative going genetically encoded voltage indicators for two-photon voltage imaging in vivo.

Authors: *M. LAND[1], X. LU[5], S. YANG[6], E. ZAABOUT[2], Z. LIU[2], X. DONG[2], A. MCDONALD[2], Y. GOU[2], V. VILLETTE[9], S. LAI[2], C. CAI[10], E. FROUDARAKIS[2,11], N. ZHOU[2], S. PATEL[2], C. SMITH[2,3], P. BIZOUARD[9], J. BRADLEY[9], A. GIOVANNUCCI[10,12], A. TOLIAS[2,7,3], J. REIMER[2,3], S. DIEUDONNÉ[9], F. ST-PIERRE[2,8,7,4];

[2]Neurosci., [1]Baylor Col. of Med., HOUSTON, TX; [3]Ctr. for Neurosci. and Artificial Intelligence, [4]Dept. of Biochem. and Mol. Biol., Baylor Col. of Med., Houston, TX; [5]Systems, Synthetic, and Physical Biol. Program, [6]Dept. of Chem. and Biomolecular Engin., Rice Univ., HOUSTON, TX; [7]Dept. of Electrical and Computer Engin., [8]Systems, Synthetic, and Physical Biol. Program, Rice Univ., Houston, TX; [9]Inst. de Biologie de l'École Normale Supérieure (IBENS), École Normale Supérieure, CNRS, INSERM, PSL Res. Univ., Paris, France; [10]Dept. of Biomed. Engin., Univ. of North Carolina at Chapel Hill and North Carolina State Univ., Chapel Hill, NC; [11]Inst. of Mol. Biol. and Biotech., Fndn. for Res. and Technol. Hellas, Heraklion 70013, Greece; [12]UNC Neurosci. Ctr., Chapel Hill, NC

Abstract: Monitoring neural activity on the millisecond time scale in genetically defined cells is a goal of neuroscience, but remains challenging to perform *in vivo*. A limitation of current techniques is not being able to quantitatively monitor neuronal electrical (voltage) dynamics with single-cell or subcellular resolution from large and genetically defined populations of neurons. Genetically Encoded Voltage Indicators (GEVIs) are a promising tool to bridge this gap. GEVIs are fluorescence-emitting protein sensors that report membrane potential (voltage) dynamics as changes in brightness. However, current GEVIs suffer from low sensitivity to voltage changes and are relatively dim, limiting our ability to image smaller transients and voltage dynamics in deeper layers of the cortex. GEVIs also suffer from poor photostability, severely limiting their ability to be used for behaviorally relevant time scale. Here, we report novel (including unpublished) GEVIs optimized for long-term *in vivo* imaging under two-photon illumination, a method of choice for deep-tissue imaging. Our sensor design is based on the published ASAP family of sensors in which a voltage-sensitive domain is coupled to an extracellular circularly permuted GFP. Using high-throughput multi-parametric screening under two-photon illumination we tested mutations at amino acid positions that are structurally and evolutionary conserved in ASAP. Using this multi-parametric approach we were able to identify our champion sensor JEDI-2P. This sensor is brighter and has improved photostability, greater sensitivity and faster kinetics than previous sensors in several *in vivo* models. Using both resonant-scanning and ULoVE random-access microscopy, we show that JEDI-2P can detect voltage dynamics of individual cortical neurons in awake behaving mice for more than 30 min. Additionally, JEDI-2P can be used to robustly detect spikes at depths exceeding 400 μm in pairs of neurons. Further screening has identified a new generation of sensors with improved response amplitude and larger dynamic range to subthreshold voltage changes compared with JEDI-2P. In addition, their slower post-spike repolarization kinetics is expected to improve action potential detection with sub-kilohertz optical recording acquisition speeds, without compromising the detection of spikes within a burst. These sensors have been cloned into a variety of vectors and are available for beta testing upon request as part of our efforts to bring new tools to the neuroscience community.

Disclosures: **M. Land:** None. **X. Lu:** None. **S. Yang:** None. **E. Zaabout:** None. **Z. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US patent for the SPOTlight screening method. **X. Dong:** None. **A. McDonald:** None. **Y. Gou:** None. **V. Villette:** None. **S. Lai:** None. **C. Cai:** None. **E. Froudarakis:** None. **N. Zhou:** None. **S. Patel:** None. **C. Smith:** None. **P. Bizouard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership shares in Karthala Systems, a commercial supplier of RAMP microscopes. **J. Bradley:** None. **A. Giovannucci:** None. **A.**

Tolias: None. **J. Reimer:** None. **S. Dieudonné:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ownership shares in Karthala Systems, a commercial supplier of RAMP microscopes. **F. St-Pierre:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US patent for a voltage sensor design #US9606100 B2 and SPOTlight screening method.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.19/WW25

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF NeuroNex 1707352
NIH R01MH124811

Title: Use of voltage-gated calcium channels as bioluminescence-based effectors to monitor neural activity

Authors: *A. ANDRADE[1], K. M. WEBSTER[1], U. HOCHGESCHWENDER[4], N. C. SHANER[5], C. I. MOORE[2], D. LIPSCOMBE[3];
[2]Neurosci., [3]Carney Inst. for Brain Sci., [1]Brown Univ., Providence, RI; [4]Central Michigan Univ., Mount Pleasant, MI; [5]UCSD, San Diego, CA

Abstract: Calcium is essential for cellular processes including neural firing and transmitter release. Fittingly, myriad calcium channels and regulators are present in distinct cellular compartments, and calcium indicators are widely used as a surrogate for activity. However, these indicators cannot track activity in specific channels. To fill this need, we are developing a novel method named 'LuMiPorins' (LMPs) by fusing proteins of voltage-gated calcium channels (Ca_Vs) to bioluminescent genetically encoded calcium indicators (BL-GECIs). We initially targeted $Ca_V2.2$ and $Ca_V3.3$ channels, which have distinct voltage-dependent properties and localize to different subregions of neurons. $Ca_V2.2$ channels activate in response to relatively large depolarizations; they are expressed throughout neurons but target to presynaptic active zones where they control transmitter release. $Ca_V3.3$ channels activate with relatively small depolarizations close to the resting membrane potential, and they predominantly localize to dendrites and soma where they drive pacemaking and contribute to rebound bursting. After fusing a BL-GECI (GeNL_Ca520) to the C-terminus of these Ca_V channels, the resulting $Ca_V2.2$-LMP and $Ca_V3.3$-LMP fusion proteins retained channel function in mammalian tsA201 cells similar to wildtype (current density in pA/pF: $Ca_V2.2$-WT = 41.8 ± 12.6, n = 6, $Ca_V2.2$-LMP = 38.25 ± 7.5, n = 6; $Ca_V3.3$-WT = 65.7 ± 15.4, n = 5, $Ca_V3.3$-LMP = 52.3 ± 8.2, n = 7). $Ca_V2.2$-LMP and $Ca_V3.3$-LMP expressed in tsA201 cells emit light above background in the presence of the luciferin coelenterazine-*h* (hCTZ) (luminescence in relative light units: $Ca_V2.2$-LMP/$Ca_V\beta_3$/$Ca_V\alpha_2\delta$-1 = 650.6 ± 48.5, n = 4; $Ca_V3.3$-LMP = 869.8 ± 32.2, n = 7). For $Ca_V3.3$-

LMP and Cav2.2-LMP, the bioluminescence signal was sensitive to intracellular calcium, as it was reduced by intracellular BAPTA. For Cav3.3-LMP, the bioluminescence signal increased in the presence of the calcium ionophore ionomycin. The calcium sensitivity of the bioluminescence signal for Cav3.3-LMP was dependent on the hCTZ concentration with optimal signal to background at sub-micromolar concentrations. These new molecules can be used to monitor calcium-dependent neural activity. Further, the light generated by GeNL_Ca520 fused to Cav channels can, in future applications, be used to drive effector molecules like opsins to provide real time and channel-specific control of neural activity.

Disclosures: A. Andrade: None. **K.M. Webster:** None. **U. Hochgeschwender:** None. **N.C. Shaner:** None. **C.I. Moore:** None. **D. Lipscombe:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.20/WW26

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Howard Hughes Medical Institute
École normale supérieure

Title: Microbial rhodopsin derived two-photon compatible voltage sensors toolbox

Authors: A. ABDELFATTAH[1,2], V. VILLETTE[3], ***S. YANG**[2], R. VALENTI[4], J. MACKLIN[2], J. BRADLEY[3], S. DIEUDONNÉ[3], E. R. SCHREITER[2];
[1]Brown Univ., Providence, RI; [2]Janelia Res. Campus, Ashburn, VA; [3]École normale supérieure Paris, Paris, France; [4]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Genetically encoded fluorescent voltage indicator proteins (GEVIs) allow observation of voltage dynamics in the brains of model organisms with cell-type specificity. Microbial rhodopsin derived GEVIs display favorable high voltage sensitivity and fast kinetics, but rhodopsin FRET GEVIs have been considered incompatible with 2-photon microscopes for deeper brain imaging. Through exploring the diversity of microbial rhodopsins, we report novel rhodopsin-based GEVIs that are compatible with 2-photon excitation and enable monitoring of sub- and suprathreshold activity in layer II/III of the visual cortex in awake behaving mice. Moreover, through optimization of imaging conditions, we further discovered that rhodopsin-based GEVIs are generally compatible with 2-photon excitation, displaying almost identical voltage sensitivity and kinetic properties under 1-photon and 2-photon illumination.

Disclosures: A. Abdelfattah: None. **V. Villette:** None. **S. Yang:** None. **R. Valenti:** None. **J. Macklin:** None. **J. Bradley:** None. **S. Dieudonné:** None. **E.R. Schreiter:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.21/WW27

Topic: I.04. Physiological Methods

Support: NIH R00 - 5R00NS107639-04
Michael J. Fox Foundation (MJFF) Aligning Science Across Parkinson's (ASAP) - ASAP-020-519

Title: Improved cranial chamber implants for high-density neurochemical and electrical neural recording in nonhuman primates

Authors: *J. CHOI[1], U. AMJAD[1], R. MURRAY[1], R. SHRIVASTAV[1], B. GOODELL[2], C. M. GRAY[2], H. N. SCHWERDT[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Gray Matter Res. LLC, Bozeman, MT

Abstract: Chronic cranial chamber implants for nonhuman primates have enabled modular electrophysiological recording, stimulation, and pharmacological manipulations of large-scale neural activity in cortical and subcortical brain regions (Feingold et al. 2012; Dotson et. al. 2015). Recently, these systems have also been adopted for providing chronic neurochemical recording functions using fast-scan cyclic voltammetry (FSCV) with an array of moveable carbon fiber (CF) electrodes (CFEs) (Schwerdt et al. 2017). Here, we designed a chamber to improve the longevity of targeted neurochemical recording functions by enhancing barriers against bacterial contamination. Bacterial contamination has posed a major challenge for chronic chamber implants as they lead to infections that can spread within the bone, and even inside the brain itself, leading to premature termination of recording experiments. These infections arise because tissue overlying the brain become exposed to external bacterial contaminants due to poor sealing of the chamber. Another channel for infection is the gap between the chamber and the bone due to tissue growth or channels created when acrylic cement is used. Commercial chamber implants (e.g., Gray Matter Research) provide hermetic sealing and have been used for chronic electrophysiological recording with stiff Pt/Ir microelectrodes. However, these systems are not compatible with fragile and flexible CF (7 μm diameter)-tipped electrodes used for FSCV-based neurochemical recording, which require guide tubes to traverse safely into the dura mater and to targeted subcortical brain structures (e.g., striatum). Without hermetic sealing, the chamber must be rinsed and disinfected semi-daily to minimize accumulation of bacteria. This cleaning becomes difficult with increasing numbers of electrodes that occlude areas within the chamber where bacteria may accumulate. We designed a chamber that implements osseo-integrating materials (polyether ketone ketone, PEKK) and design features (rubber gaskets and sealed chamber and grid ports) to enhance sealing of the chamber and protect against external bacteria. We used rubber gaskets between chamber components to create a hermetic seal. We observed bone growth across our PEKK interface, which may help seal the chamber and prevent colonization of bacteria between the chamber and bone. We also continue to record negative culture results as sampled from inside our chamber from a sealed sampling port. Future work

will evaluate the longevity of functional neurochemical recordings as well as the maintenance of sterility (i.e., negative culture results) within the chamber.

Disclosures: **J. Choi:** None. **U. Amjad:** None. **R. Murray:** None. **R. Shrivastav:** None. **B. Goodell:** A. Employment/Salary (full or part-time):; Gray Matter Research LLC. **C.M. Gray:** A. Employment/Salary (full or part-time):; Gray Matter Research LLC. **H.N. Schwerdt:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.22/WW28

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI funding

Title: Integrating EASI-FISH and Large Field of View Calcium Imaging for Multi-modal Characterization of Neuronal types during learning

Authors: *Y. WANG, W. SUN, N. SPRUSTON;
Janelia Res. Campus, Ashburn, VA

Abstract: For a holistic understanding of neuronal function and its implication in cognition and behavior, it is crucial to link the functional properties and molecular cell types of neurons. To accomplish this, we need an effective approach that allows for simultaneous and large-scale examination of both these aspects in the same brain tissue. To enable the identification and classification of different molecularly defined neuronal types, we have previously developed a method, called Expansion-Assisted Iterative Fluorescence *In Situ* Hybridization (EASI-FISH) (Wang et al., 2021). EASI-FISH is a spatial gene expression profiling method optimized for large-scale, thick brain tissues. Previously, we integrated EASI-FISH with retrograde labeling to reveal the diversity of molecular neuronal types and their axonal projections in the central amygdala (Wang et al., 2023).
In this study, we combine EASI-FISH and large-scale functional imaging using a two-photon random-access mesoscope. This approach provides a large field of view, enabling simultaneous recording of activity from thousands to tens of thousands of neurons. We developed a workflow to recover the *in vivo* imaged tissue volume for EASI-FISH, which enables correlation of cell type information with neuronal activity across a large field of view. To validate our approach, we conducted a preliminary investigation of neuronal activity in CA1 and subiculum as mice learned a linear two-alternative choice task (L2AC) in a virtual reality environment. After functional imaging, we were able to extract the brain tissue and identify the same neurons and label them using EASI-FISH. The integration of these two approaches opens up new avenues for neuroscience research, promising insights into the function of molecularly defined cell types in animals learning a complex behavioral task.
References

Wang, Y., Eddison, M., Fleishman, G., Weigert, M., Xu, S., Wang, T., Rokicki, K., Goina, C., Henry, F. E., Lemire, A. L., Schmidt, U., Yang, H., Svoboda, K., Myers, E. W., Saalfeld, S., Korff, W., Sternson, S. M., & Tillberg, P. W. (2021). EASI-FISH for thick tissue defines lateral hypothalamus spatio-molecular organization. *Cell*, *184*(26), 6361-6377 e6324. https://doi.org/10.1016/j.cell.2021.11.024
Wang, Y., Krabbe, S., Eddison, M., Henry, F. E., Fleishman, G., Lemire, A. L., Wang, L., Korff, W., Tillberg, P. W., Luthi, A., & Sternson, S. M. (2023). Multimodal mapping of cell types and projections in the central nucleus of the amygdala. *Elife*, *12*. https://doi.org/10.7554/eLife.84262

Disclosures: Y. Wang: None. **W. Sun:** None. **N. Spruston:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.23/WW29

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: HHMI

Title: Optimization of genetically encoded functional indicators for in vivo imaging: GENIE Project Team updates

Authors: *G. TURNER[1], J. P. HASSEMAN[1], A. AGGARWAL[2], B. J. ARTHUR[3], J. D. COX[1], H. FARRANTS[5], C. GUO[1], V. JAYARAMAN[5], I. KOLB[1], W. KORFF[1], A. K. LEE[1], J. LEE[6], L. LOOGER[4], J. S. MARVIN[7], M. PACHITARIU[3], K. PODGORSKI[8], D. REEP[1], M. ROZSA[1], E. R. SCHREITER[9], N. SPRUSTON[1], K. SVOBODA[1], A. G. TEBO[1], A. TSANG[1], G. TSEGAYE[1], T. WANG[1], J. ZHENG[1], J. ZHENG[1], Y. ZHANG[1];
[1]Janelia Res. Campus, Ashburn, VA; [2]Neural Dynamics, Allen Inst. for Brain Sci., Seattle, WA; [3]Janelia Res. Campus, [4]Howard Hughes Med. Inst., Ashburn, VA; [5]Janelia Res. Campus, HHMI, Ashburn, VA; [6]HHMI / Janelia Res. Campus, Ashburn, VA; [7]Janelia Farms Res. Ctr., Ashburn, VA; [8]Allen Inst. for Brain Dynamics, Seattle, WA; [9]Howard Hughes Med. Institute, Janelia Farm Res. Campus, Ashburn, VA

Abstract: GENIE is a Janelia Project Team that uses systematic mutagenesis and screening in primary neuronal culture to optimize genetically encoded sensors for neuronal activity. Our pipeline extends from biochemical characterization to in vivo validation with imaging experiments in mice, zebrafish and fruit flies. We will present the latest results of optimizing a set of genetically encoded indicators for voltage, calcium and neurotransmitter release.
WHaloCaMP: Is a hybrid chemigenetic calcium indicator. It is composed of a genetically encoded calcium-sensing domain that quenches a HaloTag-attached synthetic dye. Calcium binding relieves the quenching, resulting in a positive-signaling indicator. This sensor enables imaging at near-infrared wavelengths, depending on the choice of the synthetic dye from the JF family. Our screening pipeline identified variants with 2-5x increased sensitivity to single action

potentials, whose performance will now be tested in vivo.
iGluSnFR4: New variants of this indicator of glutamate release have higher sensitivity and diverse kinetic properties. Spontaneous synaptic release events (optical minis) in cultured neurons exhibit up to 5-fold improved signal:noise relative to the recently published iGluSnFR3 [1].
iGABASnFR2: The previous generation of this indicator was the first sensor to detect the inhibitory transmitter GABA [2]. Here we report an improved variant that has a dynamic range of 55% fluorescence change, a 2.5x improvement.
jGCaMP8: Our latest suite of genetically encoded calcium indicators is composed of three variants: **s**ensitive, **f**ast and **m**edium [3]. The f and m variants exhibit half rise times of only 7 msec, while the s variant has a 1 action potential sensitivity index (d′) of 35.
We have created and characterized transgenic mice for jGCaMP8m and 8s. A tetO-jGCaMP8s x CaMKIIa-tetTA mouse had similarly fast kinetics and sensitivity to viral expression. In parallel, we generated three knock-in strains at the TIGRE locus that co-express jGCaMP8s (or 8m) and tetTA in a Cre-dependent manner. These third generation TIGRE lines enable targeting of genetically and anatomically defined neuronal subpopulations via Cre dependence, while tetTA enables transcriptional amplification of the GCaMP indicator. Lines have been deposited at Jackson Laboratory, and can be obtained from Janelia in the interim.
[1] Aggarwal, A. et al. Nat. Methods 1-10 (2023)[2] Marvin, J. S. et al. Nat. Methods 16, 763-770 (2019)[3] Zhang, Y. et al. Nature 615, 884-891 (2023)

Disclosures: **G. Turner:** None. **J.P. Hasseman:** None. **A. Aggarwal:** None. **B.J. Arthur:** None. **J.D. Cox:** None. **H. Farrants:** None. **C. Guo:** None. **V. Jayaraman:** None. **I. Kolb:** None. **W. Korff:** None. **A.K. Lee:** None. **J. Lee:** None. **L. Looger:** None. **J.S. Marvin:** None. **M. Pachitariu:** None. **K. Podgorski:** None. **D. Reep:** None. **M. Rozsa:** None. **E.R. Schreiter:** None. **N. Spruston:** None. **K. Svoboda:** None. **A.G. Tebo:** None. **A. Tsang:** None. **G. Tsegaye:** None. **T. Wang:** None. **J. Zheng:** None. **J. Zheng:** None. **Y. Zhang:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.24/WW30

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Soma-targeted GECI constructs for Intracerebral or Intravenous administration, including jGCaMP8, NEMO and eGCaMP constructs

Authors: *S. GRØDEM[1], M. FYHN[3], K. K. LENSJØ[1], F. ROGGE[1], G. H. VATNE[1], I. NYMOEN[2];
[1]Univ. of Oslo, Oslo, Norway; [2]Univ. of Oslo, OSLO, Norway; [3]Dept. of Biosci., Oslo, Norway

Abstract: Genetically encoded Ca2+ indicators (GECIs) are widely used to measure neural activity. Here, we explore the use of systemically administered PHP.eB AAVs for brain-wide

expression of GECIs and compare the expression properties to intracerebrally injected AAVs in male mice. We show that systemic administration is a promising strategy for imaging neural activity. Next, we establish the use of EE-RR- (soma) and RPL10a (Ribo) soma-targeting peptides with the latest jGCaMP and show that EE-RR-tagged jGCaMP8 gives rise to strong expression but limited soma-targeting. In contrast, Ribo-tagged jGCaMP8 lacks neuropil signal, but the expression rate is reduced. To combat this, we modified the linker region of the Ribo-tag (RiboL1-). RiboL1-jGCaMP8 expresses faster than Ribo-jGCaMP8 but remains too dim for reliable use with systemic virus administration. However, intracerebral injections of the RiboL1-tagged jGCaMP8 constructs provide strong Ca2+ signals devoid of neuropil contamination, with remarkable labeling density. We also benchmark the recently developed, mNeonGreen based GECI NEMO, as well as eGCaMP2+, relative to jGCaMP8, and introduce soma-targeted versions of these sensors.

Disclosures: S. Grødem: None. **M. Fyhn:** None. **K.K. Lensjø:** None. **F. Rogge:** None. **G.H. Vatne:** None. **I. Nymoen:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.25/WW31

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: U19NS1237190

Title: Engineering a splitable fluorescent biosensor for circuit-specific glutamate transient at synapses

Authors: *Y. JIN[1,2], R. LIANG[2], R. G. NATAN[4], B. LYU[5], J. SUN[2], J. CHANDRA[3], S. ZHANG[6], C. K. KIM[3], H.-J. CHENG[7], K. MURRAY[8], M. MORALES[6], G. YU[5], N. JI[4], L. TIAN[2];
[2]Biochem. and Mol. Med., [1]Univ. of California, Davis, Davis, CA; [3]Ctr. for Neurosci., Univ. of California, Davis, DAVIS, CA; [4]Physics and MCB, Univ. of California, Berkeley, Berkeley, CA; [5]Bradley Dept. of Electrical and Computer Engin., Virginia Tech., Blacksburg, VA; [6]IRP, NIDA, NIH, Baltimore, MD; [7]Inst. of Mol. Biol., Academia Sinica, Nankang, Taiwan; [8]Dept. of Psychiatry and Behavioral Sci., Univ. California Davis, Davis, CA

Abstract: Glutamate is a key neurotransmitter in the brain that conducts synaptic transmission and regulates plasticity. Acting as a functional unit for learning and memory, synaptic communication through glutamate undergoes changes from internal and external stimuli. Many molecular and optical tools now allow us to piece nanoscale connectome and synaptome together to understand neural function and diseases, but imaging synaptic glutamate in circuit-defined connections hasn’t been possible. Based on an evolving series of genetically encoded intensimetric fluorescent biosensors for glutamate, iGluSnFR, here we develop a splitable

synaptic iGluSnFR (SyniGluSnFR) that can reconstitute and detect glutamate release at synaptic cleft in specific circuits. By displaying extracellularly nonfluorescent halves of the sensor to pre- and post-synaptic membrane locally in pre- and post-synaptic neurons, respectively, we can not only visualize reconstituted sensor expression at synapses using confocal fluorescent microscopy, but also record and analyze synaptic glutamate transient evoked by field stimulation in acute brain slices under two-photon microscopy in various circuits. We further apply the sensor to detect circuit-level glutamate changes from synapses in CA1 pyramidal neurons and mossy fiber boutons in CA3 in aging brain. In the awake mice, we were able to record visually driven excitatory inputs from the secondary visual cortices onto excitatory and inhibitory neuron subtypes in the primary visual cortex. he development and optimization of this sensor allow us to visualize and record glutamate activity together with two-photon microscopy. It also opens the possibility to probe the transient glutamate dynamics caused by changes in protein compositions, environmental conditions, or neurological disorders at synaptic level.

Disclosures: Y. Jin: None. **R. Liang:** None. **R.G. Natan:** None. **B. Lyu:** None. **J. Sun:** None. **J. Chandra:** None. **S. Zhang:** None. **C.K. Kim:** None. **H. Cheng:** None. **K. Murray:** None. **M. Morales:** None. **G. Yu:** None. **N. Ji:** None. **L. Tian:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.26/WW32

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U01NS113294-03/03
NIH Grant U01NS118288-02

Title: Leveraging machine learning to accelerate the protein engineering of genetically encoded voltage indicators

Authors: *A. MCDONALD[1], B. CAMPILLO[1], S. YANG[1], F. ST-PIERRE[2];
[1]Baylor Col. of Med., Houston, TX; [2]Dept. of Neurosci. and Dept. of Electrical and Computer Engin., Baylor Col. of Med. and Rice Univ., Houston, TX

Abstract: A longstanding goal in neuroscience is to decode brain functions at the molecular and circuit levels, however, the complexity of the brain and the experimental challenges make it a complex task. Current neural activity monitoring techniques such as electrophysiology, though useful, suffer from low spatial resolution and lack cell-type specificity. Similarly, calcium indicators offer low temporal resolution. Recent advancements have made it possible to directly monitor membrane potential changes in vivo using Genetically Encoded Voltage Indicators (GEVIs), providing a more in-depth understanding of brain functions compared to traditional methods. GEVIs are light-emitting proteins that report voltage dynamics as changes in brightness. Our lab has developed an automated, multiparametric screening platform to engineer

these sensors with a directed evolution approach. However, since the number of mutations vastly exceeds the number of atoms in the universe, *in vitro* methods can only sample a fraction of the whole sequence space even with high-throughput efforts. Thus, it is necessary to prioritize mutations or positions to accelerate the engineering process. Machine learning (ML) is a complementary approach to traditional wet-lab screening that benefits from the characterization of data acquired during screening which is used to train a computational model to predict sequences with improved characteristics. With the goal of creating a training dataset, we designed, sequenced, and screened saturated mutagenesis within a functionally key region of the GEVI. We then created a pipeline to train models that predict the performance of variants given their amino acid sequence. We synthesized and screened the top predicted performing variants, and found that they all had strong performance characteristics, thereby validating the ability of our models to appropriately rank previously unseen variants. Since ML benefits from more data, we utilized these novel variants to refine our models and conduct a second wave of predictions. The experimental evaluation of these variants will inform our future strategies and methodologies. Further rounds of validation are necessary to verify that the benefits of this approach outweigh the additional screening and sequencing costs. The aim of future iterations is to enhance the predictive power of our models, especially considering the small dataset sizes we are working with due to experimental limitations. We also want to assess if this method can be extended to a broader range of sensors.

Disclosures: **A. McDonald:** None. **B. Campillo:** None. **S. Yang:** None. **F. St-Pierre:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.27/WW33

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: RF1MH130391
U01NS128537
R01GM139850
F31DA056121
ISCRM Fellowship

Title: Fine-tuning of optogenetic hydrogen peroxide sensors for expanded multi-scale monitoring capabilities.

Authors: *J. D. H. LEE[1], C. NEISWANGER[5], K. KIMBALL[5], A. MOGHADASI[2], S. WAIT[1], L. TORP[3], C. I. CHAVKIN[5], A. BERNDT[4];
[1]Bioengineering/Molecular Engin., Univ. of Washington, Seattle, WA; [2]Bioengineering, Univ. of Washington, Bellevue, WA; [3]Bioengineering, [4]Univ. of Washington, Seattle, WA; [5]Pharmacol., Univ. of Washington Sch. of Med., Seattle, WA

Abstract: Reactive Oxygen Species (ROS) are inseparable companions of aerobic life forms, as their involvement spans across a wide range of cellular processes. H_2O_2 is a key ROS molecule and an oxidative stress marker, as its elevated basal level is commonly observed with disease progression. For instance, aberrant production of H_2O_2 from monoamine oxidase-B in reactive astrocytes exacerbates astrocytic reactivity and precipitates neurodegenerative hallmarks in the brain. H_2O_2 is also a pleiotropic signaling molecule that regulates a plethora of biological events. Increased H_2O_2 generation from NADPH oxidases mediated by the G-protein pathway has been reported to be responsible for a beta-arrestin-independent mechanism of opioid receptor inactivation in opioid receptor-expressing neurons. We further optimized the recently introduced oROS, a family of optogenetic H_2O_2 sensors that allows sensitive real-time measurement of H_2O_2 in biological systems. To demonstrate how oROS sensors can be applied to understand changes in H_2O_2 in diverse biological contexts, we aimed to present the use of oROS in expanded monitoring settings. Plasma membrane and mitochondria are acknowledged as the main source of endogenous H_2O_2 production. However, their role in physiology and pathology is often intertwined, which poses a challenge in effectively identifying therapeutic targets for redox medicine. oROS sensors can be targeted to various subcellular compartments to increase our understanding of the topology of H_2O_2 production in cellular environments. In addition, oROS sensors were used *in vivo* and monitored via fiber photometry, allowing H_2O_2 signal detection from opioid receptor-expressing neurons to further elucidate the H_2O_2-induced opioid receptor inactivation mechanism in a systemic context. Lastly, we acknowledge the significance of studying changes in hydrogen peroxide (H_2O_2) levels, along with the influence of other key molecules and the local environment. We present a diverse multiplexed use case of oROS with other optical tools allowing all-optical monitoring of H_2O_2 with pH, cellular redox potential, or Ca^{2+}. We envision that the oROS sensors and the use cases discussed here will stimulate new interest and discovery in the redox biology of neurological systems.

Disclosures: **J.D.H. Lee:** None. **C. Neiswanger:** None. **K. Kimball:** None. **A. Moghadasi:** None. **S. Wait:** None. **L. Torp:** None. **C.I. Chavkin:** None. **A. Berndt:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.28/WW34

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant R01NS123663
The T&C Chen Brain-Machine Interface Center
The Boswell Foundation
NEI Grant NEI F30 EY032799
Josephine de Karman Fellowship
UCLA-Caltech MSTP Grant NIGMS T32 GM008042
Della Martin Postdoctoral Fellowship
Human Frontier Science Program Cross-Disciplinary Fellowship Grant

LT000217/2020-C
USC Neurorestoration Center

Title: A window to the brain: ultrasound imaging of human neural activity through a permanent acoustic window

Authors: *C. RABUT[1], S. NORMAN[2], W. GRIGGS[2], J. RUSSIN[3], K. JANN[5], V. CHRISTOPOULOS[6], C. LIU[4], R. A. ANDERSEN[2], M. G. SHAPIRO[1];
[1]Div. of Chem. and Chem. Engin., [2]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; [3]USC Neurorestoration Ctr. and the Departments of Neurosurg. and Neurol., USC, Los Angeles,, CA; [4]USC Neurorestoration Ctr. and the Departments of Neurosurg. and Neurol., USC, Los Angeles, CA; [5]USC Stevens Neuroimaging and Informatics Inst., Los Angeles, CA; [6]Dept. of Bioengineering, Univ. of California, Riverside, Riverside, CA

Abstract: *Introduction*: Recording human brain activity is crucial for understanding normal and aberrant brain function. However, available recording methods are either highly invasive or have relatively low sensitivity. There is a clear and distinct need for neurotechnologies that optimally balance the tradeoffs between invasiveness and performance. Based on power Doppler imaging, functional ultrasound imaging (fUSI) is an emerging neuroimaging technique that visualizes neural activity by mapping local changes in cerebral blood volume (CBV). fUSI offers sensitive, large-scale, high-resolution neural imaging. However, fUSI cannot be performed through adult human skull. In the study, we demonstrate fUSI in an awake adult participant equipped with a polymeric ultrasound-transparent “acoustic window” installed as part of a skull replacement procedure following a decompressive hemicraniectomy.
Methods: We first examined the suitability of a polymeric skull replacement material for functional ultrasound imaging in an in vivo rodent model. This allowed us to design a PMMA acoustic window that could be permanently installed in a human patient as part of a skull reconstruction (Fig A). We recruited a human participant, who suffered a traumatic brain injury, underwent a left decompressive hemicraniectomy and was reconstructed with an acoustic transparent skull 30 months after his injury. We recorded fUSI data with a linear ultrasound array transmitting at 7.5 MHz positioned above the acoustic window (on top of the scalp), in the awake participant (Fig B-C).
Results: Through this window and overlaying intact scalp, in an ambulatory setting outside the operating room, we demonstrated fully noninvasive recording and decoding of functional brain signals while our human participant performed visuomotor tasks, including playing a video game and strumming a guitar (Fig D-F).
Conclusion: This study marks the first instance of high-resolution (200 μm) and large-scale (50 mmx38 mm) ultrasound brain imaging through a permanent acoustic window.

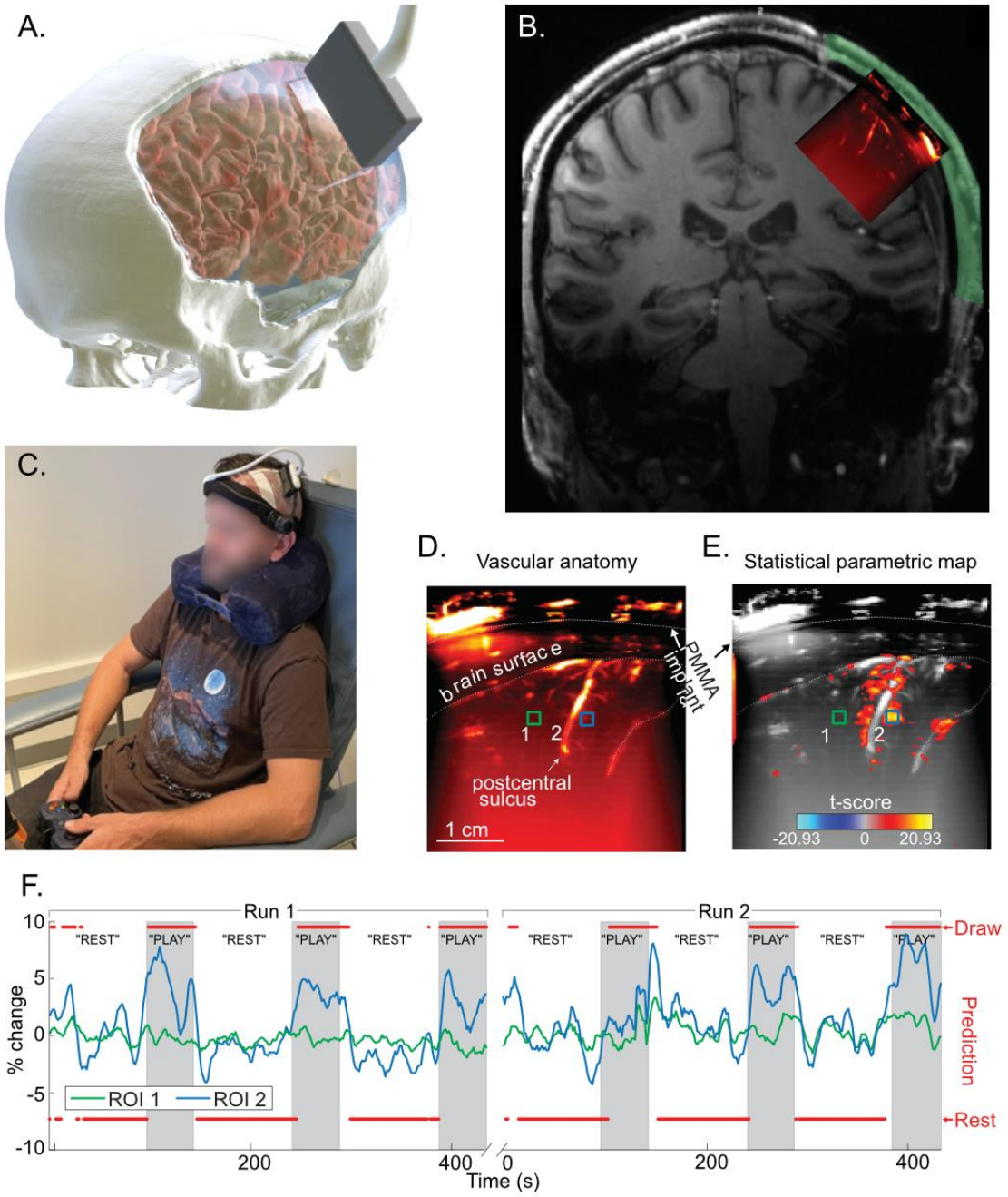


Figure : **Permanent acoustic window enables noninvasive functional brain imaging in fully reconstructed, freely behaving subjects with high spatiotemporal resolution and large coverage** (**A**) Illustration of fUSI recording through a cranial window (**B**) Co-registration of the fUSI imaging plane with an anatomical MR image of the participant (**C**) Example setup of the participant playing with a joystick during fUSI recording (**D**) Vascular anatomy of the imaging plane. Dashed lines highlight specific anatomic features, including PMMA implant surface, brain surface, and sulcal vessels. SMG: Supramarginal gyrus; PoCG: Postcentral gyrus. Colored boxes show ROIs used in part F (**E**) Task-modulated areas across two concatenated runs. T-score statistical parametric map, values shown for voxels where p(corrected) < 10e-10 (**F**) Mean scaled fUSI signal from ROIs. White regions are rest blocks; grey regions are task blocks. Red circles show prediction from linear decoder

Disclosures: **C. Rabut:** None. **S. Norman:** None. **W. Griggs:** None. **J. Russin:** None. **K. Jann:** None. **V. Christopoulos:** None. **C. Liu:** None. **R.A. Andersen:** None. **M.G. Shapiro:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.29/WW35

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant RF1MH130391
NIH Grant U01NS128537
NIH Grant R01GM139850
Herbold Fellowship (2021)
NSF Grant DGE-2140004

Title: Machine Learning Directed Engineering of Genetically Encoded Fluorescent Indicators

Authors: *S. J. WAIT[1], J. LEE[2], L. TORP[2], M. RAPPLEYE[3], A. BERNDT[2];
[1]Mol. Engin., [2]Univ. of Washington, Seattle, WA; [3]Bioengineering, Univ. of Washington, Seattle, Seattle, WA

Abstract: Genetically encoded fluorescent indicators (GEFIs) provide a means to detect and monitor neurotransmitter dynamics in vivo spatiotemporally. Their fluorescent output is modulated by the presence of a ligand of interest in neuronal circuits. To meet experimental requirements, the proteins that comprise GEFIs can be mutated to alter the biophysical characteristics of the sensor, such as dynamic range, ligand sensitivity, and kinetics. However, GEFI engineering poses a significant challenge due to each GEFI's inherent complexity and multiple dynamic states, making optimization experimentally and intellectually demanding. To expedite the engineering process, we developed a machine-learning pipeline that learns from sequence-function libraries and proposes mutations that would alter sensor functionality (Wait et al. 2023). We identified three new GCaMP variants, dubbed eGCaMPs, with this pipeline that displayed improved functional characteristics such as large response amplitudes and fast decay kinetics. We demonstrated that our machine-learning pipeline could effectively learn from complex mutational datasets and promote efficient engineering of GEFIs. We developed this platform unbiasedly, making it broadly applicable to GEFIs with sequence-to-function libraries. As such, we used the platform to generate new optimized red-shifted calcium indicators (Ex./Em.: 580nm/605nm) and dopamine sensors. In addition to the mutations obtained from the machine learning pipeline, we can also perform a retrospective analysis of the model's learning to reveal internal mechanistic protein functions. For example, in the eGCaMP study, we found that residues along the interaction face between calmodulin (CaM) and the calmodulin-binding peptide (CBP) were commonly found in the fluorescence predictions. In contrast, interactions between the residues in the cpGFP linkers and CaM were commonly found in the kinetics predictions. We believe these associations may indicate interactions within the protein that are important for the given biophysical characteristic. We used these interactions as the basis for mutational libraries and high-throughput screening for further optimization of these GEFIs). In summary, we demonstrate the versatility of our machine-learning pipeline and its ability to

complement high-throughput screening methodologies. Overall, these applications have significant implications for improving the efficiency of GEFI engineering.

Disclosures: S.J. Wait: None. **J. Lee:** None. **L. Torp:** None. **M. Rappleye:** None. **A. Berndt:** None.

Poster

PSTR507. Biochemical and Molecular Probes for Neuronal Dynamics

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR507.30/WW36

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIA grant R01AG060919
NSF grant 2030348

Title: Isolation of targeted hypothalamic neurons for studies of hormonal, metabolic, and electrical regulation

Authors: *E. FORZISI[1], F. SESTI[2], C. ROSER[3], A. GAUR[3];
[1]Rutgers Univ. Grad. Program In Neurosci., Piscataway, NJ; [2]Neurosci. and Cell Biol., [3]Rutgers Univ., Piscataway, NJ

Abstract: The hypothalamus regulates fundamental metabolic processes by controlling functions as varied as food intake, body temperature, and hormone release. As the functions of the hypothalamus are controlled by specific subset of neuronal populations, the ability to isolate them provides a major tool for studying metabolic mechanisms. In this regard, the neuronal complexity of the hypothalamus poses exceptional challenges. For these reasons, new techniques, such as Magnetic-Activated Cell Sorting (MACS), have been explored. This paper describes a new application of magnetic-activated cell sorting (MACS) using microbead technology to isolate a targeted neuronal population from prenatal mice brains. The technique is simple and guarantees a highly pure and viable primary hypothalamic neuron culture with high reproducibility. The hypothalamus is gently dissociated, neurons are selectively isolated and separated from glia cells, and finally, using a specific antibody for a cell surface marker, the population of interest is selected. Once isolated, targeted neurons can be used to investigate their morphological, electrical, and endocrine characteristics and their responses in normal or pathological conditions. Furthermore, given the variegated roles of the hypothalamus in regulating feeding, metabolism, stress, sleep, and motivation, a closer look at targeted and region-specific neurons may provide insight into their tasks in this complex environment.

Disclosures: E. Forzisi: None. **F. Sesti:** None. **C. Roser:** None. **A. Gaur:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.01/WW37

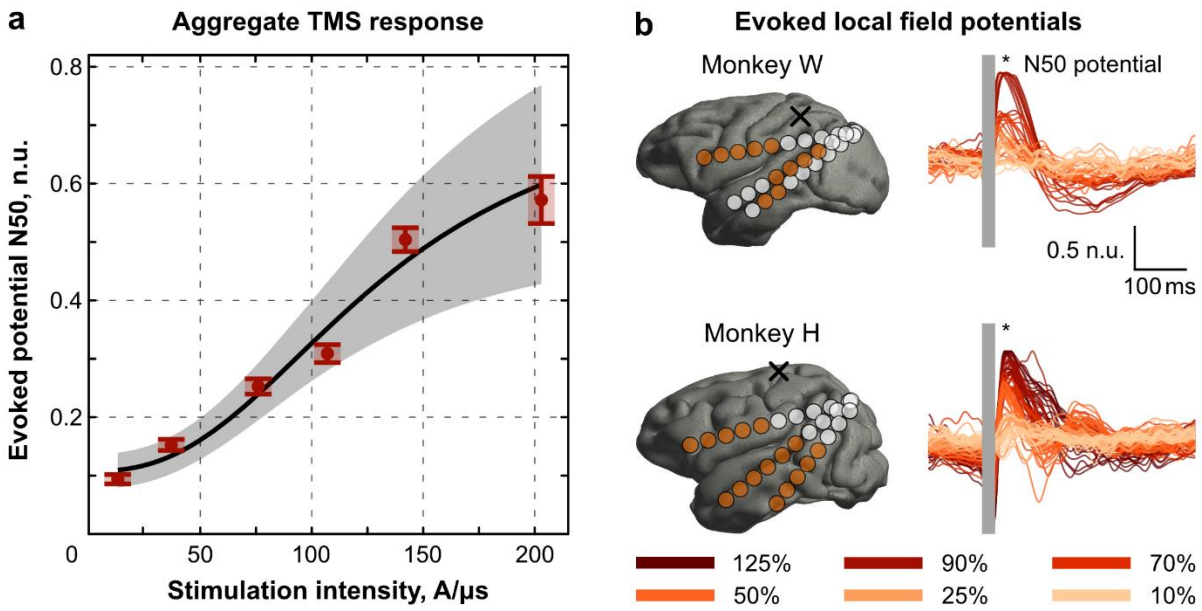
Topic: I.04. Physiological Methods

Support: NIH Grant R01NS109498
NIH Grant RF1MH117428
NIH Grant K99MH128454

Title: Dissociation of direct and indirect transcranial magnetic stimulation effects in nonhuman primates

Authors: *N. D. PERERA[1], I. ALEKSEICHUK[1], S. SHIRINPOUR[1], M. WISCHNEWSKI[1], G. LINN[2,3], K. MASIELLO[2], B. BUTLER[2], B. E. RUSS[2], C. E. SCHROEDER[2,4], A. FALCHIER[2,3], A. OPITZ[1];
[1]Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Ctr. for Biomed. Imaging and Neuromodulation, The Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY; [3]Dept. of Psychiatry, NYU Grossman Sch. of Med., New York City, NY; [4]Departments of Psychiatry and Neurosurg., Columbia Univ. Col. of Physicians and Surgeons, New York City, NY

Abstract: Transcranial Magnetic Stimulation (TMS) is a noninvasive neuromodulation technique commonly utilized in brain research and clinical applications. However, it has been debated that some of the TMS evoked responses could be the result of peripheral effects rather than direct neural activation. To this end, we recorded brain activity from two anesthetized monkeys (subjects W+H) with three implanted electrodes projecting from visual area (V2) to frontal eye field, auditory cortical and temporal regions in the left hemisphere. Biphasic single-pulse TMS was delivered over the left hemisphere to four locations in W and five locations in H. We stimulated a lateral location on the right hemisphere as the location control. Sham protocols were executed to control for auditory click (in W+H), auditory masking and somatosensory stimulation (in H). Stimulation intensities were set to 10, 25, 50, 70 and 90% for W+H with additional power mode setting at 125% of the maximum stimulator output for H. We preprocessed the neural data by removing noisy channels, TMS and muscle artifacts, interpolating, down-sampling to 1 kHz and bandpass filtering at 1-50 Hz. During analysis, we observed a negative deflection occurring 50 ms after TMS delivery in local field potentials (LFPs). This component (N50) showed a dose dependency in the main stimulation locations in W+H in subsets of contacts (Figure). These contacts also showed an inverse relationship of N50 with coil-contact distance in each monkey (R_W=0.81, R_H=0.52). LFPs from auditory control revealed responses confined to auditory region. LFPs from somatosensory control did not elicit a response comparable to active TMS in the recorded regions. Auditory masking did not alter the response in non-auditory contacts significantly. However, decreased N50 response was observed in auditory contacts. In this study, we provide evidence of dose dependency of TMS, the stimulation location dependency and the separability of direct and peripheral effects which is relevant for interpreting human TMS-EEG results.



Disclosures: **N.D. Perera:** None. **I. Alekseichuk:** None. **S. Shirinpour:** None. **M. Wischnewski:** None. **G. Linn:** None. **K. Masiello:** None. **B. Butler:** None. **B.E. Russ:** None. **C.E. Schroeder:** None. **A. Falchier:** None. **A. Opitz:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.02/WW38

Topic: I.04. Physiological Methods

Support: AFOSR

Title: Detection of neuronal modulation by impedance spectroscopy

Authors: ***S. OMIDI**[1], G. FABI[3], X. WANG[3], J. HWANG[3], Y. BERDICHEVSKY[1,2];
[1]Dept. of Bioengineering, [2]Dept. of Electrical and Computer Engin., Lehigh Univ., Bethlehem, PA; [3]Dept. of Materials Sci. and Engin., Cornell Univ., Ithaca, NY

Abstract: Examination of dielectric properties of cells and tissues at radio- and micro-wave frequencies led to identification of relaxation processes occurring in different frequency ranges. These processes have been correlated with physiological mechanisms linked with cell disease states, enabling development of novel, label-free diagnostics, such as microwave-based stroke detection. However, spectroscopy in the megahertz and gigahertz range has not, up to now, been used to examine activity-related processes in neurons. Several physiological processes occurring in active neurons, such as synaptic vesicle cycling and changes in ionic concentrations and water content, may potentially cause changes in dielectric properties in this frequency range. Ability to

detect these processes in brain tissues in a label-free manner, with high time resolution, may lead to improved understanding of neuronal function and mechanisms of neuromodulation. Here, we examined whether activity-dependent changes in neuronal properties can be detected using wideband spectroscopy. We developed a microfabricated array of eight gold-titanium grounded coplanar waveguides (GCPWs) on a glass waver. A polydimethylsiloxane (PDMS) well was mounted on top of the exposed portion of the array. We then placed 3D spheroids of rat cortical cells into the PDMS wells (one well per array chip), and allowed the spheroids to attach to the waveguides over the course of 2-3 days. Spheroids expressed channelrhodopsin-2 (ChR2) for optogenetic activation, and red fluorescent calcium indicator jRGECO1a. We then placed spheroid-containing arrays on an inverted microscope with a patterned light stimulator that allowed us to modulate activity of neurons in the spheroids. The waveguides were connected to a vector network analyzer with a bandwidth of 9 KHz to 9 GHz. Transmission and reflection spectra were taken several times per second while activity of neurons was modulated. We found that a correlation between transmission of electromagnetic waves in the gigahertz range through the waveguides and modulated activity in the spheroid. We also found that disinhibition of the spheroids with bicuculine resulted in increased amplitude of neuronal activity and changes in megahertz and gigahertz transmission. This study demonstrates first evidence that activity-associated changes in neuronal dielectric properties can be detected at this frequency range.

Disclosures: S. Omidi: None. **G. Fabi:** None. **X. Wang:** None. **J. Hwang:** None. **Y. Berdichevsky:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.03/WW39

Topic: I.04. Physiological Methods

Support: AAN Clinical Research Training Scholarship
Child Neurology Foundation Pediatric Epilepsy Research Foundation Shields Grant
NIH T32NS115705

Title: Eeg artifact in deep brain stimulation patients: the halo effect

Authors: *A. ALPERS[1], M. GOMEZ-RAMIREZ[2], K. J. LIZARRAGA[3], A. L. HEWITT[3]; [1]Neurosci., [2]Brain and Cognitive Sci., [3]Neurol., Univ. of Rochester, Rochester, NY

Abstract: Deep brain stimulation (DBS) has been shown to be an effective treatment for various disorders, including dystonia and Parkinson's disease, but it is not yet known how this therapy alters the brain networks involved. Additionally, frequency oscillations offer a potential marker for closed loop control of stimulation, which could lengthen battery life. Electroencephalography (EEG) can help study networks effects and potentially identify biomarkers of DBS response, but

little is known about how the surgery and hardware alter EEG recordings. To examine this, we compared EEG recordings from 9 adult subjects with DBS electrodes implanted in the globus pallidus internus (5M, 4F, 49.1±/-27.1 yrs): 5 newly implanted DBS, 4 battery replacement DBS, as well as 2 controls with no history of craniotomy (2F, 33+/-10 yrs). During 1 minute EEG recordings, participants rested in a chair with DBS stimulation briefly turned off. Data was collected at 2048 Hz, downsampled to 250 Hz, and high-pass filtered at 1 Hz. Peak power spectral density (PSD) frequencies were identified in sensorimotor cortex channels. Power spectra at these frequencies were visualized across all 128 electrodes using MATLAB topographic plots. For all 5 newly implanted participants, topographical plots post DBS surgery show a “halo-pattern” consisting of fronto-central excitation surrounded by a circular depression at frequencies below 30 Hz. In 5/5 subjects, this pattern was present immediately post surgery but before starting chronic stimulation. In participants with a pre-surgical EEG, 3/3 do not show this pattern. Additionally, this pattern is not present in controls (2/2) or battery replacement (4/4) participants. The pattern remained regardless of EEG reference scheme. The evidence suggests this halo pattern could be a breach artifact caused by the bilateral DBS surgery craniotomies, as opposed to a lingering stimulation artifact or a reference artifact. Understanding this artifact will enable more accurate analysis of the network effects of DBS using EEG.

Disclosures: A. Alpers: None. **M. Gomez-Ramirez:** None. **K.J. Lizarraga:** None. **A.L. Hewitt:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.04/WW40

Topic: I.04. Physiological Methods

Support: BBRF NARSAD YI 29441

Title: Invasive and noninvasive evidence of amygdala engagement to DLPFC stimulation

Authors: X. LIU, H. OYA, A. BOES, ***J. JIANG**;
Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: While invasive modulation of amygdala activity has shown promise in treating certain refractory psychiatric cases, its widespread use among millions of treatment-resistant patients is impractical and entails inherent neurosurgery-related risks. Our recent study indicates transcranial magnetic stimulation (TMS) of the dorsolateral prefrontal cortex (DLPFC) provides a potential noninvasive alternative. However, given the amygdala's deep location in the medial temporal lobe, there is a critical need for definitive causal evidence to determine whether and how DLPFC stimulation engages the amygdala. Aiming at this goal, we performed 3 experiments utilizing an unparalleled combination of invasive and noninvasive stimulation and recording methods in humans. In Exp. 1, we found single-pulse intracranial electrical stimulation

(iES) to the DLPFC evokes significant potentials in the amygdala that is concurrently recorded with intracranial EEG (iEEG) in epilepsy patients (n=11 electrodes). In contrast, single-pulse iES to the left ventrolateral prefrontal cortex did not evoke similar amygdala response (n=17 electrodes). Translating stimulation with noninvasive TMS, we observed that single-pulse TMS to the DLPFC also evokes significant amygdala responses as concurrently recorded with iEEG in epilepsy patients (n=30 electrodes, Exp. 2) and with functional MRI (fMRI) in healthy individuals (N=78 subjects, Exp. 3). In contrast, these responses are not observed when stimulating other control sites. More importantly, we identified significant correlations between these stimulation-evoked responses in the amygdala and DLPFC-amygdala connectivity assessed with resting state functional connectivity MRI. Together, these results provide compelling and conclusive causal evidence of amygdala engagement to DLPFC stimulation through a functional connectivity mechanism, highlighting the potential of personalized, circuit-guided noninvasive neuromodulatory therapies aimed at modulating the amygdala in treatment of psychiatric disorders.

Disclosures: **X. Liu:** None. **H. Oya:** None. **A. Boes:** None. **J. Jiang:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.05/WW41

Topic: I.04. Physiological Methods

Support: ERC Grant 101001448

Title: Revolutionizing neural data transfer: retinomorphic telemetry unleashes the power of BCIs

Authors: *H. P. LIAW[1], Y. HE[1,2], P. HEUBER[1,3], P. RUSSO[1,4], M. GOURDOUPARIS[1,3], C. SHI[1,4], **Y. H. LIU**[1,4,3];
[1]Stichting IMEC Nederland, Eindhoven, Netherlands; [2]Univ. of Groningen, Groningen, Netherlands; [3]Delft Univ. of Technol., Delft, Netherlands; [4]Eindhoven Univ. of Technol., Eindhoven, Netherlands

Abstract: Brain-computer interface (BCI) has shown promise in treating neurological/neuropsychiatric disorders and helping restore lost sensorimotor functions. Despite the surgical risks, intra-cortical BCIs can collect neural signals with significantly higher spatial and temporal resolution than wearable BCIs. The information-rich signal makes it possible to decode the activities in the brain accurately. However, large data from a high-channel-count recording system exceeds the capability of existing wireless telemetry systems. The high energy demand for transferring data leads to tissue overheating and large telemetry module size, including battery, while developing a fully implantable BCI system.
Inspired by how retinas encode visual information, we design a dedicated application-specific integrated circuit (ASIC) chip that converts extracellular neural signals into binary pulse trains

and transmits them via tissues with low power consumption. We reduce the data size by more than one order of magnitude and significantly reduce latency. The data size is reduced further by exploiting the spasticity of action potentials (spikes). This retinomorphic telemetry can compress data by >20X and consume power well below the tissue heating limit. Spike sorting result based on reconstructed extracellular signals shows that the waveform features are well-preserved. This retinomorphic encoded data can decode motor prediction tasks (data from neuroBench) using a neuron-inspired 'spiking neural network,' achieving similar accuracy compared to GPU-server-based neural decoding results while consuming extremely low energy.
The tethered wires connecting the micro-electrode arrays on the cortex and other electronic components on the skull, e.g., neural data processor or data telemetry, may lead to complications such as infections or bleeding. Our wireless telemetry system allows the brain implants to be 'free-floating' on the cortex, using a new tissue-coupled transdural data telemetry method. The miniature wireless communication achieves a data rate 250 times faster than standard wireless systems, such as Bluetooth, while consuming 1000 times lower power.
The presented retinomorphic BCI will be easily scalable to perform brain-wide recording and can potentially decode various tasks both locally and globally on implants. Taken together, our approach can revolutionize how neuroscientists and neurologists collect and process neural data from intra-cortical BCIs.

Disclosures: **H.P. Liaw:** None. **Y. He:** None. **P. Heuber:** None. **P. Russo:** None. **M. Gourdouparis:** None. **C. Shi:** None. **Y.H. Liu:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.06/WW42

Topic: I.04. Physiological Methods

Support: NIH (R01 NS105697)
University of Pittsburgh Brain Institute

Title: The neurophysiology of cortical resting state investigated with high resolution tools in monkeys

Authors: *K. MANIKANDAN[1], N. S. CARD[3], O. A. GHARBAWIE[2,1];
[1]Bioengineering, [2]Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [3]Dept. of Neurolog. Surgery, Univ. of California Davis, Davis, CA

Abstract: Resting state (RS) activity is commonly measured and analyzed for insight into the organization of brain networks. Correlations in infra-slow components (<0.1 Hz) of RS time series are the foundation for inferences about RS networks (RSNs). Nevertheless, the relationship between infra-slow signals and ground truth neurophysiology and neuroanatomy is not properly understood. This fundamental knowledge gap exists in part because previous

investigations have largely relied on tools with limited spatial resolution (e.g., fMRI, MEG, ECoG) to bridge RS and neural properties. Here, we deploy a multi-modal approach to investigate the neurophysiology of RS at columnar and laminar resolution. In two anesthetized squirrel monkeys, we used high-density microelectrode mapping to parcellate sensorimotor cortex into cortical areas (area 2 to premotor cortex) and somatotopic zones (leg to face). In the same hemispheres, RS was measured at high spatial resolution (~14um/pixel, 26x23 mm window) with intrinsic signal optical imaging (RS-ISOI). Seed-based correlations of RS-ISOI time series revealed functional connectivity (FC) maps corresponded exceptionally well to traced cortical connections. We used several FC maps and cortical microvasculature to guide multi-site local field potential (LFP) recordings with linear electrode arrays (32 channels, 100um spacing) that spanned the cortical depth. We obtained results from 107 site pairs, spanning 1-15 mm inter-site distance, and FC ranges from -0.3 to 0.8 (Pearson coefficients from RS-ISOI). Pairwise correlations on LFP time series (10 min/recording) revealed two main observations concerning the relationship between FC and neurophysiology - (1) Correlation strength of LFPs declined monotonically with distance between site pairs. (2) Correlation strength increased linearly with connectivity strength in FC maps. These relationships were consistent across LFP frequency bands but had a higher constant for low frequencies (<15 Hz) and infra-slow fluctuations in gamma band power. Preliminary analyses showed differential laminar organization for peak amplitude of LFP frequency bands, which suggests that fingerprints of feedforward and feedback connectivity are embedded in the LFP time series recorded during RS. Our results collectively indicate that RSNs are locked to the spatiotemporal organization of LFP. Combining high-resolution imaging and electrophysiology in the same hemisphere opens exciting possibilities for interrogating the neurobiological properties of RS at scales commensurate with cortical architecture.

Disclosures: K. Manikandan: None. **N.S. Card:** None. **O.A. Gharbawie:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.07/WW43

Topic: I.04. Physiological Methods

Support: NIH/NIAAA R01AA027269-01

Title: Alcohol exposure during the third trimester equivalent alters prefrontal - hippocampal theta coherence during a spatial working memory task in rat.

Authors: *S. KIM, J. J. STOUT, Jr., H. L. ROSENBLUM, A. Y. KLINTSOVA, A. L. GRIFFIN;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Alcohol exposure (AE) during pregnancy is known to affect medial prefrontal cortex (mPFC) and hippocampus (HPC) development. These structures are vulnerable to AE during the 3rd trimester equivalent in the rat model of Fetal Alcohol Spectrum Disorders (FASD), a period known as the brain growth spurt (BGS). The mPFC and HPC are known to connect and interact through the thalamic nucleus reuniens (Re), which has been previously demonstrated to be significantly damaged in this rat model of FASD. Recently, we have found that AE disrupts spatial working memory (SWM). Therefore, we hypothesized that these impairments could be related to changes in mPFC-HPC neural synchrony. To test this hypothesis, pups were either exposed to 5.25 g/kg/day ethanol in milk formula via two intragastric intubations 2 hours apart on postnatal days (PD) 4-9 (AE group) or were sham intubated (SI) without any liquid administered. In adulthood (>PD90), rats were implanted with stainless steel wires targeting the mPFC and HPC. Local field potentials (LFPs) were recorded as rats learned to perform a SWM-dependent delayed alternation (DA) task. Each session consisted of trials with 10 seconds, 30s, and 60s delays presented in a pseudorandomized order. We then extracted neural data surrounding the entry of the choice zone on the T-maze and performed a coherence analysis to compare the degree of mPFC-HPC oscillatory synchrony between the SI and AE groups. Analysis of theta coherence at the choice point demonstrated that AE animals exhibited significantly lower mPFC-HPC coherence in the 6-9 Hz range compared to SI animals (AE: N = 4 rats, 59 sessions, SI: N = 6 rats, 86 sessions; $z = -3.9$, $p<.001$). We then examined theta power from the mPFC and HPC at the choice point. While there was no significant difference in the mPFC theta power, AE rats had significantly higher hippocampus theta power compared to SI rats ($z = 2.7$, $p<0.01$). These findings support the hypothesis that, in addition to structural damage to the circuit, AE during BGS impairs mPFC-HPC functional interactions, which could explain why AE rats were impaired in SWM task as demonstrated in our previous study. Our findings provide a better understanding of the impact of developmental AE on HPC-Re-mPFC circuitry damage and cognitive function in adulthood.

Disclosures: S. Kim: None. **J.J. Stout:** None. **H.L. Rosenblum:** None. **A.Y. Klintsova:** None. **A.L. Griffin:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.08/WW44

Topic: I.04. Physiological Methods

Title: The suppressed do not rebound - MRBD and PMBR coincide during reversals of movement direction

Authors: *L. WINKLER[1], M. BUTZ[1], A. SHARMA[1], J. VESPER[2], A. SCHNITZLER[1], P. FISCHER[3], J. HIRSCHMANN[1];
[1]Heinrich-Heine-University, Duesseldorf, Germany; [2]Univ. Hosp. Duesseldorf, Duesseldorf, Germany; [3]Univ. of Bristol, Bristol, United Kingdom

Abstract: Neural dynamics underlying movement are typically studied by means of simple motor paradigms involving ballistic movements. However, it remains unknown how continuous movement and its changes are reflected in motor network activity. We simultaneously measured magnetoencephalography (MEG) and local field potentials (LFPs) from the subthalamic nucleus (STN) in 20 Parkinson's patients performing ongoing rotational movements with occasional reversals of movement direction. The extent to which start, stop, and reverse cues could be predicted was varied in two blocks (predictable vs. unpredictable). We observed movement-related beta desynchronization (MRBD) and a post-movement beta rebound (PMBR) in cortical motor areas and the STN. Importantly, PMBR exhibited a higher peak frequency and was more lateralized to the hemisphere contralateral to the moving hand than MRBD. During reversals of movement direction, MRBD and PMBR occurred simultaneously, resulting in almost perfect cancellation of beta power in the contralateral hemisphere and transient beta power suppression in the ipsilateral hemisphere where MRBD was stronger than PMBR. The sum of start- and stop-related beta power predicted the reversal power dynamics well, suggesting that PMBR and MRBD are independent processes that can occur in parallel during complex movement. STN-cortex beta coherence decreased at movement start and increased at stop, resembling power, but it also increased during reversals. Unpredictable movement cues were associated with higher levels of beta coherence. Regarding gamma activity, we observed increases in power at movement start and decreases at movement termination in the STN contralateral to movement. During reversals, gamma power decreased and increased in succession, reflecting the reversal motor sequence. Our findings demonstrate that the beta rhythm is not modulated in a sequential fashion; instead, prominent beta power modulations can overlap in time. Conversely, STN gamma power may be modulated successively in the reversal process, closely reflecting the movement being performed. Furthermore, beta coherence between cortical and subcortical motor areas may be crucial in complex movement contexts that require enhanced caution.

Disclosures: L. Winkler: None. **M. Butz:** None. **A. Sharma:** None. **J. Vesper:** None. **A. Schnitzler:** None. **P. Fischer:** None. **J. Hirschmann:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.09/WW45

Topic: I.04. Physiological Methods

Title: A platform for inducing spike-time dependent plasticity in patterned neural networks

Authors: *B. MAURER, S. J. IHLE, J. DURU, K. VULIć, T. RUFF, J. VÖRÖS;
Lab. of Biosensors and Bioelectronics, Inst. for Biomed. Engin., ETH Zurich, Zurich, Switzerland

Abstract: How the brain processes information to learn and form memories is still a widely unanswered question. To gain insights, neurons are studied with great effort *in vivo*. However,

the applied methods often only enable monitoring subsets of neurons for limited timespan embedded in a complex network. In bottom-up neuroscience, small neural networks of reduced complexity are engineered by seeding primary rat neurons or human induced pluripotent stem cell derived neurons into polydimethylsiloxane microstructures on top of microelectrode arrays (MEAs) to spatially confine the location of their soma and guide neurite growth [1]. Electrically evoked stimulation responses of these networks are stable and reproducible over several hours [2]. The microstructure used in this work comprises a single seeding well per network and 12 microchannels branching out from this node, where the axons can grow and action potentials can be recorded. By aligning the spike data obtained from spontaneous activity to every individual electrode, pre- and postsynaptic pairs can be identified, where a spike on the observation electrode reliably occurs with a fixed, positive latency of up to 2ms. A stimulation protocol is developed, where the axon growing from the neuron identified as “presynaptic” is stimulated with a positive or negative delay d with respect to the axon of the postsynaptic neuron. The stimulation pattern is altered between a depression-inducing phase with a positive delay, and a potentiation-inducing phase with a negative delay. Every stimulation phase is followed by a readout phase of spontaneous activity. The average latency and spike count of the postsynaptic electrode are assessed after each episode. All experiments are performed with a custom readout and incubation system, where the culture medium is kept at 37°C and water is added to the culture to account for evaporation. Preliminary experiments with a delay of d=±2ms and stimulation at 4Hz for 60s followed by 60s recording for more than 1000 iterations show statistically significant differences in relative postsynaptic spike count depending on the applied protocol. Further experiments will include a parameter search and apply different synaptic blockers to test the hypothesized underlying mechanisms. Fundamental neuroscience research is lacking a platform for stable long-term model systems with high-resolution readouts. Establishing neural networks on MEAs with reduced complexity could bridge this gap and benefit fundamental neuroscience, drug development and biohybrid technology.
References:
1. Forró, et al. (2018) *Biosensors & Bioelectronics: 122.*
2. Ihle, et al. (2021) *Biosensors & Bioelectronics: 113896.*

Disclosures: **B. Maurer:** None. **S.J. Ihle:** None. **J. Duru:** None. **K. Vulić:** None. **T. Ruff:** None. **J. Vörös:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.10/WW46

Topic: I.04. Physiological Methods

Support: IBS-R015-D1

Title: Mapping whole-brain effective connectivity of sensorimotor network in non-human primates using electrical microstimulation with 7T fMRI

Authors: *M. HAN[1,3], S. KANG[3], E. BAEG[3], S. KIM[1,3,2];
[1]Dept. of Biomed. Engin., [2]Dept. of Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon-si, Korea, Republic of; [3]CNIR, Inst. For Basic Sci. (IBS), Suwon, Korea, Republic of

Abstract: The sensorimotor system plays a crucial role in daily life, and extensive research has been devoted to its understanding. However, previous studies have primarily focused on specific regions within the sensorimotor network, particularly cortical areas, while disregarding the intricate interactions with subcortical structures. Given the fact that cortical and subcortical structures closely interact with each other, it necessitates the investigation of causal influences within the network, known as effective connectivity, at the whole-brain level. Here, to investigate the effective connectivity of sensorimotor system at the whole-brain level, we employed sensory stimulation on the forearm and applied electrical microstimulation (EM) to key nodes of the sensorimotor network, primary motor (M1) and primary somatosensory cortices (S1), in slightly anesthetized non-human primates (n = 3), while acquiring fMRI signals using ultrahigh field 7 Tesla. Sensory stimulation elicited notable activations in contralateral regions associated with the somatosensory pathway, including the ventral posterior lateral nucleus of the thalamus (VPL) and S1, as well as subsequent cortical areas such as the secondary somatosensory cortex (S2), area 5, and M1. Additionally, we observed activation in the ipsilateral cerebellum, indicative of the involvement of the spinocerebellar tract. When applying electrical microstimulation (EM) to S1, we observed significant activations in nearly identical regions as the sensory stimulation, including ipsilateral S1, S2, area 5, M1, VPL, and contralateral cerebellum, with the exception of area 7. In the case of M1 stimulation, we observed activations in sensorimotor cortices such as the premotor (PM), supplementary motor (SMA), and S1. Within subcortical areas, we noted activity in the post-commissural putamen (commonly referred to as motor putamen), ventral lateral nucleus (VL), and centromedian nucleus (CM) of the thalamus. Notably, significant activations were also observed in the contralateral cerebellum. Our data provides detailed insights into the thalamic activations associated with sensorimotor cortices. It is worth noting that the thalamus has traditionally been regarded as challenging to delineate into specific subregions. Also, the results suggest that the cerebellum plays a crucial role in processing both somatosensory and motor-related information within the sensorimotor network. The implications of these findings are significant, as they provide valuable insights into the effective connectivity of the sensorimotor network, highlighting the interactions between cortical and subcortical regions.

Disclosures: **M. Han:** None. **S. Kang:** None. **E. Baeg:** None. **S. Kim:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.11/WW47

Topic: I.04. Physiological Methods

Support: NIH 1U24NS113647-01

Title: A dynamic environment navigation task for investigating hippocampal neural dynamics and plasticity

Authors: *Z. LU, H. XU, Z. LI, D. SONG;
Biomed. Engin., USC, Los Angeles, CA

Abstract: The hippocampus plays a critical role in learning, memory, and spatial navigation. It also exhibits long-term synaptic plasticity (LTSP), which is widely postulated as the underlying mechanism of learning and memory. However, the role of hippocampal LTSP in spatial navigation remains elusive. The main challenge in studying LTSP during spatial navigation is that neural representations of space (i.e., place fields) form rapidly as animals enter a new environment. The exposure of the animal to a single environment results in limited neural and behavioral data for characterizing neural dynamics and plasticity. To address this issue, we designed a novel behavioral task called the dynamic environment navigation (DEN) task, where multiple new environments were explored by animals (rats). The DEN task took place in a four-compartment, round arena with four removable dividers. Each compartment had distinct visual cues in the form of white and black-patterned cards attached to its inner walls. By sequentially removing the dividers, animals were exposed to a series of new spaces with varying geometric shapes, sizes, and visual cues. Animals were given enough time (5-15 minutes) to fully explore each compartment. After the animals finished exploring all four compartments, the dividers were reinserted in reverse order to reintroduce the rats to previously encountered spaces. This step was to assess whether the animals had formed stable memories of the previously experienced environments. We collected unitary activities (spikes) and local field potentials (LFPs) from the hippocampal CA3 and CA1 regions, along with the movement trajectories of rats performing the DEN task (n = 4). We characterized the place fields of hippocampal neurons (84 in CA3, 68 in CA1) at each stage of the task. Our results showed that place fields formed within 5-15 minutes at each stage. Some of these place fields remained in the same location but changed in size, while others shifted in location as animals were exposed to new environments. When animals were reintroduced to previously encountered environments, some place fields remained in the same location and shape, indicating the formation of stable memories. These findings suggest that dynamically changing environments influence changes in place fields, likely through alterations in hippocampal neural dynamics. To further investigate whether LTSP is involved in these changes during the DEN task, we will further quantify the changes in place fields and conduct additional computational studies on the input-output functions of the hippocampus (e.g., CA3-CA1) using the recorded spike trains.

Disclosures: Z. Lu: None. **H. Xu:** None. **Z. Li:** None. **D. Song:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.12/WW48

Topic: I.04. Physiological Methods

Title: Developing Human Spinal Cord Methodology to Study Pain circuits

Authors: *L. S. MIRAUCOURT[1], M. GEORGIOPOULOS[2], J. OUELLET[2], M.-E. PAQUET[4], R. SHARIF NAEINI[3];
[1]Life Sci. Compex, [2]Spine Surgery Program, Dept. of Surgery, McGill University, Montreal Canada, [3]Life Sci. Complex, McGill Univ., Montreal, QC, Canada; [4]Canadian neurophotonics platform, CRIUSMQ Univ. Laval, Quebec, QC, Canada

Abstract: Chronic pain is a debilitating condition affecting more than a third of the world's population. It decreases the quality of life of patients and imposes a heavy economic burden to society. Preclinical research has provided a great understanding of the mechanisms underlying chronic pain. However, these discoveries often fail to translate to the clinical population. This may reflect the need for better preclinical tests that can predict clinical efficacy with greater accuracy, and the need to recognize that the species differences may be greater than anticipated. Recent progress in access to neuronal tissue from human organ donors has opened the door to transformational changes in pain research, and great efforts are devoted to validating discoveries made in preclinical models in human dorsal root ganglia and spinal cord neurons. In this study, we examine the viability of spinal cord sections obtained from human organ donors, when processed as 300 microns sections in organotypic cultures for up to one week. This preparation can be co-incubated with adeno-associated viral vectors to drive the expression of fluorescent proteins under the control of cell-specific promoters. The incubation of live human spinal cord sections with cell-targeted fluorescent proteins enables electrophysiological recording from visually identified cells. This work has the potential to provide insights that are critical for understanding pathological pain mechanisms and evaluating analgesic drug candidates.

Disclosures: L.S. Miraucourt: None. **M. Georgiopoulos:** None. **J. Ouellet:** None. **M. Paquet:** None. **R. Sharif Naeini:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.13/WW49

Topic: I.04. Physiological Methods

Support: JSPS KAKENHI “Out of Eurasia” Grant JP19H05736

Title: 3D printed cranial implants and automated behavioral measurements to streamline electrophysiology experiments in macaques

Authors: *R. BRETAS, B. TIA, A. IRIKI;
Innovation Design Office, RIKEN, Kobe, Japan

Abstract: Electrophysiology is a powerful technique for investigating the neural basis of behavior. However, conducting experiments in awake animals can be time-consuming, labor-intensive, and prone to errors. To address these challenges, we have developed a system that streamlines electrophysiology experiments in awake macaques by employing 3D-printed cranial implants and automated behavioral measurements.
Our 3D-printed cranial implant incorporates multiple recording chambers and head fixation attachments into a single device. We utilize biocompatible materials and a low-cost 3D printer to enhance accessibility and reduce the risk of infection. Each implant is customized for individual animals using MRI or PET scans, featuring a rigid frame that connects the chambers and head-fixation devices. Additionally, a surrounding wall provides a smooth interface with the skin, further reducing the risk of infection. All components of the implant, including the recording grids and chamber lids, are 3D printed, fully autoclavable, and compatible with dental cement for in situ repairs, if necessary. The recording chambers are hermetically sealed with O-rings and undergo pressure testing to ensure reliability.
In order to enable comprehensive data collection, we have also automated two behavioral experiments involving motor and sensory tasks. The first task focuses on mirror self-recognition in a minimally restrained macaque. For this setup, a piezoelectric sensor is integrated into the experimenter's hand to accurately detect touch onset while lightly touching the subject in various body areas. DeepLabCut is employed to automate the identification of body parts and touched areas using video data, while eye tracking monitors gaze position. The second task investigates how posture influences grasping. It involves the macaque grasping objects using different grips while alternating between sitting and quadrupedal postures. DeepLabCut captures upper limb locations in 3D, and an actuator automatically cycles through grasping targets. Grasping force is measured in six axes using a force sensor, and capacitive pads and additional force sensors aid in identifying hand location and exerted force during resting periods. Data processing for both experiments is streamlined by synchronizing and combining the entire dataset using Matlab. This approach enhances objectivity, reduces confounding variables, and minimizes repetitive manual labor. Our methods aim to facilitate complex behavioral experiments in awake animals, while ensuring reliable and safe acute neuronal recordings.

Disclosures: **R. Bretas:** None. **B. Tia:** None. **A. Iriki:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.14/WW50

Topic: I.04. Physiological Methods

Title: Cranial implants for targeted brain-wide Neuropixels recordings in mice

Authors: *C. BENNETT[1], T. RAMIREZ[2], B. OUELLETTE[1], A. CAHOON[1], C. GRASSO[1], A. WILLIFORD[1], R. HOWARD[1], B. HARDCASTLE[1], A. SRIDHAR[1], H. CABASCO[1], R.

GILLIS[1], S. DURAND[1], E. MCBRIDE[1], P. A. GROBLEWSKI[1], S. R. OLSEN[1];
[1]Allen Inst., Seattle, WA; [2]Columbia Univ., New York, NY

Abstract: Understanding the neural basis of behavior and cognition requires measuring the coordinated activity of many interconnected regions distributed throughout the brain. Technical advances in large-scale electrophysiology, such as Neuropixels probes, enable dense multi-regional recordings, but significant surgical and procedural hurdles remain for these experiments to achieve their full potential. Most previous studies in mice have used multiple small craniotomies to access the brain, but this approach has several drawbacks, including potential tissue damage, the requirement for anesthesia before the recording session, and limited viability over multiple recording days. Here we describe a novel 3D-printed cranial implant and associated workflows for making high-quality multi-probe recordings in the mouse brain. Our cranial implant can be flexibly designed with custom insertion holes to target cortical and subcortical structures for multi-probe recordings. We demonstrate the high success rate of the surgical procedure, biocompatibility of the implant, and stable Neuropixels recordings from distributed brain regions over several consecutive recording days. Moreover, we show that the transparent cranial implant is compatible with optical imaging and optogenetics in the cortex. Finally, behavioral training times on a visually guided task are not impacted by the cranial implant. We demonstrate the utility of this new methodology by making high-yield, multi-Neuropixels recordings from behaving mice. Overall, this study provides a powerful methodology for large-scale multi-region electrophysiology to uncover distributed neural computation in the mouse brain.

Disclosures: C. Bennett: None. **T. Ramirez:** None. **B. Ouellette:** None. **A. Cahoon:** None. **C. Grasso:** None. **A. Williford:** None. **R. Howard:** None. **B. Hardcastle:** None. **A. Sridhar:** None. **H. Cabasco:** None. **R. Gillis:** None. **S. Durand:** None. **E. McBride:** None. **P.A. Groblewski:** None. **S.R. Olsen:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.15/WW51

Topic: I.04. Physiological Methods

Support: NINDS 1R21NS116519-01
NIAAA 5R01AA016852-13

Title: A dispersed electrode approach for measuring brain wide dynamics

Authors: *D. KLORIG;
Wake Forest Univ. Hlth. Sci., Winston-salem, NC

Abstract: State-of-the-art neurophysiology systems are optimized for dense recordings in one or two brain areas. The functional units of the brain, however, are not constrained to a particular

anatomical area, but involve widespread networks with complex dynamics. Precise recordings from many connected structures in a widespread network would offer a unique opportunity to capture those dynamics with high fidelity, providing information about the networks themselves but also about the moment to moment state of the brain. This information is critical for understanding normal fluctuations in brain activity and for the abnormal activity associated with pathological conditions. In order to gain access to brain wide dynamics, new approaches are needed. We have developed a dispersed electrode technique (DET) paired with optogenetic stimulation (oDET) specifically for this purpose. Micro-electrodes are individually placed in a variety of structures that are part of a known or suspected functional network. Optogenetic stimulation of one or more subsets of the functional network are used to query the state of the network at any given time. Versions of this system have been used extensively to characterize seizure risk on a timescale ranging from minutes to months. Tungsten microwires, widely considered to be obsolete following the development of high density silicon probes, have distinct advantages for the dispersed approach. They are inexpensive, small (15-35 μm), flexible, produce minimal brain damage, and when individually placed and bent out of the way they are very low profile allowing them to be paired with other chronic systems such as fiber optics, micro-lenses, fluidics, or silicon probes. As an example of the utility of this approach, we present data from a dispersed 16 channel array with optogenetic stimulation used to track network excitability over a 3 month period in chronically implanted mice, as well as seizure data collected using a kainic acid model of epilepsy. Targeted structures include unilaterally: prefrontal cortex, lateral septum, piriform cortex, reuniens nucleus, and bilaterally: amygdala, entorhinal cortex, dentate gyrus, CA3, CA1, and subiculum. Low noise wideband (0.01 Hz - 10 kHz) recordings were obtained including LFPs and multiunit activity. We aim to illustrate the general utility and flexibility of such a system for advancing our understanding of network dynamics in freely behaving animals.

Disclosures: D. Klorig: None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.16/WW52

Topic: I.04. Physiological Methods

Title: Behaviour and sleep-EEG effects of psilocybin in the mouse, as measured with TaiNi ultra-light wireless telemetry

Authors: *J. R. HUXTER[1], A. BERNARDO[2], N. CARROLL[2], M. BROWN[2], L. DLUGOSZ[2], M. DUXON[1], G. HIGGINS[2];
[1]Transpharmation, Ltd., London, United Kingdom; [2]Transpharmation Canada, Fergus, ON, Canada

Abstract: Sleep-electroencephalography (EEG) is an important tool for bridging the species gap in drug discovery. However, EEG is challenging in small species such as mice because of the impact that tethers or bulky implants have on animal behaviour. Here we demonstrate the application of an ultra-lightweight head-mounted EEG (Tainitec, London UK) to explore the dose-dependent effects of psilocybin on sleep and EEG. Male, C57BL/6J mice (20-45g body wt.) were used in all experiments. First, we established an appropriate psilocybin dose range using the head twitch (HT) response as a proxy for hallucinogenic experience. Next, a separate cohort were implanted with skull-screw electrodes over the frontal and occipital/parietal cortex and EMG electrodes under the nuchal muscle, and a permanent Omnetics connector compatible with the TaiNi head-mounted transmitter system. After recovery the mice were treated with vehicle or psilocybin (0.3-3 mg/kg s.c.) in a Latin square design with a 1-week washout period. After each dosing session, EEG was recorded during normal home-cage activity for 24h, under a 12h light-dark cycle. Sleep architecture and EEG were analysed using proprietary software. Psilocybin induced a HT response with a calculated ED50 (95% CI) of 0.3 (0.1-1.1) mg/kg. At doses 3-10 mg/kg the HT response declined with an emergence of hypolocomotion and hypothermia (e.g., pre-1h post: Vehicle: -0.4±0.2ºC; Psilocybin 10: -5.6±0.5ºC; $p<0.01$). In contrast to the HT response, these latter effects were not antagonised by the selective 5-HT2A antagonist M100907 (0.5 mg/kg i.p.), rather a partial attenuation was recorded following 5-HT1A antagonist WAY100635 (1 mg/kg s.c.) pretreatment.Using an equivalent psilocybin dose range for sleep-EEG analysis, we observed a dose-dependent reduction in the amount of REM sleep during the first 4h post dose, and a significant increase in the latency to the first REM sleep bout. Psilocybin also produced clear reductions in lower-frequency oscillations (delta, theta, beta) which were specific to non-REM sleep, and a reduction in gamma oscillations which was most evident during waking. These results demonstrate a pattern of sleep-EEG responses in mice which are consistent with psilocybin's effects in humans. Parallel behavioural studies enabled the distinction between psilocybin doses associated with on-target effects at the 5-HT2A receptor, and non-specific or off-target effects. Moreover, these studies demonstrate that wireless sleep-EEG recordings can be used to reliably profile both known and novel psychedelics in the mouse, with the added potential for probing multi-site surface and depth recordings.

Disclosures: **J.R. Huxter:** A. Employment/Salary (full or part-time):; Transpharmation, Ltd. **A. Bernardo:** A. Employment/Salary (full or part-time):; Transpharmation Canada. **N. Carroll:** A. Employment/Salary (full or part-time):; Transpharmation Canada. **M. Brown:** A. Employment/Salary (full or part-time):; Transpharmation Canada. **L. Dlugosz:** A. Employment/Salary (full or part-time):; Transpharmation Canada. **M. Duxon:** A. Employment/Salary (full or part-time):; Transpharmation, Ltd. **G. Higgins:** A. Employment/Salary (full or part-time):; Transpharmation Canada.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.17/WW53

Topic: C.06. Neuromuscular Diseases

Title: Brain mapping using ultra-high-density electroencephalography

Authors: *L. SCHREINER, C. GUGER, M. CHING, K. MAYR;
g.tec medical engineering GmbH, Schiedlberg, Austria

Abstract: Intraoperatively neurophysiology (ION) techniques are the gold standard for monitoring brain function and spatial mapping of brain areas. Using median nerve stimulation (MNS) and the underlying principle of phase reversal (PR) at the central sulcus (CS) is a widely applied methodology to identify the somatosensory cortex. As ECoG is only applicable in operating room environments, its usage is inevitably connected to risks for the patient and increased costs in general. On the other hand, brain research using the non-invasive EEG has other limiting factors, such as low spatial resolution. So-called multichannel or high-density EEG has proven helpful in clinical neurological applications such as preoperative localization of epileptogenic lesions using source localization. The current work shows the application of a novel ultra-high-density EEG (uHD EEG) system used to record somatosensory evoked potentials (SSEPs). MNS was performed on one healthy male subject to generate the potentials. Right- and left-hand stimulation was applied in separate measurements. The SEP phase inversion from anterior to the CS to posterior to the CS was adopted to locate the primary motor (M1) and the somatosensory (S1) cortex. The identification was achieved using a simple peak detection method as well as classification using k-means, categorizing the waveform as either originating from the sensory or motor area. method, categorizing the waveform as either originating from the sensory or motor area. 256 channels of uHD EEG were placed on the scalp over both hemispheres of the somatosensory-motor cortex for data acquisition. Our topography plots of the spatial distribution from the SSEPs demonstrate a detailed distinction between channels located above the somatosensory and motor cortex and the central sulcus. The distinction of anterior and posterior channels to the central sulcus by adopting spectral clustering resulted in an average accuracy of 95.2%, comparable to applying those methods using electrocorticogram (ECoG) data. The conduction of additional investigations employing the system with higher channel capacity and performing comparative assessments with invasive techniques like electrocorticography can substantially improve preoperative brain mapping procedures.

Disclosures: **L. Schreiner:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **C. Guger:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **M. Ching:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH. **K. Mayr:** A. Employment/Salary (full or part-time):; g.tec medical engineering GmbH.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.18/WW54

Topic: I.04. Physiological Methods

Support: Ayudas Joan Oró para la contratación de personal investigador predoctoral en formación (2023 FI-2 00197)

Title: A game-inspired analysis of connectivity changes identifies epilepsy surgery targets

Authors: *K. IVANKOVIC[1,2], A. PRINCIPE[1,2], J. MONTOYA[1,3], L. MANUBENS-GIL[4], R. ZUCCA[3,1], M. DIERSSEN[5,1], R. ROCAMORA[2,1];
[1]Univ. Pompeu Fabra, Barcelona, Spain; [2]Hosp. del Mar Res. Inst., Barcelona, Spain; [3]Ctr. for Brain and Cognition (CBC), Barcelona, Spain; [4]SEU-Allen Joint Ctr. for Neuron Morphology, Southeast Univ. (SEU), Jiangsu, China; [5]CRG - Ctr. For Genomic Regulation, Barcelona, Spain

Abstract: Epileptogenic network dynamics depend on the regulatory inputs from other brain networks. As the regulation ceases, the brain enters a connectivity state critical for the seizure to occur. We hypothesized that the epileptogenic nodes present maximal connectivity change from the interictal to the critical state. We describe the dynamics between network nodes as a competition and analyze connectivity changes within a game-theoretical framework. We implemented a game of cards between multiple players, in which the highest card wins a turn. The concept of this game was transferred to estimate the winners among brain nodes, based on their connectivity change.
Neural populations recorded by intracranial EEG were network nodes. Node connectivity states were quantified in interictal epochs and 1 minute before seizure, using several connectivity measures. A support vector machine with K-fold cross-validation produced scores used to approximate node connectivity change. The scores were used as cards comprising a deck of each player. Players used one of four strategies to select a card (maximum, minimax, average, or random). The competition hypothesis was validated by comparing the winning nodes to surgical resections. To overcome the confounding effect of resection size and the number of available nodes, random groups of nodes were generated, with a size of 10% of the resection. The groups were sorted based on the number of wins. The winners were the groups scoring above four standard deviations from the mean score. Resection overlap ratio between winners and losers was used to predict surgery outcomes. The validation cohort was 21 consecutive patients with a post-surgical follow-up of minimum 3 years.
The winners had significantly higher resection overlap than the losers. The game strategies maximum and minimax produced the best results, confirming our hypothesis. Using the resection overlap ratio between winners and losers, patients' post-surgical outcomes were perfectly classified (AUC = 100%). The most informative connectivity measures were spectral coherence in δ, θ, α, and β frequency bands, phase lag index in δ and γ bands, and cross-correlation in δ band. The combination of these six measures provided the most accurate outcome prediction in our cohort.
The epileptogenic network presents maximal connectivity changes during seizure generation. Analyzing the connectivity change as a competition between network nodes allows for identifying surgical resections with unprecedented accuracy. This work provides a tool that may aid surgical decision-making and adds insight into seizure generation mechanisms, supporting competition-like network dynamics.

Disclosures: K. Ivankovic: None. **A. Principe:** None. **J. Montoya:** None. **L. Manubens-Gil:** None. **R. Zucca:** None. **M. Dierssen:** None. **R. Rocamora:** None.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.19/WW55

Topic: I.04. Physiological Methods

Title: Development of fully automatic EEG preprocessing - a vital step for sustained progress in the field

Authors: *J. DREO, J. JUG, B. ALJAŽ, F. AGATIć, T. PAVLOVčIč, A. OGRIN, D. SAKIć; BrainTrip Limited, Ljubljana, Slovenia

Abstract: Based on our review of 300 EEG papers published since the 1960s, the typical modern EEG study collects about 85x more data compared to 50 years ago. This is mostly due to increased subject and channel counts. Since EEG data is extremely prone to both physiological (ocular, sweating, muscle) and non-physiological (electrode failure, EM) artifacts, any quantitative EEG analysis requires extensive pre-processing to either exclude or correct for these artifacts. However, EEG data is also notoriously difficult to preprocess relying mostly on manual artifact selection, manual guidance of cleaning algorithms, and subjective judgements. EEG preprocessing is thus not only labor intensive but also idiosyncratic. This hinders progress in the field since EEG results are difficult to replicate and compare across different labs. Collecting orders of magnitude more EEG data than in the past, coupled with most researchers still using purely manual EEG preprocessing, represents a clear bottle-neck in EEG research posing a major obstacle to progress. It is therefore vital to develop a fully automated EEG preprocessing pipeline with a performance comparable to human raters. While several semi-automatic preprocessing pipelines have been developed over the years, mostly for MATLAB, none are 1) fully automatic (require absolutely no manual input), 2) offer comparable or better performance than expert raters, 3) are designed to deal with the full range of EEG artifacts, and 4) are built to offer scalable processing of arbitrarily large EEG datasets irrespective of local hardware limitations. We have created a novel pipeline (aPIPE) that is fully generalized (its output may be used for a wide variety of EEG analyses), fully automated, minimally invasive in terms of data changes, and its results rival expert human EEG raters in terms of specificity and sensitivity of finding EEG artifacts. The pipeline is implemented in the cloud, obviating the need for large local computational resources, and offers virtually unlimited scalability accommodating even the largest EEG datasets. The new aPIPE algorithm has the potential to significantly accelerate progress of EEG analysis by offering easy to use, standardized, computationally attainable, and fully automatic preprocessing of EEG data.

Disclosures: **J. Dreo:** A. Employment/Salary (full or part-time):; BrainTrip Limited. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BrainTrip Limited. **J. Jug:** A. Employment/Salary (full or part-time):; BrainTrip Limited. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

BrainTrip Limited. **B. Aljaž:** A. Employment/Salary (full or part-time):; BrainTrip Limited. **F. Agatić:** A. Employment/Salary (full or part-time):; BrainTrip Limited. **T. Pavlovčič:** A. Employment/Salary (full or part-time):; BrainTrip Limited. **A. Ogrin:** A. Employment/Salary (full or part-time):; BrainTrip Limited. **D. Sakić:** A. Employment/Salary (full or part-time):; BrainTrip Limited.

Poster

PSTR508. Electrophysiology-Neural Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR508.20/WW56

Topic: I.04. Physiological Methods

Title: Engineered in vitro neuronal networks exhibit Boolean logic behavior

Authors: *K. VULIC, J. KÜCHLER, H. YAO, S. WEAVER, S. J. IHLE, J. DURU, B. MAURER, J. VÖRÖS;
ETH Zurich, Zurich, Switzerland

Abstract: Studying information storage and processing directly in the brain is challenging. Hence, we adopt a bottom-up approach, focusing on small neural networks cultured in a controlled environment. By culturing neurons in polydimethylsiloxane (PDMS) microstructures on multielectrode arrays (MEAs), we can guide the growth of neurons, stimulate them andrecord their activity. These cultured networks exhibit directionality and stability over time [1], enabling us to explore their input-output relationships.
In this work, we constructed networks of primary rat neurons consisting of two input nodes and one output node. We investigated the natural activation function of these biological networks (a biological analogue of an artificial neural network (ANN) activation function). Through the use of PDMS microstructures and nanochannels [2], we customized the strength and direction of the synaptic transmission. To stimulate the network, we applied voltage spike trains of different amplitudes and frequencies to the two pre-synaptic input nodes. The immediate resulting activity of the post-synaptic output node was recorded. We determined the average spike frequency as a measure of the network's activation function. Initially, we provided the same stimulation amplitude to both input nodes and observed that the average output spike frequency increased in a sigmoid fashion as the stimulation amplitude increased. The response reached a saturation point beyond a threshold amplitude of 300 mV. We used this amplitude to investigate the frequency response of the network. By applying identical frequency stimulation to the two inputs, we discovered a reversed-sigmoid-like behavior as the frequency increased. We observed a threshold frequency of 50 Hz, beyond which the network's activity was silenced. Next, we explored various combinations of different amplitudes and frequencies for the input stimulation. Each stimulation pattern exhibited stable and reproducible responses which we identified as logic operations AND, NOT, and OR. Furthermore, we found that the networks displayed resilience to noise introduced by varying amplitudes and frequencies, as well as resilience across different days in vitro.

In addition to its importance in fundamental neuroscience research, these networks have the potential to be integrated with ANNs to create closed-loop systems for future hybrid computing. To enable more complex logic operations like XOR, we can either introduce plasticity through specific stimulation patterns or strategically confine neurons within PDMS to generate more intricate circuits.
Ref:
[1] Ihle et al, Biosens. Bioelectron., 2022
[2] Mateus et al, ACS Nano, 2022

Disclosures: **K. Vulic:** None. **J. Küchler:** None. **H. Yao:** None. **S. Weaver:** None. **S.J. Ihle:** None. **J. Duru:** None. **B. Maurer:** None. **J. Vörös:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.01/WW57

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: The effect of ICV dosing method, rate, and volume on biodistribution and inflammation

Authors: *T. Y. YANG, S. SABHLOK, D. HENDERSON, R. KPETE, G. DELANEY, M. A. BOWMAN, J. ROESER, C. PERITORE;
Charles River Labs., South San Francisco, CA

Abstract: Small molecules, such as adeno-associated viruses (AAVs), hold great therapeutic potential for the treatment of central nervous system (CNS) disorders but have limited efficacy when administered systemically due to the inability of most AAVs to cross the blood-brain barrier (BBB). Intracerebroventricular (ICV) injections are a common method for drug administration into the cerebrospinal fluid (CSF), bypassing the BBB, ensuring local delivery to the CNS and target region. Most studies examining the ICV route of administration have focused on the relationship between dosing route and efficacy, however there is a need to understand whether various methods, rates, or volumes can induce CNS inflammation or brain injury. To this end, the present study investigated the effect of various ICV injection methods (direct infusion vs. catheter), volumes (small vs. large), and rates (slow infusion vs. bolus) on biodistribution and inflammatory markers in the CNS. Male C57Bl/6 mice received an ICV injection of AAV9 either through unilateral infusion, bilateral infusion, or guide cannula with different volumes and rates. To assess acute and chronic inflammatory markers, CSF and blood samples were collected 48h and 4 weeks post-dose, respectively. Biodistribution of AAV9 was examined in the brain using quantitative PCR while inflammatory markers were analyzed in blood and CSF using V-PLEX MSD kits. Bilateral infusion of AAV9 produced the greatest biodistribution in the brain compared to unilateral infusion. Moreover, proinflammatory cytokine levels did not increase in response to administration of AAV9 for any route of delivery. Results

from this study give a better understanding of how different ICV dosing methods, rates, and volumes are tolerated and potentially influence neuroinflammation and gene expression.

Disclosures: **T.Y. Yang:** None. **S. Sabhlok:** None. **D. Henderson:** None. **R. Kpete:** None. **G. Delaney:** None. **M.A. Bowman:** None. **J. Roeser:** None. **C. Peritore:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.02/WW58

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Isoflurane anaesthesia for ICV administration in rodent pups

Authors: *M. HEINS, K. HUININK, M. OLTHUIS, G. FLIK;
Charles River Labs., Groningen, Netherlands

Abstract: New therapeutic modalities as gene therapy and anti-sense oligonucleotides have emerged as promising treatments for (rare) genetic disorders. Since the approval of Gendicine in 2003, several more AAVs and ASOs have become available for treatment. For CNS genetic disorders, as the effects of the aberrant genes are present from conception, treatment as early as possible.
In the preclinical setting, the default procedure for *in vivo* assessment of tolerability and dose response is intracerebroventricular (ICV) administration of the therapeutic in P0-P2 rodent pups. These procedures are typically performed in a stereotaxic frame under injection or thermal anaesthesia. Injection anaesthesia presents a risk of either over- or underdosing of the injection anaesthetic. Thermal or cryo-anaesthesia comes with risks of freeze tissue damage. In addition, both these techniques are quite time consuming. Finally, more and more, cryo-anesthesia is observed as a major welfare impact on the animals and in some countries already forbidden to be used.
The generally preferred route of anaesthesia for adult animals is by application of an inhalation gas application, such as isoflurane. Transferring this method of anaesthesia to rodent pups requires the available equipment to be adapted and/or modified. In general, this tends to result in a suboptimal fit with either isoflurane loss or limited accessibility of the target region. To overcome these challenges, we have designed and manufactured an isoflurane platform for rodent pups.
Pilot studies confirmed the functionality and usability of the platform for rodent pup ICV administrations. The platform demonstrated to be robust and easy to use for the intended purpose. Next, comparative studies to assess the differential effects of isoflurane inhalation anaesthesia with the current standard of thermal anaesthesia were performed. The isoflurane platform anaesthesia showed improved or at least similar results to the cryo-anaesthesia. Notably, the duration of the ICV administration procedure was demonstrated to be significantly

reduced. Providing a refinement that is beneficial for the welfare of both the pup(s) and the mother.

Disclosures: **M. Heins:** A. Employment/Salary (full or part-time):; CRL. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CRL. **K. Huinink:** A. Employment/Salary (full or part-time):; CRL. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CRL. **M. Olthuis:** A. Employment/Salary (full or part-time):; CRL. **G. Flik:** A. Employment/Salary (full or part-time):; CRL. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CRL.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.03/WW59

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Polyethylene glycol interaction with different cells: an in vitro study

Authors: *I. OLADELE[1], S. E. BOURDO[2], J. A. KAMYKOWSKI[3], F. WATANABE[2], B. P. CHHETRI[2], M. A. MUHI[2], S. ALI[2], K. B. VANG[2], A. S. BIRIS[2];
[1]Applied Sci., Univ. of Arkansas, Little Rock, Little Rock, AR; [2]Ctr. for Integrative Nanotechnology Sci., Little Rock, AR; [3]Digital Microscopy Lab., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Polyethylene glycol interaction with different cell types: an in vitro study
Authors
***Ibrahim Oladele[1]**, Shawn E Bourdo[2], Jeff A Kamykowski[3], Fumiya Watanabe[2], Bijay P Chhetri[2], Malek A Muhi[2], Syed F Ali[2], Kieng Vang Bao[2], Alexandru Biris[2].
[1]Applied Sci., Univ. of Arkansas at Little Rock, Little Rock, AR; [2]Ctr. for Integrative Nanotechnology Sci., Little Rock AR; [3]Digital Microscopy Lab., Univ. of Arkansas for Medical Sciences, Little Rock, AR.
Disclosures
Ibrahim Oladele: None. **Shawn E Bourdo:** None. **Jeff A Kamykowski:** None. **Fumiya Watanabe:** None. **Bijay P Chhetri:** None. **Malek Muhi:** None. **Syed F Ali:** None. **Kieng Bao Vang:** None. **Alexandru Biris:** None.
Abstract
The application of conjugated therapeutics covalently bound to polyethylene glycol (PEG) has been well documented in the field of nanomedicine, but much is yet to be known about the interaction between free PEG and cells within the body. This study aimed to characterize the interaction of free PEG with macrophage (RAW 264.7) immune system cells, and PC12 non-immune system cells, to further understand PEG behavior at the cellular level. FITC tagged PEG

of three different molecular weights (MWs) (1, 5, and 10 KDa) were exposed to cultured RAW 264.7 and PC12 cells. All experiments were conducted three times in triplicates. Following the treatment, PEG-FITC fluorescence intensity in both cell lines was qualitatively and quantitatively measured as a function of cellular internalization of the polymer using Laser Scanning Confocal Microscopy (LSCM) and a microplate reader respectively. The results indicate that PEG was internalized by both cell lines independent of its respective molecular weights. Interestingly, the RAW 264.7 cells were found to have a higher capacity to internalize the PEG (all MWs) compared to the PC12 cells. In summary, the results of this study suggest that PEG is more efficiently interacting with the macrophage cells compared to the PC12 cells, indicating the possible interaction of the immune cells with the PEG chains before the polymer chains reach the non-immune system cells targeted for various therapeutic applications. More research is needed to understand the biodistribution and the complex interaction of PEG within the human body.

Disclosures: I. Oladele: None. **S.E. Bourdo:** None. **J.A. Kamykowski:** None. **F. Watanabe:** None. **B.P. Chhetri:** None. **M.A. Muhi:** None. **S. Ali:** None. **K.B. Vang:** None. **A.S. Biris:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.04/WW60

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Houston Methodist Foundation
Houston Methodist Research Institute Clinician-Scientist award
Paula and Rusty Walter Endowment at Houston Methodist
Walter Oil & Gas Corp Endowment at Houston Methodist

Title: Electrokinetic convection enhanced delivery of solutes to the brain from a surface hydrogel

Authors: *J. CRUZ-GARZA[1], L. S. BHENDERU[1], K. M. TAGHLABI[1], K. P. FRAZEE[1], J. R. GUERRERO[1], M. K. HOGAN[2], F. HUMES[2], R. ROSTOMILY[1], P. J. HORNER[2], A. H. FARAJI[1];
[1]Dept. of Neurosurg., [2]Ctr. for Neuroregeneration, Houston Methodist Res. Inst., Houston, TX

Abstract: Introduction: Electrokinetic convection enhanced delivery (ECED) utilizes an external electric field to induce electroosmosis and electrophoresis, allowing for directional control of infused therapeutic agents into the brain. ECED allows for enhanced control of infused solutes and overcomes many limitations of pressure-driven drug delivery. We studied the ability of ECED to induce solute infusion from the cortical surface using doped biocompatible hydrogels. **Methods:** A biocompatible hydrogel doped with neutral 0.4 mM Texas Red 3,000

MW dextran conjugate fluorophore was placed on the cortical surface of the brain in both *ex vivo* (N = 16) and *in vivo* (N = 13) rat brains. A positive electrode was placed inside the hydrogel and a negative counter electrode, a hollow cannula filled with a saline solution, was placed 2.5 mm intraparenchymally underneath the hydrogel. ECED trials were performed with a 50 µA current for 30 minutes, and diffusion-only control trials were performed with a 0 µA current for 30 minutes. The brains were sectioned immediately after the trials and fluorescent microscopy images were taken to determine the linear distance of infusion from the brain surface, displacement of peak fluorescence intensity, and the area of infusion. **Results:** The distance to 10% of maximum fluorescence intensity in ECED trials was statistically greater than in control trials *ex vivo* (0.57 ± 0.09 vs. 0.24 ± 0.09, p=0.03 mm), and *in vivo* (1.17 ± 0.13 vs. 0.36 ± 0.12 mm, p<0.01). The displacement of peak fluorescence intensity along the direction of infusion in ECED trials compared to control trials was significant in *in vivo* trials (0.23 ± 0.02 vs. 0.09 ± 0.02 mm, p<0.01), but not for *ex vivo* trials (p=0.41). The area of infusion containing fluorescence with a minimum of 10% of maximum intensity was not significantly different in ECED vs. control *ex vivo* (p=0.12) but was significant *in vivo* (1.30 ± 0.26 vs 0.29 ± 0.18 mm^2, p=0.01). **Conclusion:** ECED allows for significant intraparenchymal penetration of macromolecules from a doped hydrogel bank on the cortical surface. The effect of the ECED intervention was more pronounced for *in vivo* trials than *ex vivo*. This technique provides an alternative to pressure-driven brain infusions techniques, and it allows for different infusion configurations, as a hydrogel bank has the potential to conform to brain lesion areas for infusion of therapeutic agents or priming cortical or subcortical targets for neural interfaces. Future studies will investigate the optimal parameters for running ECED and for ECED to occur with varying electrode placements with and without a hydrogel bank.

Disclosures: J. Cruz-Garza: None. **L.S. Bhenderu:** None. **K.M. Taghlabi:** None. **K.P. Frazee:** None. **J.R. Guerrero:** None. **M.K. Hogan:** None. **F. Humes:** None. **R. Rostomily:** None. **P.J. Horner:** None. **A.H. Faraji:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent number 11471674.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.05/WW61

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Houston Methodist Clinician Scientist Award (20210001)

Title: Electrokinetic Convection-Enhanced Delivery to Pig Brains in the MRI Environment

Authors: *K. M. TAGHLABI[1], *K. M. TAGHLABI[1], L. S. BHENDERU[1], J. G. CRUZ-GARZA[1], A. TAHANIS[1], J. R. GUERRERO[1], C. KARMONIK[2], A. H. FARAJI[1];

[1]Neurolog. Surgery, Houston Methodist Res. Inst., HOUSTON, TX; [2]Houston Methodist Res. Inst., Houston, TX

Abstract: Background Electrokinetic convection-enhanced delivery (ECED) can effectively infuse macromolecules into the brain parenchyma with directional control along a current path. This technique utilizes an external electric field to direct an infusion via electroosmosis and electrophoresis. Technological advances in intra-operative Magnetic Resonance Imaging (MRI) for neurosurgical interventions provide the possibility of studying the infusion of agents in the brain in real-time.
Objective The present research investigates the feasibility of ECED in the presence of concurrent real-time MR imaging, in a large animal model.
Methods Four *ex vivo* pig brains were placed on a custom frame inside a 3T MRI scanner in a standard knee coil. A HEPES-based saline solution and two 200 µm inner diameter cannulas were used to infuse Gadodiamide contrast via ECED. The cannulas were placed in the parietal and frontal lobes of the pig brain. We assessed the infusion volume of the contrast agent with a current of 100 µA for 30 min of ECED. Concurrently, real-time T1-weighted pointwise encoding time reduction with radial acquisition (PETRA) MRI scans were taken at 10, 20, and 30 mins from the start of the infusion. Baseline and control scans were acquired at the same time points without application the electric field.
Results Control experiments showed no appreciable infusion of the Gadodiamide contrast agent. ECED produced localized infusions into the brain parenchyma. The infusion volume increased from 0 µL at baseline to 27.87 ± 15.64 µL at 30 min (p &lt0.001). There was a significant increase in volume of infusion between 10 min ECED and 30 min ECED (4.91 µL vs 27.87 µL, p=0.004) as well as between 20 min ECED and 30 min ECED (11.3 µL vs 27.87 µL, p=0.04).
Conclusion ECED-based infusion of a Gadodiamide contrast agent can be visualized in real-time in the MRI environment effectively. ECED, coupled with intraoperative real-time MRI scans, can allow for the precise and directed infusions of therapeutic agents inside the brain. Future trials will investigate the ability of ECED to control infusion directionality, optimize technical parameters of current, cannula design, and infusion times, and evaluate the safety of this novel intervention using *in vivo* models.

Disclosures: K.M. Taghlabi: None. **K.M. Taghlabi:** None. **L.S. Bhenderu:** None. **J.G. Cruz-Garza:** None. **A. Tahanis:** None. **J.R. Guerrero:** None. **C. Karmonik:** None. **A.H. Faraji:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.06/WW62

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Innovation Fund Denmark Grant 9065-00237B

Title: Uncovering CNS access of lipidated exendin-4 analogues by quantitative whole-brain 3D light sheet imaging

Authors: *A. PARKA[1], G. SKOVBJERG[1], U. ROOSTALU[1], C. GRAVESEN SALINAS[1], J. LERCKE SKYTTE[1], J. PERENS[1], C. CLEMMENSEN[2], L. ELSTER[1], C. KAAS FRICH[1], H. HANSEN[1], J. HECKSHER-SØRENSEN[1];
[1]Gubra, Hørsholm, Denmark; [2]Novo Nordisk Fndn. Ctr. for Basic Metabolic Res., Copenhagen, Denmark

Abstract: Peptide-based drug development for CNS disorders is challenged by poor blood-brain barrier (BBB) penetrability of peptides. While acylation protractions (lipidation) have been successfully applied to increase circulating half-life of therapeutic peptides, little is known about the CNS accessibility of lipidated peptide drugs. Light-sheet fluorescence microscopy (LSFM) has emerged as a powerful method to visualize whole-brain 3D distribution of fluorescently labelled therapeutic peptides at single-cell resolution. Here, we applied LSFM to map CNS distribution of the clinically relevant GLP-1 receptor agonist (GLP-1RA) exendin-4 (Ex4) and lipidated analogues following peripheral administration. Mice received an intravenous dose (100 nmol/kg) of IR800 fluorophore-labelled Ex4(Ex4), Ex4 acylated with a C16-monoacid (Ex4_C16MA) or C18-diacid (Ex4_C18DA). Other mice were administered C16MA-acylated exendin 9-39 (Ex9-39_C16MA), a selective GLP-1R antagonist, serving as negative control for GLP-1R mediated agonist internalization. Two hours post-dosing, brain distribution of Ex4 and analogues was predominantly restricted to the circumventricular organs, notably area postrema and nucleus of the solitary tract. Ex4_C16MA and Ex9-39_C16MA also distributed to the paraventricular hypothalamic nucleus and medial habenula. Notably, Ex4_C18DAwas detected in deeper-lying brain structures such as dorsomedial/ventromedial hypothalamic nuclei and the dentate gyrus. Similar CNS distribution maps of Ex4-C16MA and Ex9-39_C16MA suggest that brain access of lipidated Ex4 analogues is independent on GLP-1 receptor internalization. The cerebrovasculature was devoid of specific labelling, hence not supporting a direct role of GLP-1RAs in BBB function. In conclusion, peptide lipidation increases CNS accessibility of Ex4. Our fully automated LSFM pipeline is suitable for mapping whole-brain distribution of fluorescently labelled drugs.

Disclosures: **A. Parka:** A. Employment/Salary (full or part-time):; Gubra. **G. Skovbjerg:** A. Employment/Salary (full or part-time):; Gubra. **U. Roostalu:** A. Employment/Salary (full or part-time):; Gubra. **C. Gravesen Salinas:** A. Employment/Salary (full or part-time):; Gubra. **J. Lercke Skytte:** A. Employment/Salary (full or part-time):; Gubra. **J. Perens:** A. Employment/Salary (full or part-time):; Gubra. **C. Clemmensen:** None. **L. Elster:** A. Employment/Salary (full or part-time):; Gubra. **C. Kaas Frich:** None. **H. Hansen:** A. Employment/Salary (full or part-time):; Gubra. **J. Hecksher-Sørensen:** A. Employment/Salary (full or part-time):; Gubra.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.07/WW63

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of in vitro assays to evaluate the impact of SGSH enzyme replacement therapy for Sanfilippo Syndrome type A in different brain cell types

Authors: *A. ASENJO MARTINEZ[1], C. GUIJAS[2], A. NIELSEN[1], K. RUSS[1], F. SOTTY[1], K. FOG[1], M. ROHE[1];
[1]H. Lundbeck A/S, Copenhagen, Denmark; [2]H Lundbeck A/S, Lundbeck La Jolla Res. Ctr., San Diego, CA

Abstract: Since the first enzyme replacement therapy (ERT) for Gaucher disease was approved in 1991, ERT has become standard of care for several lysosomal storage diseases (LSD), where substrates accumulate in the lysosomes due to misfunctioning enzymes that cannot degrade the substrates. In traditional ERT, enzymes are administered intravenously with the aim to restore lost or altered enzymatic activity. However, in LSD affecting the central nervous system, the peripherally administered enzymes will not cross the blood-brain-barrier and hence not provide the needed enzyme replacement in brain. This is the case of Sanfilippo Syndrome type A (MPS IIIA), a monogenetic disease caused by ~150 different loss-of-function mutations in the lysosomal enzyme N-Sulfoglucosamine Sulfohydrolase (SGSH), resulting in accumulation of heparan sulfate (HS) in lysosomes. This accumulation disrupts cellular homeostasis with consequences for autophagy and mitochondrial and synaptic functions, leading to neuroinflammation and neurodegeneration. To develop an efficient ERT approach to MPS IIIA, it is key to understand cellular and intracellular distribution of HS accumulation and SGSH activity. To that end, we have generated SGSH KO HEK cell lines to study intracellular accumulation of HS. As HS quantification by immunocytochemistry is challenging due to its similarity to other glycosaminoglycans and poor antibody specificity, we have established a high-throughput enzymatic digestion assay coupled with liquid chromatography-tandem mass spectrometry (LC-MS) that allows accurate quantification of HS disaccharides. Using this method, we confirmed HS accumulation in KO cells compared to control, and that HS can be degraded *in vitro* with recombinant SGSH treatment. To study SGSH function in brain relevant cell types, we differentiated induced pluripotent stem cells (iPSCs) into neurons, microglia and astrocytes to assess SGSH expression and activity by RNAseq and enzyme activity assay, respectively. We found that SGSH expression and activity is enhanced in microglia and astrocytes compared to neurons, suggesting their major contribution to MPS IIIA neuropathology. To characterize HS accumulation in brain relevant cell types in a disease relevant setting, we generated iPSC SGSH KO clones and differentiated these to neurons, microglia and astrocytes with the purpose to evaluate cell specific HS accumulation and degradation after *in vitro* treatment with recombinant SGSH. Thus, we have developed *in vitro* assays that can be used to evaluate the efficacy of various SGSH ERTs in different brain cells paving the way for efficient drug development for patients living with MPS IIIA.

Disclosures: **A. Asenjo Martinez:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **C. Guijas:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **A. Nielsen:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **K. Russ:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **F. Sotty:** A. Employment/Salary (full or part-time):; H.

Lundbeck A/S. **K. Fog:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **M. Rohe:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.08/WW64

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH PIONEER DP1OD025535 (to V.G.)
NIH BRAIN Initiative Armamentarium UF1MH128336 (to V.G. and T.F.S.)
Caltech Merkin Institute for Translational Research (to V.G. and T.F.S)
Beckman Institute for CLARITY, Optogenetics and Vector Engineering Research (CLOVER) for technology development and dissemination (to V.G. and T.F.S.)

Title: Cell surface interactomes of primate brain-enriched engineered viral vectors reveal determinants of blood-brain barrier permissivity

Authors: *T. F. SHAY[1], X. CHEN[1], S. JANG[1], T. J. BRITTAIN[1], B. WALKER[2], C. TEBBUTT[2], D. A. WOLFE[1], C. M. AROKIARAJ[1], E. E. SULLIVAN[1], X. DING[1], Y. LEI[1], M. R. CHUAPOCO[1], V. GRADINARU[1];
[1]Caltech, Pasadena, CA; [2]Charles River Labs., Chinley, United Kingdom

Abstract: The blood-brain barrier (BBB) is a complex cellular structure whose behavior in both health and disease remains incompletely understood. Directed evolution of adeno-associated virus (AAV) vectors with enhanced BBB crossing suggests that a broader array of proteins may serve as efficient transcytosis receptors than previously suspected[1]. Understanding the properties and mechanisms of effective BBB receptors may enable the design of improved molecules to study and treat the brain and allow surveillance of known pathogens for potential evolution of brain penetrance. Here, we adapted an unbiased human cell microarray platform to determine the extracellular and cell surface interactomes of natural and engineered AAVs. We identified a naturally-evolved and serotype-specific interaction of AAV9 with possible roles in host immune modulation as well as lab-evolved interactions specific to engineered capsids that govern blood-brain barrier crossing in non-human primates. Our unbiased screening approach also allowed us to identify off-tissue binding interactions of engineered brain-enriched AAVs that may inform vector peripheral tropism and side effects. These results allow confident application of engineered AAVs in diverse model organisms and unlock future target-informed engineering of improved viral vectors for non-invasive genetic access to the brain. They also reveal potential active roles for AAV capsids in host immune modulation and fundamental biology of the blood-brain barrier.

1. Shay, T. F. et al. Primate-conserved carbonic anhydrase IV and murine-restricted LY6C1 enable blood-brain barrier crossing by engineered viral vectors. Sci. Adv. 9, eadg6618 (2023).

Disclosures: **T.F. Shay:** Other; The California Institute of Technology has filed a patent for this work with T.F.S., X.C., and V.G. listed as inventors. **X. Chen:** Other; The California Institute of Technology has filed a patent for this work with T.F.S., X.C., and V.G. listed as inventors. **S. Jang:** None. **T.J. Brittain:** None. **B. Walker:** A. Employment/Salary (full or part-time):; B.W. is an employee of Charles River Laboratories. **C. Tebbutt:** A. Employment/Salary (full or part-time):; C.T. is an employee of Charles River Laboratories.. **D.A. Wolfe:** None. **C.M. Arokiaraj:** None. **E.E. Sullivan:** None. **X. Ding:** None. **Y. Lei:** None. **M.R. Chuapoco:** None. **V. Gradinaru:** Other; V.G. is a cofounder and board member of Capsida Biotherapeutics, a fully integrated AAV engineering and gene therapy company, The California Institute of Technology has filed a patent for this work with T.F.S., X.C., and V.G. listed as inventors.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.09/WW65

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development and Refinement of Surgical Procedures for Administration of Therapeutics to the Peripheral Nervous System with Considerations for Large Animal Species Selection

Authors: D. LINIHAN, J. GESAMAN, S. WILSON, *B. GUNTER;
Charles River Labs., Mattawan, MI

Abstract: Delivery of therapeutics with peripheral nervous system (PNS) indications such as neuralgia, nerve injury, and neuropathies, may require a surgical approach for targeted administration. Often in preclinical drug assessment, the selection of the animal model utilized is based on comparable anatomy to humans. However, there are subtle anatomical differences between large animal models that must be considered prior to species selection. Recently, Charles River-Mattawan developed techniques for administering therapeutics within the PNS, specifically the dorsal root ganglia (DRG). The Non-Human Primate (NHP) has been the preferred large animal model for direct administration of therapeutics to the DRG, primarily due to PNS anatomic similarity compared to humans. However, the size of the NHP DRG limits the volume of therapeutic that can be administered, therefore an alternate large animal model is required. Administration to the lumbar DRG in farm swine is advantageous as the DRG are comparable in size to humans, thus a representative clinical dose volume can be delivered. Furthermore, farm swine are more cost effective and easier to procure than NHPs. However, farm swine present surgical challenges (in comparison to the NHP procedure) as accessing the lumbar DRG involves significant tissue dissection, a higher rate of intra-operative bleeding, and increased anesthesia time. Pilot work was initiated in farm swine to assess suitability as an alternate large animal model. To perform the hemilaminectomy necessary to access the DRG,

extensive tissue dissection using electrocautery is required as well as the use of intra-operative intravenous administration of an antifibrinolytic agent (tranexamic acid) and hypotensive anesthesia to decrease procedural bleeding. Assessments of the maximum achievable dose volume to the L4 and L5 DRG of farm swine were then evaluated. Successful administration of up to 200 µL via bilateral concurrent injections without leakage from the DRG and animal recovery out to 8 days post injection indicates farm swine are a suitable model for intra-DRG administration. Future studies utilizing this procedure will assess distribution in the target DRG as well as systemic distribution when adjusting for dose volume and ideal flow rate.

Disclosures: **D. Linihan:** None. **J. Gesaman:** None. **S. Wilson:** None. **B. Gunter:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.10/WW66

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Blood-brain barrier Penetrating siRNA Nanomedicine Biodistribution throughout Real-time Tracking in Mouse Cortex under the Live Animal Body

Authors: H. KWON[1], K. AKYILDIZ[1], H. KIM[1], ***S. HONG**[2];
[1]IVIM Technol., Seoul, Korea, Republic of; [2]KAIST, Daejeon, Korea, Republic of

Abstract: Neurodegenerative diseases, including Alzheimer's or Parkinson's Diseases, have been reported to announce no precise pathological mechanisms yet. This reason leads to tardy progress in developing neurodegenerative disease therapy. These huddles originated from various physiological barriers, especially the blood-brain barrier (BBB), blocking the brain's effective therapeutic substance target region. For these reasons, some research has been performed on developing therapeutical agents regarding small molecules for BBB penetration. Small interfering RNA (siRNA), one of the small molecules, is being raised in the neurogenerative disease field according to high targeting specificity, low effectiveness doses, and a relatively simple drug development process. However, research on the systematic and effective methods for delivering siRNA to the brain is still needed. We investigated the distribution of siRNA penetrating BBB. Nano-encapsulation technology was applied to increase the delivery efficiency. After injecting the nano-encapsuled siRNA through intraperitoneal cavity to cranial imaging window implanted model after 24 hours fast to boost BBB penetration with glycemic control system, we imaged with intravital microscope at the several timepoints from before drug injection to 72 hour-timepoint. In this study, about 40 minutes after boosting, the siRNAs started to be observed in brain cortex, outside of the brain vessel. After 24 hours, all siRNAs penetrated the vessel to the cortex and only small residual siRNAs are observed in the blood vessel as dot. So, we could concluded that only nano-encapsuled siRNAs have difficulty to penetrate the BBB, but with fast and glycemic control system, the siRNAs were more scattered in the brain tissue.siRNA nanomedicine is becoming a new trend in the field of

biopharmaceutical compounds for neurodegenerative diseases. Still, difficulties still remained in solving the problem of delivery of the drugs to show therapeutic effects in the brain. However, we confirmed in a live animal model that nano-encapsuled siRNA boosts BBB penetration using a glycemic control system with intravital microscope. In other words, we are confident that our research results will ultimately become an important starting point for the development of drugs that can treat neurodegenerative diseases.

Disclosures: **H. Kwon:** None. **K. Akyildiz:** None. **H. Kim:** None. **S. Hong:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.11/WW67

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Mannitol-induced blood brain barrier (BBB) disruption in rat induces no behavioral deficits and offers a ten minute dosing window

Authors: R. IMMONEN[1], J. UHARI-VÄÄNÄNEN[1], F. MAN[2], S. HALONEN[1], T. MIETTINEN[1], T.-K. STENIUS[1], K. LEHTIMÄKI[1], J. RYTKÖNEN[1], ***A. NURMI**[1], A. SMITH[2], S. BÄCK[1];
[1]Charles River Discovery Services, Kuopio, Finland; [2]GE HealthCare, Chalfont St Giles, United Kingdom

Abstract: Controlled blood brain barrier (BBB) disruption serves as a route for targeted drug administration as well as a model for disease states where BBB is compromised. Such a model is an imperative testing platform for drugs targeted at brain diseases where leaky BBB can be a comorbidity, e.g., after trauma, stroke, or tumors. The aim of this study was to 1) optimize the surgery and infusion paradigm for a rat model for mannitol induced BBB disruption, 2) assess the time window of BBB opening, 3) characterize the behavioral outcome on day 7, and 4) assess neurofilament (NfL) levels in cerebrospinal fluid (CSF) on day 8.
BBB disruption in male Sprague Dawley rats was achieved by an intracarotic artery (i.a.) mannitol infusion. BBB disruption was verified by comparing the contrast enhancement using magnetic resonance imaging (MRI) on a 11.7T system (Bruker BioSpec), and ex-vivo by Evans Blue (EB) staining. For contrast enhancement, a gadolinium-based contrast agent (Gadovist) was injected via the tail vein 2 min prior and 2% EB 1 min prior to the i.a. mannitol infusion to ensure their availability in the blood stream at the time of BBB disruption. The rats were imaged immediately after the mannitol infusion, followed by sectioning of the brains for detection of EB staining. For assessment of BBB disruption duration, contrast agents (Gd+EB, tail vein) were dosed with 5-, 10- or 20 min delay after the mannitol infusion (n=2 each), and the amount of contrast agent and dye penetrating the BBB into the parenchyma was evaluated. To study potential neurotoxic effects of the BBB disruption, the mannitol model was induced in 18 rats and BBB disruption verified by MRI. On day 7 after the infusion, behavioral tests (20-point

Neuroscore and open field) were performed, and one day later CSF was collected for NfL analysis.
The extent and intensity of the contrast enhancement corresponded well with EB staining. BBB permeability was observed with both contrast enhancement and EB staining after a 5-10 min dosing delay. Negligible staining was detected with a 20 min dosing delay. No behavioral deficits were observed 7 days after the mannitol infusion. NfL concentration in the CSF of mannitol-infused animals was 1208 ± 2358 pg/mg (n=18) compared to 300-500 pg/mg in naïve rats (n=2). NfL levels <500 pg/mg were observed in 9/18 rats, and the animals with largest BBB disruption areas had NfL >1000 pg/mg (3/18).
In summary, the established clinically relevant model can be used for safe, targeted drug delivery through BBB with a dosing window of 10 min. The described mannitol infusion itself was not observed to cause any adverse effects but shows elevated NfL levels.

Disclosures: **R. Immonen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **J. Uhari-Väänänen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **F. Man:** A. Employment/Salary (full or part-time):; GE HealthCare. **S. Halonen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **T. Miettinen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **T. Stenius:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **K. Lehtimäki:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **J. Rytkönen:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **A. Nurmi:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **A. Smith:** A. Employment/Salary (full or part-time):; Charles River Discovery Services. **S. Bäck:** A. Employment/Salary (full or part-time):; Charles River Discovery Services.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.12/WW68

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

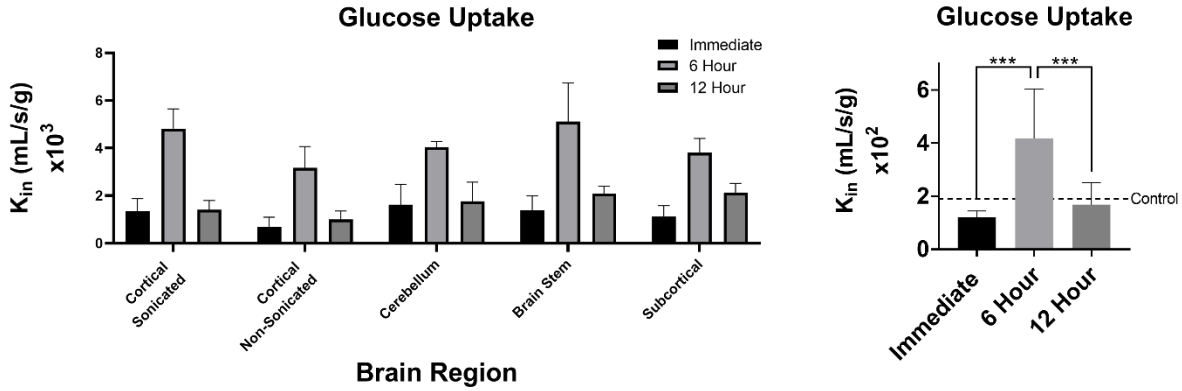
Support: 1F99CA264445-01
P20GM121322
P20GM121322-03S1
Mylan Chair Endowment Fund
METAvivor

Title: Focused ultrasound induced Blood-brain barrier opening alters tight junction expression and efflux transport

Authors: *T. ARSIWALA[1], K. BLETHEN[2], C. WOLFORD[2], B. KIELKOWSKI[2], P. WANG[2], M. RANJAN[3], J. CARPENTER[3], V. S. FINOMORE, Jr[2], A. REZAI[3], P. R. LOCKMAN[2];

[1]Blanchette Rockefeller Neurosci. Inst., Morgantown, WV; [3]Rockefeller Neurosci. Inst., [2]West Virginia Univ., Morgantown, WV

Abstract: The blood-brain barrier (BBB) is an important physical and functional barrier that prevents drugs from entering the brain. In recent years, low intensity focused ultrasound (LiFU) has emerged as a method to enhance drug efficacy in the central nervous system (CNS). Focused ultrasound presents a distinctive and noninvasive method of temporarily disrupting the BBB, thereby facilitating enhanced drug accumulation in targeted regions of the brain. Initial studies focused on determining optimal parameters for safety and therapeutic feasibility. Preclinical research paved the way for clinical trials using LiFU in various CNS diseases. However, the mechanistic effects of LiFU on the BBB and downstream processes remain poorly understood. This study investigated the relationship between LiFU-induced permeability and secondary changes in the BBB over time. We examined changes in vascular volume and glucose uptake at different time intervals after LiFU. We found an immediate increase in permeability in cortical regions, followed by a secondary increase at 6 hours post-sonication, which returned to baseline at 12 hours. Glucose uptake also increased at 6 hours, indicating a heightened energy demand for repair processes. Further, we observed that P-gp function declined gradually after LiFU, peaking at 6 hours. We also found an increase in inflammatory cytokines at 6-hr post-sonication. This transient change in tight junction protein expression could be used to enhance drug delivery through the BBB.



Disclosures: T. Arsiwala: None. **K. Blethen:** None. **C. Wolford:** None. **B. Kielkowski:** None. **P. Wang:** None. **M. Ranjan:** None. **J. Carpenter:** None. **V.S. Finomore:** None. **A. Rezai:** None. **P.R. Lockman:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.13/WW69

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Focused Ultrasound Surgery Foundation

Title: Focused ultrasound achieves delivery of mrna therapy to the mouse brain for lysosomal storage disorder niemann pick type c

Authors: *P. NOWLIN[1], B. FUNK[2], Y. ZHANG[1], C. HUNG[2], O. BODAMER[2], N. TODD[1]; [1]Radiology, Brigham and Women's Hosp., Boston, MA; [2]Genet., Boston Children's Hosp., Boston, MA

Abstract: The objective of this study is to achieve the delivery of lipid-nanoparticle (LNP) packaged NPC1 protein mRNA (NPC-modRNA) into the brain for treatment of the lysosomal storage disorder Niemann Pick Disease Type C (NPC). LNP-packaged mRNA therapies have shown the ability to increase their target protein's expression following systemic injection, in peripheral organs, but are unable to cross the blood brain barrier (BBB). Focused Ultrasound (FUS) blood brain barrier opening is a promising treatment for enhancing the delivery of normally non-penetrant drugs to specific brain structures in a focused region of only a few millimeters.We explored the feasibility of this by treating 24 NPC1 knockout (KO) mice in four experimental groups (n=6 per group). Equal numbers of male and female mice were divided into groups for FUS vs NO FUS, and NPC1 vs eGFP (enhanced green fluorescent protein). FUS groups received sonication prior to retro-orbital (R.O) injection of therapy and NO FUS groups did not. Each mouse received an injection at 7 and 9 weeks old, of either NPC1 or eGFP targeted mRNA therapy at a dose of 1.0 mg/kg. BBB opening was targeted to the cerebellum and microbubbles (20 uL/kg) were administered via tail vein catheter. FUS sonications were applied at 10ms bursts and 1Hz repetition frequency over 120 seconds at 0.32MPa; BBB opening was confirmed with contrast MRI following treatments. We observed higher mortality in the FUS group - it is unclear whether this was due to formulation or overall stress of the procedure. Mice were sacrificed 48 hours following the last injection, brain and peripheral organs were perfused, extracted, and prepared for immunofluorescent analysis. Western blots showed no cerebellar expression of NPC1 or GFP in the NO FUS group, while expression of each was observed in the spleen. Data analysis for the FUS group is ongoing, but we expect similar results to our pilot study of wild-type mice treated with FUS (n=5) that showed GFP expression following cerebellum targeted FUS with similar parameters and LNP-eGFP mRNA therapy R.O injection following treatment.Cerebellar ataxia is a consistent phenotype of NPC KO mice, this is due to progressive Purkinje cell loss. To evaluate therapy mediated cell rescue, sections of cerebellum underwent Nissl and DAPI staining, and Purkinje cells were manually counted. Full analysis is ongoing.

Disclosures: P. Nowlin: None. **B. Funk:** None. **Y. Zhang:** None. **C. Hung:** None. **O. Bodamer:** None. **N. Todd:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.14/WW70

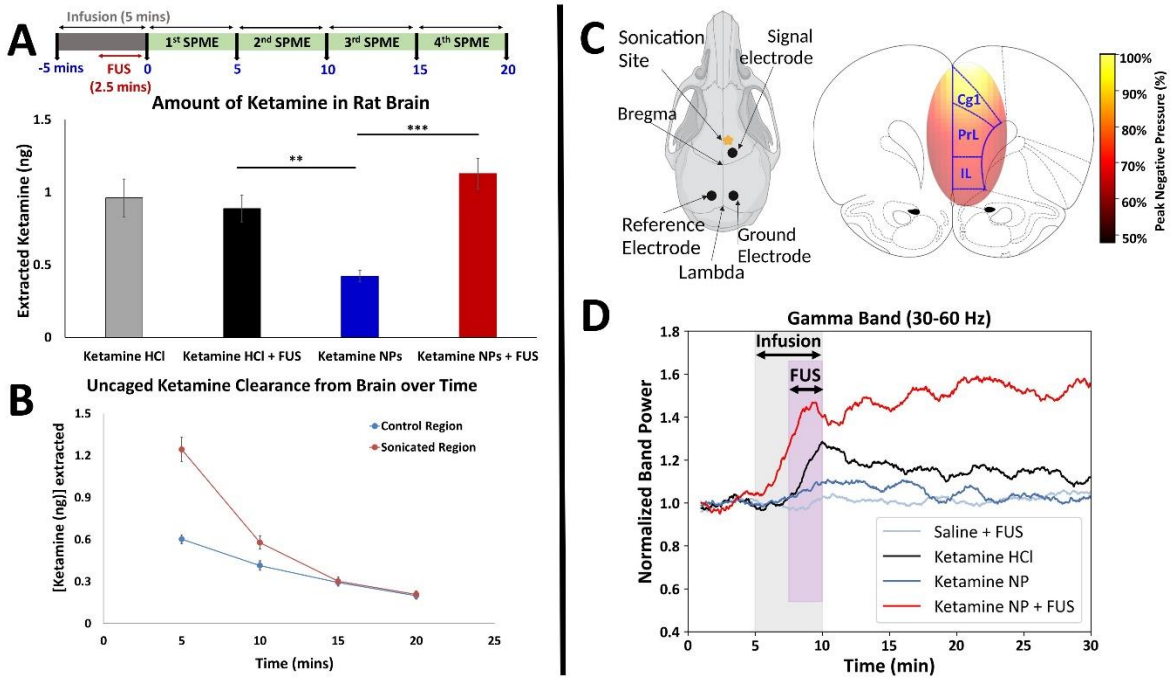
Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSF GRFP
NINDS BRAIN Initiative RF1 MH114252
NINDS BRAIN Initiative UG3 NS114438
NINDS HEAL Initiative UG3 NS115637
Stanford Wu Tsai Neurosciences Institute
NIH/NIMH

Title: Ultrasonic ketamine uncaging in rat medial prefrontal cortex induces an electrophysiologic pattern that outlasts its pharmacokinetics

Authors: *B. YU[1], K. SINHA ROY[1], M. PUROHIT[1], Y. XIANG[1], J. B. WANG[1,3], M. AZADIAN[1], A. K. TAOUBE[1], A. A. KWAN[1], D. GOMEZ LOPEZ[4], R. D. AIRAN[2]; [2]Neuroradiology, [1]Stanford Univ., Stanford, CA; [3]Dept of Anesthesia and Critical Care Med., Johns Hopkins Hosp., Baltimore, MD; [4]Vanderbilt Univ., Nashville, TN

Abstract: Ketamine, a dissociative anesthetic and recreational drug, has seen recent interest due to its approval as an antidepressant. To elucidate how ketamine affects plasticity in different brain regions, and how physiologic biomarkers may relate to that plasticity, we used a nascent technology for noninvasive localized drug administration: ultrasonic drug uncaging, in which focused ultrasound (FUS) induces drug release from IV-administered nanoparticles (NP) in target brain regions. We first used solid phase microextraction (SPME) to quantify brain ultrasonic ketamine uncaging. Without FUS, there was minimal brain ketamine exposure following 1.5 mg/kg ketamine NP infused IV over 5 min; but with FUS (250 kHz; 0.9 MPa in situ peak neg. pressure; 50 ms/5 Hz PRF/2.5 min) brain ketamine increased to match that of dose-matched ketamine-HCl (A; N=4-5/group, **:$p<0.01$, ***:$p<0.001$). Uncaging yielded higher brain ketamine than the contralateral control site indicating that uncaging was spatially resolved to <5 mm (B). The uncaged ketamine cleared with expected timing, equalizing to control at 15 min post FUS (B; N=10/group). We then applied ketamine uncaging to the rat medial prefrontal cortex (mPFC) while recording electrocorticography (ECoG; C) in adult male Long Evans rats during awake restraint stress. While saline infusion and FUS produced no specific ECoG change, a minimal increase in gamma (30-60 Hz) band power was seen with 0.75 mg/kg NP infused IV over 5 min without FUS (D). A more prominent increase in gamma band power was seen with 0.75 mg/kg ketamine-HCl infusion, which decreased proportionate to the clearance of ketamine from the brain (B). Surprisingly, a dose-matched NP infusion with FUS applied to the mPFC not only produced even higher gamma band power, but this power was sustained through 20 min post FUS/infusion, surpassing the pharmacokinetics of ketamine in the brain (B,D). These results indicate that the mPFC is uniquely sensitive to the physiologic plasticity induced by ketamine in an acute stress model, and that gamma band EEG/ECoG may serve as a biomarker for these changes.



Disclosures: **B. Yu:** None. **K. Sinha Roy:** None. **M. Purohit:** None. **Y. Xiang:** None. **J.B. Wang:** None. **M. Azadian:** None. **A.K. Taoube:** None. **A.A. Kwan:** None. **D. Gomez Lopez:** None. **R.D. Airan:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.15/WW71

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of an Electrochemiluminescence-Based Pharmacokinetics Assay for an Antisense Oligonucleotide Drug for the Treatment of Amyotrophic Lateral Sclerosis

Authors: ***S. B. HARKINS**, A. SZABOLCS, I. SHIN, T. J. BREAK, J. DEBAD, J. N. WOHLSTADTER;
R&D, Meso Scale Diagnostics, Rockville, MD

Abstract: Antisense oligonucleotide (ASO) drugs provide treatment options for some debilitating diseases that were previously considered undruggable. One example of such an ASO drug is tofersen, which is used in the treatment of Amyotrophic Lateral Sclerosis. With the emergence of this new class of drugs comes the challenge of building highly sensitive, reliable, and high-throughput pharmacokinetics (PK) assays. Here, we demonstrate the feasibility of developing a sensitive, extraction-free, plate-based assay for the detection of the ASO drug

tofersen in biological fluids and tissue samples. A dual-probe hybridization assay with an antibody-based detection method was developed using an electrochemiluminescent (ECL) platform to achieve high sensitivity. In brief, a biotin-labeled DNA probe complementary to a portion of the analyte was coated on the surface of a streptavidin plate, and a digoxigenin-labeled detection probe was hybridized to the analyte in biological matrices with a thermal cycler. Samples were then added to the plate where the analyte-detection probe complexes were bound by the coated capture probes. A SULFO-TAG labeled anti-digoxigenin antibody and read buffer were used for the generation of the ECL signal. In addition to this standard assay format, an augmented reporter system was established (N-PLEX ULTRA) to further improve assay sensitivity through signal amplification. We found that the use of locked nucleic acids in the probes was essential for improving the detection of this ASO drug. After further optimization of assay conditions, our test method showed excellent sensitivity for tofersen, with a lower limit of detection (LLOD) between 30 and 70 fM (0.2-0.5 pg/mL) in diluent and rabbit plasma. In rat liver lysates, high, mid, and low analyte spikes demonstrated recoveries between 88-105% against a plasma-based calibration curve and linearity of dilution over the dynamic range of the assay. The use of the N-PLEX ULTRA system enabled a further improvement in sensitivity, with LLODs approaching 1 fM (7 fg/mL) in matrices. This ECL-based assay method offers a simple, highly sensitive, extraction-free workflow for PK assay development of ASO drugs. The short, on-instrument time makes this assay compatible with high-throughput screening. The assay is compatible with highly modified oligonucleotide drugs, and the improvement in sensitivity with the N-PLEX ULTRA format sets a new standard for ultrasensitive oligonucleotide PK assay development.

Disclosures: **S.B. Harkins:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **A. Szabolcs:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **I. Shin:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **T.J. Break:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **J. Debad:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics. **J.N. Wohlstadter:** A. Employment/Salary (full or part-time):; Meso Scale Diagnostics.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.16/WW72

Topic: I.04. Physiological Methods

Support: PhD-fellowship strategisch basisonderzoek 1S93622N

Title: Bidirectional fluidic interfacing in rodents.

Authors: ***S. MARCIGAGLIA**[1], R. DE PLUS[1], C. VANDENDRIESSCHE[2], R. VANDENBROUCKE[2], S. HAESLER[3];

[1]NERF, Leuven, Belgium; [2]VIB, Gent, Belgium; [3]Neuroelectronics Res. Flanders, Leuven, Belgium

Abstract: Fluidic access to the brain is a requirement for many neuroscientific studies. This can include the delivery of chemicals or drugs to specific regions or the sampling of ventricular cerebrospinal fluid (CSF). However, the brain's innate defense system to xenobiotics is a huge obstacle in this bi-directional exchange. The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB), in particular, restrict access so effectively that 98% of small-molecule drugs and nearly all large-molecule drugs cannot cross from the blood circulation[1]. Convection-enhanced delivery (CED) is one technique that aims to circumvent the problem by injecting the desired chemical directly in contact with the tissue. Beside the invasiveness, this technique suffers from one major problem, the spread of the infusate can be affected by many factors and is often biased towards the insertion track (a phenomenon known as backflow). We have here developed a miniaturized delivery system that combines all known desirable features for brain infusions. This 140um catheter was first tested in a brain phantom model (agarose gel) and shown to perform better in terms of backflow and overall distribution than an equally sized control catheter. Furthermore, *in vivo* infusions in the striatum and Anterior Olfactory Nucleus (AON) of mice (n=12) showed respectively better predictability and targetability. We further adapted the system for chronic implantation. This results in a plug-and-play delivery system, which, in a chemogenic odor-perception task, proved to be less disruptive to neural circuitry than the previously available method. In order to improve the fluidic access in the opposite direction, we also developed a catheter for chronic CSF sampling in rodents. We quantified the presence of nucleic acids and a range of cytokines in multiple samples and established long-term (200 days), high-frequency (daily) intraventricular CSF sampling. A newly developed implantable chamber also allows the co-housing of animals without damage to the catheter. We also proved that the sampling procedure is not damaging to the surrounding tissue by evaluating the presence of blood in CSF before and after BBB disruption. In conclusion, this work presents considerable progress towards the achievement of a minimally invasive bidirectional platform for both chronic drug delivery and CSF sampling in rodents.
1.Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx*. 2005;2(1):3-14. doi:10.1602/neurorx.2.1.3

Disclosures: S. Marcigaglia: None. **R. De Plus:** None. **C. Vandendriessche:** None. **R. Vandenbroucke:** None. **S. Haesler:** None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.17/WW73

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Brain distribution by use of MetaQuant microdialysis in rat and minipig

Authors: *G. FLIK[1], K. HUININK[1], **M. HEINS**[1], G. QUESSEVEUR[2], M. VERSET[2], C. BUNDGAARD[3];
[1]Charles River Labs., Groningen, Netherlands; [2]Charles River Labs., Lyon, France; [3]Lundbeck A/S, Valby, Denmark

Abstract: The development process of new chemical entities targeting the CNS has an intrinsic need for assays that allow a clear evaluation of the biodistribution. Microdialysis is a minimally invasive sampling technique that allows for accurate determination of compound levels in the extracellular fluid of the brain. The use of intracerebral microdialysis in rodents is well-established, but development in non-rodents has been limited so far. To gain a better understanding of the NCE biodistribution, we have previously developed the quantitative MetaQuant microdialysis technology and can combine this technique with blood collection and CSF collection in rodents. We have now extended two of these sampling capabilities, MetaQuant microdialysis and simultaneous blood collection, to the Göttingen minipig, a freely-moving non-rodent model. We determined the biodistribution of the brain penetrant anti-epileptic carbamazepine in both rats and minipigs. To this end, the rats underwent surgery to position a probe and a jugular vein cannula for microdialysate and blood sample collection. The minipigs had a guide and custom-made protection and collection box as well as a jugular vein cannulation and vascular access port surgically placed. After the appropriate recovery time for each species the probes were connected to microperfusion pumps and sample collection was initiated. Once flow speed was confirmed and the system equilibrated, the test compound was dosed and microdialysate samples were collected in 30-minute intervals. In addition, blood samples were collected at specified timepoints and processed to plasma. All collected samples were analysed for carbamazepine concentrations. Levels of carbamazepine in rat and minipig MetaQuant microdialysate and plasma samples over time demonstrate the added value of the microdialysis technology itself. The comparison of the levels in the two species illustrates how this new non-rodent minipig microdialysis model can bring additional value during the preclinical research phase. Potential differences or similarities in biodistribution between these species can be used to support translation to human biodistribution and brain penetration in the clinical phase.

Disclosures: **G. Flik:** A. Employment/Salary (full or part-time):; Employed by CRL. **K. Huinink:** A. Employment/Salary (full or part-time):; Employed by CRL. **M. Heins:** A. Employment/Salary (full or part-time):; Employed by CRL. **G. Quesseveur:** A. Employment/Salary (full or part-time):; Employed by CRL. **M. Verset:** A. Employment/Salary (full or part-time):; Employed by CRL. **C. Bundgaard:** A. Employment/Salary (full or part-time):; Employed by Lundbeck.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.18/WW74

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: A Novel Therapy for Glioblastoma Using Human Stem Cell Derived Interneurons as Therapeutic Vectors

Authors: *W. MANLEY;
CHOP, PHILADELPHIA, PA

Abstract: Glioblastoma Multiforme (GBM), while rare in children, is the third most common CNS tumor diagnosisand represents the majority of all malignant CNS diagnoses. Prognosis remains poor with a 5.6% 5-yearaverage survival rate, since GBMs are treatment resistant and highly invasive and vascularized. Thesecharacteristics are attributed in part to GBM's overexpression of the chemoattractant stromal derivedfactor 1(SDF-1)/CXCL12. To improve the ever-static survival rate of the GBM diagnosis, there is a needfor novel therapeutics. Manipulation of the SDF-1 chemoattractant system may prove to serve as anecessary novel therapeutic advancement. SDF-1 interacts with the chemokine receptor CXCR4 withinthe tumor microenvironment to mediate tumor growth and vasculaturization. Since the SDF-1/CXCR4signaling pathway is a powerful chemoattractant for the migration of cortical inhibitory neurons (INs)during brain development, we hypothesize that cortical interneuron transplants near the periphery ofGBMs known to express SDF-1 will migrate into that periphery. We will test this hypothesis in vivo byinjecting U87-MG cells into neonatal nude mice, transplanting INs after tumor growth, and imaging todetect migration and ex vivo using mouse cortical interneurons and GBM cell lines imaged via Incucyte.

Disclosures: W. Manley: None.

Poster

PSTR509. Drug Delivery

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR509.19/WW75

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: VA RR&D RX002305

Title: Targeted drug delivery using leukomimetic nanoparticles in experimental autoimmune neuritis, a model for Guillain-Barre Syndrome.

Authors: *C. KAUR[1,3,4], M. CABE[1,4], K. LANGERT[2,4];
[1]Loyola Univ. Chicago, Maywood, IL; [2]Dept. of Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, maywood, IL; [3]Pharmacol. and Neurosci. graduate program, Loyola Univ. Chicago, Stritch Sch. of Med., Maywood, IL; [4]Res. service, Edward Hines Jr. VA Hosp., Hines, IL

Abstract: Acute inflammatory demyelinating polyneuropathy (AIDP) is a subtype of Guillain-Barré syndrome (GBS) and the leading cause of acute autoimmune flaccid paralysis. Localized inflammation of the microvasculature within affected nerves (the blood-nerve barrier (BNB)) enables the transendothelial migration of circulating autoreactive leukocytes and is a key

pathogenic event. While experimental autoimmune neuritis (EAN), a well-established rat model of GBS, has increased our understanding of AIDP pathophysiology, treatment options remain palliative and ineffective. To address this need for novel treatments, previous studies demonstrated that high-dose, systemic administration of lovastatin therapeutically attenuates EAN. Lovastatin is generally safe, and well-tolerated; however, the high doses required to achieve anti-inflammatory pleiotropic effects are associated with serious side effects. Targeted delivery using biodegradable nanoparticles (NPs) is a promising strategy to administer therapeutics to affected tissue while avoiding off target toxicity. Our current work focuses on development of a statin-loaded NP coated with macrophage-derived plasma membrane vesicles (mNPs) as a novel drug delivery system that will promote accumulation of intravenously administered statins in inflamed nerves. We hypothesize that mNPs will use mechanisms similar to autoreactive leukocytes to migrate across the BNB and access affected nerves. We culture rat NR8383 macrophages in high yield (3x10^8) and gently lyse cells using nitrogen cavitation. Plasma membrane vesicles are isolated by differential centrifugation and are shown to retain CD11 and CD18 proteins using western immunoblot. We surface functionalize polymeric NP cores with macrophage plasma membrane using sonication and demonstrate colloidal stability compared with bare NPs. We demonstrate specificity of mNPs for inflamed over quiescent endothelium *in vitro* using a functional adhesion assay and primary peripheral nerve microvascular endoneurial endothelial cells (PNMECs). Preliminary *in vivo* experiments demonstrate specificity of I.V.-administered mNPs to inflamed nerves in rats with EAN compared with healthy controls.

Disclosures: C. Kaur: None. **M. Cabe:** None. **K. Langert:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.01/WW76

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

Support: NSF DBI2015317
NSF IIS1704436

Title: A Neural Circuitry Model for Aplysia Feeding Control - Model Development and Verification

Authors: *Y. LI[1], V. A. WEBSTER-WOOD[5], J. P. GILL[2], H. CHIEL[2,3,4], G. SUTTON[6], R. QUINN[1];
[2]Dept. of Biol., [3]Dept. of Neurosciences, [4]Dept. of Biomed. Engin., [1]Case Western Reserve Univ., Cleveland, OH; [5]Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA; [6]Lincoln, Univ. of Lincoln, LINCOLN, United Kingdom

Abstract: As a basic behavior, feeding has been widely studied in animal motor control. Understanding the mechanisms underlying feeding can further shed light on how animals achieve multifunctional, adaptive, and robust behaviors in an ever-changing environment. To study this topic, we focus on *Aplysia* californica, a species of sea slug which can generate complex feeding behaviors with a relatively small number of neurons. We developed a computational model of *Aplysia* feeding control circuitry based on known synaptic connections and measured neuronal activities. The model contains buccal motor neurons B31, B8, B38, B7, B6/B9, buccal interneurons B63, B64, B52, B34, B40, B65, B30, B20, B4, and cerebral-buccal interneurons CBI-2, CBI-4 and CBI-3, which have been identified as key elements for *Aplysia* feeding control. We adopted Synthetic Nervous Systems (SNSs) to model the overall neural dynamics. An SNS is a neural network model inspired by the biophysics of neurons. It treats synaptic inputs to a neuron as conductance changes and uses neural activity to reflect temporal firing frequency. Neurons in the model are organized into three layers. In the motor neuron layer and buccal ganglion layer, neurons are further divided into nine subnetworks according to their functions. In addition, the model integrates sensory feedback loops. Such feedback mechanisms are considered vital to the generation of multifunctional and adaptive behaviors. We have quantitively compared the SNS model, a spiking model, and animal data for B64, B4, B34, and B52. The results demonstrated that the SNS model captures the intrinsic dynamics of neurons in the animal. We qualitatively showed the model can generate two ingestive behaviors (biting and swallowing) and one egestive behavior (rejection) when integrated with a biomechanical model of *Aplysia*'s feeding apparatus. Moreover, we showed the integrated neuromuscular model shares similar behavior durations with animals for in vivo biting, ingestive patterns generated by an isolated ganglion, swallowing loose seaweed, and swallowing unbreakable seaweed. Comparison of the simulated swallowing behavior with animal data further showed that the kinetics and neural activities of the model are similar to those of animals. These results suggested that the model reflects the dynamics of *Aplysia* feeding control circuitry. This model can be used as a platform to test neuromechanical hypotheses, and predictions generated by the model can guide future experimental work.

Disclosures: Y. Li: None. **V.A. Webster-Wood:** None. **J.P. Gill:** None. **H. Chiel:** None. **G. Sutton:** None. **R. Quinn:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.02/WW77

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

Support: NSF DBI 2015317

Title: Multimodal Parameter Inference for a Canonical Motor Microcircuit Controlling Rat Hindlimb Motion

Authors: *C. JACKSON[1], R. QUINN[1], M. C. TRESCH[2], M. CHARDON[3], C. J. HECKMAN[4]; [1]Case Western Reserve Univ., Cleveland, OH; [2]Biomed. Eng, Physical Med. and Rehab, Physiol., [4]Dept. of Physical Therapy and Human Movement Sci., [3]Northwestern Univ., Chicago, IL

Abstract: This work explored synaptic strengths in a computational neuroscience model of a controller for the hip joint of a rat which consists of Ia interneurons, Renshaw cells, and the associated motor neurons.
This circuit has been referred to as the Canonical Motor Microcircuit (CMM). It is thought that the CMM acts to modulate motor neuron activity at the output stage. We first created a biomechanical model of a rat hindlimb consisting of a pelvis, femur, shin, foot, and flexor-extensor muscle pairs modeled with a Hill muscle model. We then modeled the CMM using non-spiking leaky-integrator neural models connected with conductance-based synapses. To tune the parameters in the network, we implemented an automated approach for parameter search using the Markov chain Monte Carlo (MCMC) method to solve a parameter estimation problem in a Bayesian inference framework. As opposed to traditional optimization techniques, the MCMC method identifies probability densities over the multidimensional space of parameters. This allows us to see a range of likely parameters that produce model outcomes consistent with animal data, determine if the distribution of likely parameters is uni- or multi-modal, as well as evaluate the significance and sensitivity of each parameter. We then add the descending commands from pattern generator networks and perform a perturbation analysis to compare the MCMC results.

Disclosures: C. Jackson: None. **R. Quinn:** None. **M.C. Tresch:** None. **M. Chardon:** None. **C.J. Heckman:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.03/WW78

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

Support: NSF DBI 2015317

Title: Advancing an SNS-driven bipedal balance controller

Authors: *J. S. MCNEAL, A. J. HUNT;
Portland State Univ., Portland, OR

Abstract: The task of explaining human balance using engineering control theory has been explored several times over the last few decades. Some of these studies have indicated that

balance control can be explained by a traditional position-derivative feedback controller operating on errors from the vision, vestibular, and proprioceptive sensory systems. However, there are few theories of how the nervous system might be arranged to produce this control behavior, and how the nervous system might weight the importance of competing demands, such as energy expenditure and postural alignment. In this work, we present the results of a model for human balance control tested on a synthetic nervous system (SNS) controlling an inverted pendulum. The modeled body is actuated by linear-Hill type muscle models and mimics human balance trials reported in the literature. Our specific advancements in this work are the deployment and coordinated control of simulated muscles, implementation of Ib positive force feedback loops, and testing of the relative importance of energy expenditure and postural alignment in creating controllers that match human balance control. Our process pairs the explorative nature of analytic methods with the exploitative benefits of particle swarm optimization to create an automated approach that rapidly estimates biologically plausible coefficient values. Frequency analysis shows that controllers that were optimized to weigh the importance of energy expenditure more than postural alignment produce a closer match with published human data.

Disclosures: **J.S. McNeal:** None. **A.J. Hunt:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.04/WW79

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

Support: National Key R&D Program of China (No. 2020AAA0130400).

Title: A GPU-based computational framework that bridges neuron simulation and artificial intelligence

Authors: G. HE, **Y. HE**, *K. DU;
Peking Univ., Beijing, China

Abstract: Biophysically detailed multi-compartment models are powerful tools to explore computational principles of the brain and also serve as a theoretical framework to generate algorithms for artificial intelligence (AI) systems. However, the expensive computational cost severely limits the applications in both the neuroscience and AI fields. The major bottleneck during simulating detailed compartment models is the ability of a simulator to solve large systems of linear equations. Here, we present a novel Dendritic Hierarchical Scheduling (DHS) method to markedly accelerate such process. We theoretically prove that the DHS implementation is computationally optimal and accurate. This GPU-based method performs at 2-3 orders of magnitude higher speed than that of the classic serial Hines method in the conventional CPU platform. We build a DeepDendrite framework, which integrates the DHS

method and the GPU computing engine of the NEURON simulator and demonstrate applications of DeepDendrite in neuroscience tasks. We investigate how spatial patterns of spine inputs affect neuronal excitability in a detailed human pyramidal neuron model with 25,000 spines. Furthermore, we provide an in-depth discussion on the potential of the DHS method for AI, specifically highlighting its ability to enable the efficient training of biophysically detailed models in typical image classification tasks.

Disclosures: **G. He:** None. **Y. He:** None. **K. Du:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.05/WW80

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

Support: Bordeaux Neurocampus junior chair program, co-funded by Région Nouvelle-Aquitaine and the University of Bordeaux Initiative of Excellence (IdEx) (F.B.W. Neurocampus junior chair)
European Union's Horizon Europe research and innovation program under European Research Council (ERC) Starting Grant agreement N° 101040391 (F.B.W. MEMOPROSTHETICS)

Title: A dynamical computational model of theta generation in hippocampal circuits to study theta-gamma oscillations during neurostimulation

Authors: *N. VARDALAKIS[1], A. AUSSEL[2], N. P. ROUGIER[2], F. B. WAGNER[3];
[1]Univ. de Bordeaux, Bordeaux, France; [2]Inst. Des Maladies Neurodegeneratives, Bordeaux, France; [3]Ctr. Natl. de la Recherche Scientifique, Bordeaux, France

Abstract: Neurostimulation of the hippocampal formation has shown promising results for modulating memory, but the underlying mechanisms remain unclear. These results could be linked to the mechanisms of theta-nested gamma oscillations and theta phase reset, which are both crucial for memory processes. We can use computational models to investigate the effects of neurostimulation on hippocampal oscillations, however, current computational models suffer from two major limitations: theta oscillations are assumed to have a fixed amplitude and phase velocity. To overcome these issues, we developed a novel computational model of a coronal slice of the human hippocampal formation composed of Hodgkin-Huxley neurons coupled to a model of the medial septum, represented as a set of abstract Kuramoto oscillators producing a dynamical theta rhythm and exhibiting phase reset. This model generated theta-nested gamma oscillations under theta drive and exhibited stimulation-induced theta phase reset. Under certain conditions, a single stimulation pulse switched the network behavior from non-oscillatory to a state producing theta-nested gamma oscillations. Furthermore, under weak theta input unable to produce oscillations, pulse train stimulation at the theta frequency could restore theta-nested

gamma oscillations. We also discovered that the inclusion of phase reset affected the results differentially based on the phase at which stimulation was delivered. This framework opens new avenues for studying the effects of hippocampal neurostimulation, targeting specific phases of ongoing theta oscillations and specific subparts of the hippocampal formation.

Disclosures: **N. Vardalakis:** None. **A. Aussel:** None. **N.P. Rougier:** None. **F.B. Wagner:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.06/WW81

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

Support: NIH Grant R01MH130415
NIH Grant R01AG076227
NIH Grant U24EB029005
NIH Grant S10 OD025181
NSF Grant 1935771
NSF Grant 1935749

Title: Hnn-core: a python software for cellular and circuit-level interpretation of human meg/eeg

Authors: ***N. TOLLEY**[1], R. THORPE[1], M. JAS[2], C. J. BAILEY[4], H. CHENG[5], B. CALDWELL[1], R. PARTANI[6], C. FERNANDEZ PUJOL[7], M. HÄMÄLÄINEN[3], S. JONES[1]; [1]Neurosci., Brown Univ., Providence, RI; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Boston, MA; [3]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Charlestown, MA; [4]Ctr. of Functionally Integrative Neurosci., Aarhus Univ., Aarhus C, Denmark; [5]Dept. of Psychological and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [6]Dept. of Computer Sci. and Engin., Natl. Inst. of Technol. Karnataka, Karnataka, India; [7]Dept. of Biomed. Engin., Univ. of Miami, Miami, FL

Abstract: The Human Neocortical Neurosolver (HNN) is a user-friendly neural modeling software designed to provide a cell and microcircuit level interpretation of macroscale magneto- and electroencephalography (M/EEG) signals (hnn.brown.edu). The foundation of HNN is a biophysically-detailed neocortical model, representing a patch of neocortex receiving thalamic and corticocortical drive. The HNN model was designed to simulate the time course of primary current dipoles and enables direct comparison, in nAm units, to source-localized M/EEG data, along with layer-specific cellular activity. HNN-core is a Python package containing the core functionality of HNN, and is implemented with a clear application programming interface (API). A new graphical user interface (GUI) has been developed that accesses HNN-core functions, and is distributed with tutorials on the simulation of ERPs that mimic the steps in the original HNN GUI. HNN-core was created with best practices in open-source software to allow the

computational and human neuroscience communities to understand and contribute to its development. The package is available to install with a single command on PyPI, is unit tested and extensively documented.
Tutorials are also provided that describe how to use Python code to simulate ERPs and low frequency brain rhythms in the alpha, beta and gamma bands. Additionally, “How” To examples describe how to use Python code to optimize parameters to reproduce ERPs, with a specific example on median nerve evoked responses. HNN-core also offers new functionality, including the ability to modify the local network connectivity, modify additional biophysical properties of individual cells, and record local field potentials (LFP) and cell intrinsic currents/voltages. HNN-core is distributed with three different pre-tuned template models, including that in the original HNN GUI (Jones et al. 2009), and two slightly modified networks that contain (i) updated calcium dynamics as used to investigate auditory evoked potentials (Kohl et al. 2022) and (ii) updated GABAb dynamics applied to study the functional implications of transient beta events (Law et al. 2022). Ongoing expansions will include methods to compare multi-scale model output to different data types (e.g. cell spiking activity, LFP/CSD) and to improve parameter estimation by fitting to these multiscale data types. These additional constraints will help constrain the number of parameter configurations that can accurately reproduce the empirical data. Overall, HNN is a one of a kind openly distributed tool designed for a broad user community to develop and test hypotheses on the multiscale origin of human M/EEG.

Disclosures: **N. Tolley:** None. **R. Thorpe:** None. **M. Jas:** None. **C.J. Bailey:** None. **H. Cheng:** None. **B. Caldwell:** None. **R. Partani:** None. **C. Fernandez Pujol:** None. **M. Hämäläinen:** None. **S. Jones:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.07/WW82

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

Support: UNAM-DGAPA Postdoctoral Fellowship

Title: Variability of topological and graph features in high sampling resting-state fMRI.

Authors: *J. DIAZ-PATINO[1], I. ARELIO[2], S. ALCAUTER[1];
[1]Inst. de Neurobiología, [2]Inst. de Matemáticas, Univ. Nacional Autónoma de México, Queretaro, Mexico

Abstract: Resting-state fMRI (rs-fMRI) reveals essential features of the functional brain connectome, with Topological Data Analysis recently providing new perspectives to its characterization. However, a natural question is how much of these brain characteristics vary in the same subject within a short time. This work aims to quantify such variability when exploring two properties: the minimum spanning tree (MST), i.e., the minimum number of strongest

connections to get all the nodes in a single component, and the sum of its connection strength using the area under the Betti curves of dimension 0 (AUC B_0).
We used the Midnight Scanning Club (MSC) preprocessed dataset containing rs-fMRI from 10 healthy subjects in 10 sessions of 30 minutes long. First, we segmented the interval sessions in sliding windows (window size 10 min, step size 2.2 min). We only used windows with at least 80% of motion-free frames (Gordon E.M. et al., 2017). Connectivity matrices were estimated using the Atlas Power264 (Power J. et al., 2011). We measured AUC B_0 and the Manhattan distance for each MST normalized to the maximum distance between MSTs. The table shows the standard deviation for the AUC B_0 and the average normalized Manhattan distance within-subject (NMD).
SD AUC B0: MSC01=5.19, MSC02=5.72, MSC03=4.4, MSC04=7.91, MSC05=3.66, MSC06=4.44, MSC07=3.56, MSC08=36.4, MSC09=25.35, MSC10=19.48, All=21.29.
Mean NMD: MSC01=0.69, MSC02=0.72, MSC03=0.72, MSC04=0.72, MSC05=0.66, MSC06=0.69, MSC07=0.71, MSC08=0.68, MSC09=0.65, MSC10=0.65, All=0.86.
We observe that the variability (SD) for subjects MSC08-10 is higher than for the other subjects; they also retained fewer frames after exclusion from framewise displacement information, and it has been reported that they were more prone to fall asleep during the scanning session (REF). MST distances are lower within the same subject but higher between subjects. These results suggest that MSTs may be characteristic for each subject.
The approach given in this work gave us information about the variability of topological and graph features within and between subjects. Under normal conditions, the features of individual networks do not vary significantly in a short time. These results suggest that the MST is characteristic for most subjects, although it seems to be more similar between subjects under certain conditions (high motion, potentially consciousness state). Considering our previous reports (Gracia-Tabuenca et al., 2020 & 2023) and these findings, further research on exploring these properties as potential biomarkers of normal and altered development are warranted.

Disclosures: **J. Diaz-Patino:** None. **I. Arelio:** None. **S. Alcauter:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.08/WW83

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

Support: 1 DP2 MH126377

Title: Exploring Individual Network Signatures of Stress Using a Novel Machine Learning Model

Authors: *M. A. MATKOVICH[1], S. MITCHELL[2], M. JOHNSON[2], Y. FILALI[2], M. EBERLE[1], I. HULTMAN[3], B. W. HING[4], A. C. CHAN[4], S. SRIVASTAVA[3], I. ABDUS-SABOOR[5], R. HULTMAN[1];

[1]Mol. Physiol. and Biophysics, [2]Neurosci. Grad. Program, [3]Dept. of Statistics, [4]Psychiatry, The Univ. Of Iowa, Iowa City, IA; [5]Biol. Sci., Columbia Univ., New York City, NY

Abstract: Stress is a physiological and psychological state prompted by threats to one's health and safety. Maladaptive responses to stress can exacerbate various diseases and degrade overall health. Stress vulnerability refers to the concept that some individuals have an inherent predisposition to suffering stress' negative effects. The neural circuits which underlie individual variations in stress vulnerability can serve as predictive indicators of susceptibility to the detrimental consequences of stress. Previous research led to the development of a probabilistic machine learning model that utilizes local field potential (LFP) activity data from seven brain regions encompassing multiple frequencies and outputs six "electome factor" (EF) activity scores. Electome factor one (EF1) was shown to be able to differentiate the neural changes that confer vulnerability to major depressive disorder before experiencing stress from the alterations that occur during the genesis of behavioral dysfunction after stress. The utility of the remaining EFs is still being explored. Recent developments have led to several potential hypotheses. Implanted CD1 mice (n=10) who underwent an experimental model of panic wherein 10-20% CO_2 was breathed for ten minutes followed by ten minutes of compressed air consistently displayed higher EF2 activity scores in the epoch following termination of the CO_2 challenge. Thus, elevated EF2 scores may indicate rebound from acute stress. Mice with Mrgprb4-lineage neurons ablated do not experience the dopaminergic releases prompted by positive social touch leading to combative behaviors. When implanted and recorded during sucrose splash and forced interaction tests it was found that one can discriminate the ablated cohort (n=7) from their non-ablated counterparts (n=10) as the former group's EF5 scores consistently exceed those of controls ($P<.01$). Therefore, it is possible that EF5 encodes a network capable of distinguishing neural alterations which diminish the ability to experience positive social touch. Furthermore, electome factors have the potential to explain brain states not prompted by subjection to stress. Following birth, implanted dams have been found to display significantly higher EF6 scores while on the nest versus when they are off nest (Wilcoxon matched pairs $P=.0156$). This suggests these EFs may be used to discern states of maternal engagement as well as states of stress. While these findings are promising, further analyses must be performed in order to verify and confirm the ability of EFs to discern individual behaviors and brain states before, during, and after stress-inducing experiences and other life changes.

Disclosures: M.A. Matkovich: None. **S. Mitchell:** None. **M. Johnson:** None. **Y. Filali:** None. **M. Eberle:** None. **I. Hultman:** None. **B.W. Hing:** None. **A.C. Chan:** None. **S. Srivastava:** None. **I. Abdus-Saboor:** None. **R. Hultman:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.09/XX1

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

Title: Connectome architecture favours communication via within-module diffusion and between-module routing

Authors: *C. SEGUIN[1], M. PUXEDDU[2], O. SPORNS[1];
[2]Indiana Univ., [1]Indiana Univ., Bloomington, IN

Abstract: Introduction: To date, models of connectome communication have assumed signalling takes place according to a single transmission policy. Here, we explore how the modular structure of brain connectivity—the propensity of neural elements to form tightly interconnected clusters—may engender preferences to distinct policies of network communication. We hypothesise that within-module communication is best modelled by diffusive communication, while between-module signalling is more accurately captured by routing via selective paths**.**
Methods: We explored structural brain networks from 6 species (human, marmoset, rat, mouse, fruit fly, and C. Elegans). Two network measures were computed on each connectome to derive graph-theoretical estimates of communication between neural elements: routing via shortest paths and diffusion via communicability (Fig 1A).
Results: We used piecewise regression to compute G, a measure of inter-nodal preferences for communication via routing or diffusion (Fig 1B). Across all species, G was statistically larger within modules than between modules (Fig 1C-H), indicating a preference for intra-module diffusion and inter-module routing.
Next, we investigated whether diffusion and routing models, computed on the human connectome, differed in their predictive utility of intra- and inter-module functional connectivity (FC). For both BOLD fMRI time series and intracranial EEG recordings, we found that diffusion led to significantly larger correlations to intra-module FC, while routing led to larger correlations for inter-module FC.
Conclusions: Our findings provide multiple lines of empirical evidence that within-module neural communication is best modelled by diffusive processes while between-module signalling is more accurately captured by routing protocols.

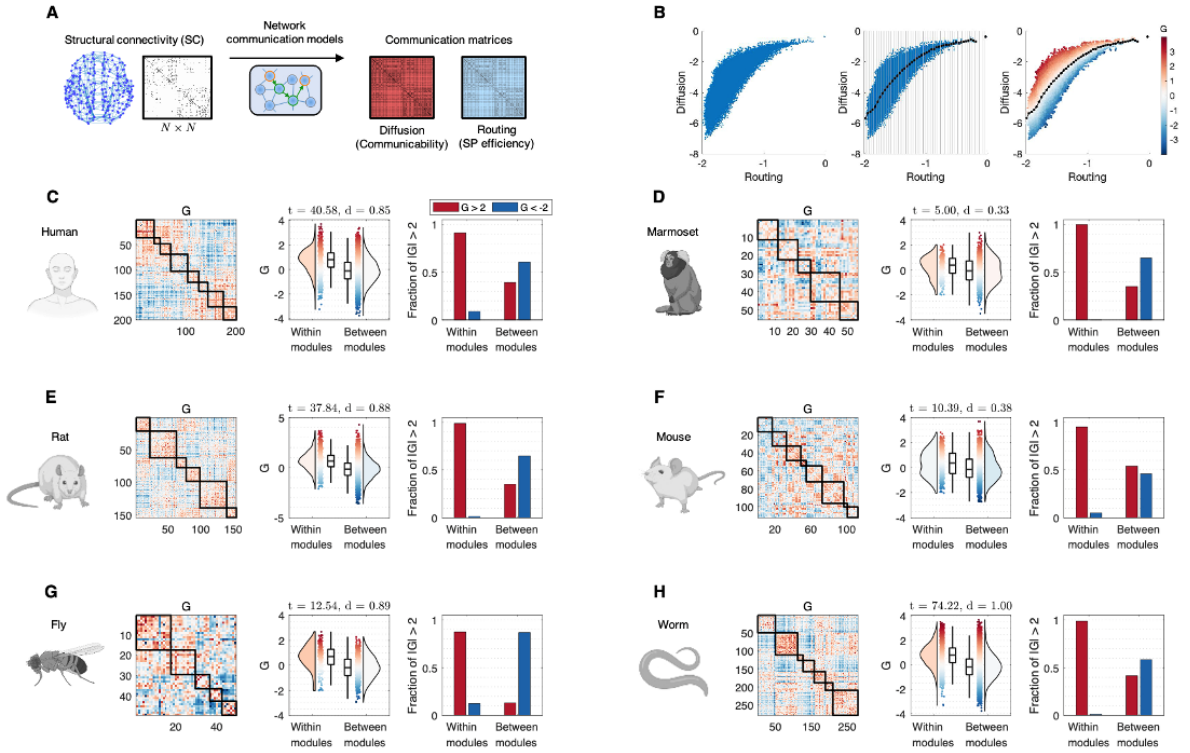


Figure 1. **(A)** Communicability (diffusion) and shortest path efficiency (routing) matrices were computed on structural connectomes. **(B)** Right: Scatter plot of the relationship between communicability and shortest path efficiency in the human connectome, where each data point represents a pair of network nodes. Center: Node pairs were divided into 100 bins based on their shortest path efficiency. Right: For each pair, we computed the difference between their communicability and the mean communicability within their bin (black dots), divided by the within-bin standard deviation. The resultant standardised residuals (*G*) measure whether the communicability of a node pair is higher (G > 0; red) or lower (G < 0; blue) than expected, based on their shortest path efficiency. A positive (negative) Gij suggests that i and j are better positioned in connectome topology to communicate via diffusion, relative to routing (routing, relative to diffusion). In particular, |Gij| > 2 indicates a statistically significant preference towards routing or diffusion. **(C-H)** Left: *G* arranged according to the modular organisation of each species' structural connectome (black lines delineate modular boundaries). Center: Two-sample *t*-test comparing within- and between-module values of *G*. The resulting *t*-statistics and Cohen's *d* effect size are reported (all *p*-values < 10^{-8}). Right: Computed separately for within- and between-module node pairs, bars show the number of element pairs for which G > 2 (red) and G < -2 (blue), divided by the number of elements for which |G| > 2.

Disclosures: **C. Seguin:** None. **M. Puxeddu:** None. **O. Sporns:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.10/XX2

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

Support: 5U01EB025830-09

Title: Multi-scale simulation of dentate gyrus granule cells activity in response to exogenous electrical stimulation guides the design of a memory prosthesis

Authors: ***S. FARZAD**, T. WEI, J. M. C. BOUTEILLER, T. W. BERGER, G. LAZZI; Biomed. Engin., USC, LOS ANGELES, CA

Abstract: Recently, closed-loop neuroprosthesis systems that can both record signals during behavioral tasks and electrically stimulate hippocampal formation appear to hold potential in improving and restoring memory functions. However, the complex structure of neural tissue and a lack of understanding of the effects of electrical stimulation on the hippocampal network pose substantial hurdles. To overcome these issues, we developed a computational model that precisely represents hippocampal stimulation. This model could serve as a tool to guide the design and positioning of electrodes, as well as determining the most suitable stimulation parameters, thus facilitating the identification of optimal stimulation strategies and broadening our understanding of the variables that influence neural response to electrical stimulation. Previously, Bingham et al., 2018 developed a multi-scale computational model for simulating electrical stimulation of a 400um thick slice of the hippocampus. The current model incorporates intricate details to realistically depict the septal to temporal extent of the dentate gyrus and its granule cell (GC) neurons and their dendritic morphologies. This modeling technique integrates two stages. First, a 3D model of the hippocampal tissue was employed to calculate the electric field distribution within the tissue following stimulation using bipolar electrodes. Subsequently, the field is applied to the cells in the neuronal network to calculate their activity. The admittance method (AM) was used to determine the field distribution, and the neural network was set up using the NEURON simulation platform. The 3D structure of the rat dentate gyrus is reconstructed using comprehensive anatomical data from thin histological sections. Our neural network model includes dentate gyrus's GCs and EC axon arbors, which project to the granule cells via the perforant path. We are using the Ruled-Optimum Ordered Tree System (ROOTS) algorithm to create realistic axon arbor structures.
Our multi-scale computational model enables investigating the effect of various electrical stimulation waveforms at diverse stimulation strengths on the hippocampal neurons. Results suggest the electrodes positioning and stimulation waveform and parameters significantly impact the EC axons' activation threshold. Maximum axon activation at low amplitudes (5uA) was observed near the infrapyramidal blade, while the minimum was noticed on the suprapyramidal blade under anodic first and anodic-monophasic stimulations. Future work will explore the influence of electrodes placement on axonal activation and subsequent granule cells activation.

Disclosures: S. Farzad: None. **T. Wei:** None. **J.M.C. Bouteiller:** None. **T.W. Berger:** None. **G. Lazzi:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.11/XX3

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

Support: NIMH IRP ZIA-MH002783

Title: Evaluating the predictive power of dynamic fMRI connectivity summary statistics

Authors: *M. A. SPURNEY, J. FASKOWITZ, J. GONZALEZ-CASTILLO, D. A. HANDWERKER, P. A. BANDETTINI;
Natl. Inst. of Mental Hlth. Div. of Intramural Res., Bethesda, MD

Abstract: Brain-behavior models often use resting-state fMRI data in the form of functional connectivity (FC) matrices, where each entry corresponds to the correlation between time series for a pair of regions (or nodes). This useful approach is limited in that a typical FC matrix is unable to capture the changes of connectivity that the brain experiences during a typical time series duration. To access precise dynamic connectivity information, we generated a time series for each node pair (or edge) that captured how the two nodes co-fluctuate from moment to moment (i.e., edge time series) (Zamani Esfahlani, 2020). Here, we explore multiple approaches to summarizing fMRI connectivity dynamics using resting-state fMRI scans from the NKI-Rockland sample (N=971, 59.4% Female, ages 6-85). In addition to the mean of the edge time series, which is equivalent to the more standard FC, we also computed the standard deviation, entropy, and several other time-dependent measures, to form new matrices for each subject. We then evaluated the predictive ability of these alternative brain representations using Connectome-Based Predictive Modeling. We produced significant predictions for measures of attention and intelligence, respectively, through a general linear model, using the edge time series mean ($r=0.26$, $p<0.001$; $r=0.35$, $p<0.001$), standard deviation ($r=0.15$, $p<0.01$; $r=0.11$, $p<0.01$), and entropy ($r=0.22$, $p<0.001$; $r=0.29$, $p<0.001$). Next, we predicted attention and intelligence using a ridge regression model that included these three representations of the data. This model performed better than our individual models for attention ($r=0.31$, $p<0.0001$) and intelligence ($r=0.43$, $p<0.0001$). We found that, across fitting iterations, the model framework repeatedly selected the mean of the edge time series in building these predictions, suggesting that the mean (or the FC) is relatively most predictive. Finally, we computed several other temporally sensitive summary metrics, including autocorrelation and dynamic entropy. Their predictive value proved to be not as significant as that of the mean of edge time series. In sum, our results demonstrated that mean co-fluctuation, i.e., functional connectivity, showed significant predictive power that was unmatched compared to a variety of other summary statistics, suggesting perhaps, that what the brain is doing over 10 minute periods is more predictive of traits than the specific dynamics of how it changes from moment to moment. Future work will focus on exploring spatial and temporal aspects of these edge time series that may either be more predictive of traits or more informative of the functional organization of the brain.

Disclosures: M.A. Spurney: None. **J. Faskowitz:** None. **J. Gonzalez-Castillo:** None. **D.A. Handwerker:** None. **P.A. Bandettini:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.12/XX4

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

Support: JSPS KAKENHI Grant Number JP21H05137
This work used computational resources of the Supercomputer Fugaku provided by RIKEN Center for Computational Science through the HPCI System Research Project (Project ID: hp220162, hp230206).

Title: Propagation of gamma oscillations in a connectome based spiking neural network model of the mouse cortico-cerebellar circuit

Authors: *J. IGARASHI[1], T. YAMAZAKI[2];
[1]Ctr. for Computat. Sci., RIKEN, Wako, Japan; [2]The Univ. of Electro-Communications, The Univ. of Electro-Communications, Tokyo, Japan

Abstract: Gamma oscillation occurs in the cerebral cortex during specific states, which is considered to work for information processing. However, it remains unknown how it interacts among layers in the cerebral cortex and among regions. To examine it, we developed a spiking neural network model of the mouse brain consisting of the primary and secondary motor cortices (M1 and M2), thalamus, pons, and cerebellum.
We set the intra-regional connections based on anatomical and electrophysiological data [1] and the inter-regional connections based on connectome data provided by Allen Institute [2]. We used the leaky integrate-and-fire neuron model and conductance synapse models for modeling all neurons and synapses.
First, we examined how gamma oscillation could appear in M1. We stimulated excitatory and inhibitory cells within one voxel of layer 2/3 (L2/3) with a bias current input for 500 ms, assuming sensory afferent input. Oscillatory neural activities occurred in the gamma frequency range (~40 Hz) across L2/3 to L6. The neurons in the L2/3, L5, and L6 showed spike phase lock to gamma oscillation that was bandpass-filtered mean firing rate of all neurons in M1. Within these same layers, the excitatory cells and fast-spiking interneurons fired at earlier phases than low-threshold spiking neurons. The peaks of the mean spike phase appeared in the order of L2/3, L5, and L6, reflecting the primary excitatory connections from superficial to deep layers.
Next, to investigate how the gamma oscillation of M1 reflects to other brain regions, we analyzed simulated neural activities of M2, pons, thalamus, and cerebellum. The gamma oscillation propagated from M1 to L5 of M2 via inter-regional connections and propagated to L2/3 and L6 from L5 in M2. The gamma oscillation of M1 also propagated to the cerebellar cortex via pons. The pontine neurons and granule cells exhibited strong spike phase lock to gamma oscillations, while the Purkinje cells and cerebellar nucleus neurons showed very weak ones.
These results suggest that each neuron type in the brain region responds differently to gamma oscillations and may be dynamically regulated by the responsive neuron type.
[1] Igarashi, et al., (2019) Frontiers in Neuroinformatics, 13, 1-15
[2] Knox, et al., (2019) Network Neuroscience, 3, 217

Disclosures: J. Igarashi: None. **T. Yamazaki:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.13/XX5

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

Support: NSF PIRE Award 1743475
NSF DBI 2015317

Title: A Dynamic Simulation of a Compliant Worm Robot Amenable to Neural Control

Authors: *S. RIDDLE, C. JACKSON, K. A. DALTORIO, R. D. QUINN;
Case Western Reserve Univ., Cleveland, OH

Abstract: This work details the development and validation of a computational model of a dynamic 3D compliant worm-like robot controlled by a Synthetic Nervous System (SNS) [1]. An SNS is a dynamical neural network comprised of computational models of neurons and synapses which have been implemented as controllers for several other biologically inspired robots. The model was built and simulated in Mujoco, a physics engine oriented towards robotics and biomechanics research. This physics engine is able to approximate soft bodied dynamics and generate contact, gravitational, frictional, and internal forces. These capabilities allow the model to realistically simulate the movements and dynamic behavior of a physical soft-bodied worm-robot. For validation, the results of this simulation were compared to data gathered from a physical worm robot [2] and found to closely match key behaviors such as deformation propagation along the compliant structure and actuator efficiency losses in the middle segments due to ground contact friction. In addition to physics modeling, Mujoco can handle control signals and produce sensor feedback making it relatively easy to interface with the SNS controller. A simplified controller was previously developed for a simple 2D kinematic model and was successfully implemented on this 3D model with little alteration [3]. The new SNS uses central pattern generators composed of mutually inhibitory leaky-integrator neurons to generate coordinated actuator control signals and works with feedback to induce peristaltic locomotion. This model will be useful for analyzing dynamic effects during peristaltic locomotion like contact forces and slip as well as developing and improving control algorithms that avoid unwanted slip.
[1] - Szczecinski, N.S., Hunt, A.J., Quinn, R.D.: A functional subnetwork approach to designing synthetic nervous systems that control legged robot locomotion. Frontiers in Neurorobotics 11 (2017)[2] - Wang, Y.: Preparing worm-like robots for unknown environments: Perception and path planning. In: CWRU Dissertation (2022)[3] - Riddle, S., Nourse, W.R.P., Yu, Z., Thomas, P.J., Quinn, R.D.: A synthetic nervous system with coupled oscillators controls peristaltic locomotion. In: Biomimetic and Biohybrid Systems. pp. 249-261. Springer International Publishing, Cham (2022)

Disclosures: S. Riddle: None. **C. Jackson:** None. **K.A. Daltorio:** None. **R.D. Quinn:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.14/XX6

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

Support: NIH Grant R01AA029926
NIH Grant P50AA026117

Title: Causal functional connectivity networks in the study of alcohol use in heavy drinking adults

Authors: *C. C. MCINTYRE[1], H. PETERSON-SOCKWELL[1], R. G. LYDAY[2], M. BAHRAMI[2], J. A. FISH[3,4], E. M. BOLLT[3,4], P. J. LAURIENTI[2];
[1]Neurosci., [2]Radiology, Wake Forest Univ. Sch. Of Med., Winston Salem, NC; [3]Electrical and Computer Engin., [4]Clarkson Ctr. for Complex Systems Sci., Clarkson Univ., Potsdam, NY

Abstract: Network neuroscience has made significant contributions to our understanding of brain function. One of the most popular methods for brain network analysis includes use of functional connectivity networks, which are typically built from temporal correlations of blood oxygen level dependent (BOLD) signal from distinct brain regions measured with functional magnetic resonance imaging (fMRI). We refer to these as cooperative functional networks (cFNs) as they highlight brain regions with synchronized activity. However, cFNs only tell part of the functional connectivity story as they cannot identify causal connections between brain regions. These causal, directed connections are likely crucial to the brain's dynamic shifts in functional connectivity, which would allow for quick adaptation to environmental cues, cognitively demanding tasks, etc. However, there is currently no generally accepted method to identify whole-brain causal networks. To address this gap, we used optimal causation entropy (oCSE) to identify causal connections resulting in whole brain networks with regions that are resistant to synchronization, which we call impervious functional networks (iFNs). Causation entropy is a measure of conditional information flow and can recover the direct connections in a functional network, where other methods such as correlation or transfer entropy are unable to distinguish between direct versus indirect interactions. To assess the biological relevance of iFNs, we analyzed fMRI scan data from 39 heavy-drinking adults aged 24-60 years old who had abstained from alcohol consumption for three days prior to their scan. During their scan, each participant viewed alcohol-related and neutral images. Both cFNs and iFNs were constructed for each participant using the Shen 268 parcellation atlas. Permutation tests were used to determine if network topology differed between viewing of the alcohol and neutral cue for cFNs and iFNs. The cFNs only differed in assortativity ($p = .011$) between the two conditions. The iFNs exhibited significant or near-significant differences in several topological measures, including sink count ($p = .040$), source count ($p = .056$), and giant component size ($p = .067$). These findings support the biological relevance of iFNs and serve as a starting point for further elucidation of their role in directing shifts in functional connectivity. We propose that rather than assessing cFNs alone, future functional network analyses should consider cFNs and iFNs together so that both the synchronized and the unsynchronized, shift-directing relationships in the brain are captured.

Disclosures: **C.C. McIntyre:** None. **H. Peterson-Sockwell:** None. **R.G. Lyday:** None. **M. Bahrami:** None. **J.A. Fish:** None. **E.M. Bollt:** None. **P.J. Laurienti:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.15/XX7

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

Support: JSPS KAKENHI Grant JP21K15610

Title: Effects of signal packetization on whole-brain communication dynamics simulated in the connectome

Authors: *M. FUKUSHIMA[1], K. LEIBNITZ[2,3];
[1]Hiroshima Univ., Higashi-Hiroshima City, Japan; [2]Natl. Inst. of Information and Communications Technol., Suita City, Japan; [3]Osaka Univ., Suita City, Japan

Abstract: Methodological advances have made it possible to map the complete set of white matter structural connections (connectome) in the mammalian brain. Existing computational research has studied the dynamics of whole-brain network communication by simulating the flow of abstract discrete signals in the connectome. These studies approximate the communication dynamics in the connectome by assuming some propagation strategies and switching architectures in simulations. Random walk, shortest path routing, and their intermediates have been assumed as propagation strategies and message switching and packet switching as switching architectures in models of brain network communication. However, the relationships between propagation strategies and switching architectures have not been sufficiently explored in the literature. Here, we investigate how the difference between packet switching and message switching (i.e., whether a signal [message] is split into packets or not) affects the transmission efficiency of propagation strategies when simulating the communication dynamics in the connectome. As the connectome data, we used a structural network of the macaque brain derived from the CoCoMac database. With this network, we ran discrete-event simulations of message switching or packet switching under each of the following propagation strategies: random walk (RW), shortest path (SP), and informed and biased versions of RW (iRW and bRW), which have intermediate properties between RW and SP in terms of communication speed and information cost. We evaluated the transmission efficiency of each propagation strategy by measuring the elapsed time to transmit 100 messages or packet sets between randomly selected pairs of source and destination nodes in the connectome. We found that the elapsed time with packet switching was longer under RW but shorter under iRW. There was no significant difference for SP. In bRW, the elapsed time with packet switching was shorter when a parameter controlling the propagation behavior was specified in an intermediate range. These results show that packetization degrades the transmission efficiency of RW but improves that of iRW and bRW, i.e., propagation strategies that balance communication speed and

information cost. Since such intermediate strategies are more physiologically plausible than RW and SP, our results indicate a novel advantage of considering packet switching for communication in brain networks and provide new insights for modeling the whole-brain communication dynamics in the connectome.

Disclosures: M. Fukushima: None. **K. Leibnitz:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.16/XX8

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

Support: NIH Grant R01 NS117405
Duke Compute Cluster

Title: Dissecting the neural basis of short-latency responses to transcranial magnetic stimulation of motor cortex: a computational modeling study

Authors: *K. KUMARAVELU[1], G. J. YU[2], M. A. SOMMER[1], A. V. PETERCHEV[2], W. M. GRILL[1];
[1]Biomed. Engin., [2]Psychiatry and Behavioral Sci., Duke Univ., Durham, NC

Abstract: Transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) elicits a series of high frequency volleys termed D- and I-waves measured epidurally in the corticospinal tract (CST) of awake humans. The D-wave is thought to be caused by direct activation of CST axons, while I-waves are believed to be generated by trans-synaptic activation of CST axons. The cortical circuits and mechanisms involved in generating I-waves by TMS of M1 remain unclear. We used published network models of the cortical microcircuit to study the circuit mechanisms of TMS-induced I-waves. Previously, we used a computational model of a cortical column (Traub et al., 2005) to study the I-wave response to TMS over M1. Next, we implemented a biophysically detailed network model of the M1 to quantify the I-wave response to TMS (Dura-Bernal et al., 2022). The model comprised 10,000 cortical neurons with synaptic properties constrained based on experimental data. The pyramidal tract neurons (PTN) from Layer (L)5 were represented using detailed morphology and ion channel properties, whereas simpler models were used for neurons in other layers. The TMS field was represented using intracellular current injection of monophasic TMS pulses into the axon terminals of different proportions of intratelencephalic (IT) neurons. Spiking activity from axons of PTNs was used as the model-based proxy for the response of CST axons. We constructed dose-response curves of spiking activity averaged across L5 PTN as a function of the proportion of activated IT neurons from different layers. The M1 model reproduced I-waves only during the activation of axon terminals of L2/3 IT neurons and not in response to activation of axon terminals of L2/3 or L6 IT neurons. Further, the magnitude of I-waves increased with a higher proportion of activation of

L2/3 IT neurons. Virtual lesions of specific neural populations revealed the I1 wave was generated by the monosynaptic connection from L2/3 IT neurons to L5b PTN. However, the later I-waves (I2, I3 & I4) were generated due to recurrent connections between the PTN. Finally, we implemented a computational model of the somatosensory cortex (S1) based on the Blue Brain project (Borges et al. 2022; Markram et al. 2015). The S1 model did not exhibit I-waves after activation of pyramidal cells in any layer. We used detailed biophysical-based models of the cortex to explore the network effects of TMS induced I-waves. A better understanding of the circuit mechanisms of TMS over the motor cortex will enable the optimization of stimulation paradigms.

Disclosures: K. Kumaravelu: None. **G.J. Yu:** None. **M.A. Sommer:** None. **A.V. Peterchev:** None. **W.M. Grill:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.17/XX9

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

Support: NIH U24EB028998
NSF 1904444-1042C
NYS SCIRB DOH01-C32250GG-3450000

Title: Computational model of the ventral posteromedial thalamic circuit

Authors: *J. MOREIRA[1], S. DURA-BERNAL[1,2];
[1]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., BROOKLYN, NY; [2]Ctr. for Biomed. Imaging and Neuromodulation, Nathan Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: Recent experimental studies provided new insights about thalamic function and how its interaction with the cortex is state-dependent. They revealed a short-latency feedback pathway from layer 6 corticothalamic (L6 CT) to thalamocortical (TC) neurons, which is more evident in the awake state (Constantinople, 2013; Hirai et al, 2018; Whilden, 2021). Additionally, detailed reconstructions of neuronal projections and circuit mapping studies have uncovered new cell-type-specific pathways of information flow and integration across cortical and thalamic regions (Lam et al, 2010-11; Shepherd & Yamawaki, 2021). This opened new avenues for computational models, which can investigate cellular and network mechanisms not accessible via experiments. In this study we are building a detailed multiscale mechanistic model of the mouse barreloid thalamus circuits, using the NetPyNE tool and the NEURON simulator. Our goal is to develop a framework to study the thalamus and its interactions with the cortex, such as the influence of direct L6 CT feedback in the regulation of network excitability (Hirai et al, 2018), and the effect of modulatory L6 CT projections, which have an inhibitory effect on

thalamic relay cells at lower frequencies (0.1 Hz) and excitatory at higher frequencies (10 Hz) (Crandall et al, 2015). The model includes biophysically detailed neurons and projections between the thalamic ventral posteromedial (VPM) and reticular (TRN) nuclei, and barrel cortex L6 CT neurons. We based the thalamic cell morphology and biophysics on a recent model of the mouse somatosensory thalamus (Iavarone, 2023), and the connectivity on the latest experimental data available (Jones, 2002; Meyer, 2010; Lam et al, 2010-11; Hou, 2016; O'Reilly, 2021). The model is driven by sensory feedforward inputs from the medial lemniscus, simulated as spike generators. We validated the model's biophysical properties, including cell intrinsic dynamics and synaptic depression and facilitation, which are key components of thalamic driver and modulatory synapses. We also included a topological distribution of synaptic inputs (Jones, 2002), to account for the interaction between synaptic and cable properties present in the thalamus. Our model results showed that the specific combination of inputs dictated the dynamic responses of TC and TRN cells, which switched between burst and tonic firing, a characteristic feature of thalamic neurons. Ultimately, our model will provide insights into the mechanisms involved in the regulation of thalamocortical excitability and how interactions between L6 CT neurons and thalamus can shape the information arriving at the cortex.

Disclosures: **J. Moreira:** None. **S. Dura-Bernal:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.18/XX10

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

Support: Howard Hughes Medical Institute

Title: A simplified model of high-dimensional neural responses to stimuli

Authors: ***F. DU**[1,2], M. NUÑEZ[1], M. PACHITARIU[1], C. STRINGER[1];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Deep convolutional neural networks (CNNs) have demonstrated human-level success on visual tasks, suggesting that CNNs may be good models of the visual system. Various studies have thus used CNNs to predict neural responses to visual stimuli. Despite significant improvements over classical visual models, most CNN-based encoding models still have limitations: they typically use human-centric training datasets like ImageNet and there is still a large gap between their accuracy in fitting neural data and the achievable upper bound. Further, the deep neural networks are hard to interpret which makes it challenging to define neuron feature selectivity from the model.
We used calcium imaging in the mouse primary visual cortex of transgenic mice expressing jGCaMP8s, and successfully recorded neural responses to over 40k natural texture images per session. Our trained models had a significant increase in the variance explained compared to

previous approaches, highlighting the substantial impact of employing a larger image dataset. Moreover, we developed a new shallow model comprising two convolutional layers and a neuron-specific pooling layer, which achieved state-of-the-art performance, matching or even exceeding the performance of the deeper models. In addition, in our shallow model we found that the first layer could be substantially compressed as long as the second layer was wider; this wider architecture better reproduced the high-dimensional aspects of the neural activity. Also, the performance of the model did not increase significantly with increasing the number of neurons, suggesting that most neurons encoded distinct visual features. These findings allowed us to construct a simplified "minimodel" for each individual neuron to investigate the high-dimensional visual computations. Next, we found that visual texture class could be invariantly decoded from both mouse visual cortex and our model. Using our simplified model, we developed novel and robust methods to visualize neuron receptive fields of the neurons selective to each texture classes. The results revealed that these neurons represented many diverse visual features pooled selectively in space, which provides insight into the mechanisms underlying invariant texture recognition in mice.
Our shallow model serves as a first step towards understanding high-dimensional visual computations in the mouse cortex. We demonstrate the feasibility of constructing accurate models using a limited number of neurons and parameters, offering a quantitative approach for understanding of visual properties across diverse experimental contexts.

Disclosures: **F. Du:** None. **M. Nuñez:** None. **M. Pachitariu:** None. **C. Stringer:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.19/XX11

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

Support: RIKEN Center for Brain Science
Brain/MINDS from AMED under Grant no. JP15dm0207001
JSPS KAKENHI Grant No. JP18H05432
JSPS KAKENHI Grant No. JP21J10564
Masason Foundation

Title: A biological model of nonlinear dimensionality reduction

Authors: *K. YOSHIDA[1,2], T. TOYOIZUMI[1,2];
[1]RIKEN Ctr. for Brain Sci., Wako, Saitama, Japan; [2]The Univ. of Tokyo, Tokyo, Japan

Abstract: Animals make decisions based on high-dimensional sensory inputs. Obtaining its low-dimensional representation is crucial for efficient downstream information processing. Although the machine learning field developed useful nonlinear dimensionality reduction methods such as t-distributed stochastic neighbor embedding (t-SNE), their biological implementations are

unknown. Here, we develop a biologically-plausible algorithm that approximates t-SNE. The proposed algorithm, named Hebbian t-SNE, is implemented in a simple three-layer feedforward neural network consisting of a large number of middle-layer neurons with sparse activities, being inspired by the Drosophila olfactory circuit consisting of projection neurons, Kenyon cells, and mushroom body output neurons. The synaptic weights from the middle to output layers are updated according to a three-factor Hebbian learning rule, depending on the product of presynaptic, postsynaptic, and global factors so that similar (high-dimensional) inputs evoke similar (low-dimensional) outputs and vice versa. Hebbian t-SNE obtains low-dimensional representations of input clusters, such as ones distributed according to entangled rings or MNIST data, that are more linearly separable than those learned by principal component analysis (PCA). We further show that the low-dimensional representation learned by Hebbian t-SNE is useful for learning the association between inputs and rewards. Hebbian t-SNE separates reward and non-rewarded patterns in a low-dimensional space well reflecting the original input structures and, thereby, endows good generalization for inputs that are not yet associated with rewards. We finally explore with the model the possibility that Hebbian t-SNE could be implemented in Drosophila olfactory circuits. First, odor functional groups based on chemical structures are better captured in the representation learned by Hebbian t-SNE than that by PCA. Next, the obtained representation by applying Hebbian t-SNE for the activities of projections neurons explains the behaviourally measured valence index better than the PCA and kernel PCA. In summary, we argue that nonlinear dimensionality reduction learning, such as t-SNE, could be implemented in a biological circuit.

Disclosures: **K. Yoshida:** None. **T. Toyoizumi:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.20/XX12

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

Support: DIRP, NIMH, USA, ZIAMH002797, ZIAMH002971
BRAIN initiative Grant U19 NS107464-01, ZIAMH00279

Title: Unveiling the impact of spatial subsampling on critical dynamics in neural networks

Authors: *K. SRINIVASAN, T. L. RIBEIRO, D. PLENZ;
Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Complex systems operating at criticality produce intricate, system-wide events that are described by power laws and scaling relationships. In the brain, neuronal avalanches—synchronized cascades of neuronal activity—have long been regarded as a key indication of criticality in the superficial layers of the cortex. These avalanches exhibit power laws in size, duration, and inter-avalanche organization, with respective exponents close to -3/2, -2, and -1.

However, identifying the critical "crackling-noise relationship" proposed by Sethna et al. (2001), which suggests that the average size of avalanches scales with duration by a factor of $\chi = 2$, has proven challenging.
Recent studies utilizing 2-photon imaging in awake transgenic mice (Capek, Lins Ribeiro et al., 2023) and recording local field potentials (LFP) in nonhuman primates (Miller et al., 2019), have provided evidence that neuronal avalanches exhibit $\chi = 2$ in vivo. These "parabolic avalanches" are observed within the confined spatial window of the recording and can effectively account for spatial subsampling by employing temporal coarse-graining techniques.
In this study, we delve further into the limitations imposed by spatial subsampling on recovering the Sethna relationship in critically balanced neuronal networks. Our network consisted of $N = 10^6$ stochastic, non-leaky Integrate-and-Fire neurons with a fully connected architecture (80% excitatory & 20% inhibitory neurons). We illustrate how subsampling in such a network affects avalanche duration, size, and scaling exponents, with larger deviations becoming more pronounced as subsampling increases. Additionally, we demonstrate how appropriate levels of temporal coarse-graining restore the scaling exponent χ to its value of 2 and unveil a quadratic scaling relationship between avalanche size and duration.
To visually represent this scaling, we introduce novel cell contribution plots, which depict the quadratic increase in the number of cells contributing to avalanches when accurately resolved. However, we acknowledge the limitations of this approach, as it underestimates other avalanche exponents and eventually leads to a singularity in the crackling noise ratio, hindering its direct relationship with the scaling exponent. Our findings underscore the constraints imposed by spatial subsampling when accurately estimating exponents and scaling relationships from experimental observations. They also highlight the importance of identifying robust signatures of criticality that remain invariant despite such experimental conditions.

Disclosures: K. Srinivasan: None. **T.L. Ribeiro:** None. **D. Plenz:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.21/XX13

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

Title: Structural disconnections in brain tumors: the normative modeling framework

Authors: H. YOUSEF, *B. MALAGURSKI TORTEI;
Biotech. Res. Ctr., Technol. Innovation Inst., Abu Dhabi, United Arab Emirates

Abstract: Introduction: The emergence of network neuroscience has indicated that brain tumors lead to brain-wide disruptions in structural connectivity. Thus, our aim was to estimate the relationship between tumor-related structural alterations and cognitive performance using normative brain atlases. The advantage of this approach is that it enables the analysis of retrospective routine MRI patient data, despite the absence of patient-specific DWI. **Methods:**

We used pre-operative data of 11 glioma (mean age = 47.5y, SD = 11.3) and 14 meningioma patients (mean age = 47.5y, SD = 11.3), obtained from the OpenNeuro database (Aerts & Marinazzo, 2022). The dataset contained T1w images and corresponding tumor masks segmented by a combination of manual and automated delineation. Information processing speed was assessed using the Reaction Time (RTI) tasks from the CANTAB battery (Cambridge Cognition, 2017). For each patient, the T1w and the tumor mask were normalized to MNI152 space using a customized HD-BET and ANTS-based processing pipeline, implemented in Nipype (Gorgolewski et al., 2011). The structural impact of tumors was quantified with LQT (Griffis et al., 2021), using normative atlas-based metrics to estimate tract-level damage and parcel-wise disconnections. Latent correlations were computed between estimated structural damage and RTI tasks, with their statistical significance estimated with permutation testing. FDR was applied to adjust for multiple comparisons. **Results:** No significant correlations were found for RTI measures with tumor size. For parcel-wise structural disconnections, significant correlations were obtained for disconnection severity between left hemisphere visual and prefrontal cortical parcels. These disconnection severities positively correlated with the standard deviation (SD) of reaction time (RTI) simple (SMT) and five-choice movement times (5MT) tasks, with correlation coefficients in the range of .63 to .79 ($p < .05$). For tract disconnection severity, damage to the left inferior fronto-occipital fasciculus (IFOF-L) was significantly correlated with the SD of 5MT ($\rho = .69$, $p = .042$). **Conclusions:** These preliminary results point to the importance of assessing the impact of brain tumors on whole-brain structural connectivity and its association with cognitive performance. In particular, the association between prefrontal-visual disconnections and RTI performance alludes to the role of the prefrontal cortex in visual processing and response. Further studies should compare the patient-specific and normative-based quantification of structural damage, particularly in the context of clinical outcomes.

Disclosures: H. Yousef: None. **B. Malagurski Tortei:** None.

Poster

PSTR510. Computational Modeling of Networks

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR510.22/XX14

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

Support: This study was supported by funding to the Blue Brain Project, a research center of the École polytechnique fédérale de Lausanne (EPFL), from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology.
Funding has been provided in part for Nexus Forge from the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 785907 (Human Brain Project SGA2)

Title: Databasing the brain to accelerate simulation neuroscience

Authors: *S. KERRIEN[1,2], D. BELL[2], S. DUMAS[2], M. DUPONT[2], P.-A. FONTA[2], H. GENET[2], C. GONZALEZ[2], O. GRABINSKI[2], A.-K. KAUFMANN[2], C. LINDQVIST[2], H. LU[2], J. LURIE[2], J. MACHON[2], A. GARCIA ROJAS MARTINEZ[2], B. MEDDAH[2], D. MONTERO MENDEZ[2], S. MOUFFOK[2], D. DHANESH[2], L. OLIVIER[2], K. PIRMAN[2], K. PLATIS[2], B. ROMAN[2], D. SAXENA[2], N. SMIT[2], N. STAFEEVA[2], M. F. SY[2], A. ULBRICH[2], W. WAJEROWICZ[2], H. MARKRAM[2], S. HILL[2,3,4];
[1]Neuroinformatics Software Engin., EPFL - Blue Brain, Geneva, Switzerland; [2]École polytechnique fédérale de Lausanne (EPFL) - Blue Brain, Geneva, Switzerland; [3]Ctr. for Addiction and Mental Hlth. (CAMH), Krembil Ctr. for Neuroinformatics, Toronto, ON, Canada; [4]Dept. of Psychiatry – Neurosci. and Clin. Translation, Univ. of Toronto, Toronto, ON, Canada

Abstract: Advancements in neuroscience present challenges in managing and organizing data, and capturing data provenance. Implementing FAIR data principles (doi:10.1038/sdata.2016.18) remains complex. The Blue Brain project utilizes an iterative process involving data acquisition, cataloging, analysis, model building, simulation, and validation for data-driven modeling and simulation of the mouse brain. Over the years, Blue Brain has been consolidating its database of the brain across a broad variety of data types and scales of the brain. Connecting together s transcriptomic, proteomic, cellular, synaptic, morphological, and electrophysiological data from internal and collaborative research as well as literature into brain atlases enables us to build detailed digital models and run large scale simulation experiments to further our understanding of the brain. This could not happen without Blue Brain Nexus, a critical technology enabling Blue Brain to achieve its data-driven simulation neuroscience mission.
Blue Brain Nexus (https://bluebrainnexus.io) is an actively developed open-source semantic data management ecosystem. It offers a secure, scalable service to manage knowledge graphs, accommodating various formats (JSON, JSON-LD) for metadata interaction. Data quality is guaranteed via W3C SHACL validation, and the platform's domain-agnostic design encourages the use of already defined data models (https://neuroshapes.org).
The Nexus ecosystem also includes a user interface for data discovery, reuse, and dissemination. It features a robust search interface, data dissemination studios, and a plugin architecture enabling user-specific web extensions, such as data visualization. The Nexus ecosystem is completed with a Python framework simplifying the management and use of the knowledge graph.
At Blue Brain, Nexus plays a pivotal role in accelerating data-driven modeling and simulation of the mouse brain. The Blue Brain knowledge graph encompasses over 2.1 million scientific entities and offers scientists data discoverability and reusability, enables engineers to automate scientific pipelines for brain model building and large-scale simulations, and streamlines the data dissemination process to fulfill our open science commitment.
Blue Brain Nexus is instrumental in large-scale data management in several global organizations, demonstrating its versatility and adaptability across varied data management landscapes.

Disclosures: S. Kerrien: None. **D. Bell:** None. **S. Dumas:** None. **M. Dupont:** None. **P. Fonta:** None. **H. Genet:** None. **C. Gonzalez:** None. **O. Grabinski:** None. **A. Kaufmann:** None. **C. Lindqvist:** None. **H. Lu:** None. **J. Lurie:** None. **J. Machon:** None. **A. Garcia Rojas Martinez:** None. **B. Meddah:** None. **D. Montero Mendez:** None. **S. Mouffok:** None. **D. Dhanesh:** None. **L. Olivier:** None. **K. Pirman:** None. **K. Platis:** None. **B. Roman:** None. **D. Saxena:** None. **N. Smit:** None. **N. Stafeeva:** None. **M.F. Sy:** None. **A. Ulbrich:** None. **W. Wajerowicz:** None. **H. Markram:** None. **S. Hill:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.01/XX15

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

Support: The European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)
ANR; ANR-17-RHUS-0004, EPINOV

Title: Virtual brain twins for personalized medicine in epilepsy

Authors: *H. WANG[1], P. TRIEBKORN[1], J. MAKHALOVA[2], J.-D. LEMARECHAL[1], B. DOLLOMAJA[1], A. WILLIAMSON[3], D. DEPANNEMAECKER[1], J. TERRY[4], F. BARTOLOMEI[1], V. JIRSA[1];
[1]INS AMU INSERM U1106, Marseille, France; [2]APHM, Epileptology and Clin. Neurophysiol. Department, Timone Hosp., Marseille,, France; [3]INS, AMU, Marseille, France; [4]Univ. of Birmingham, Birmingham, United Kingdom

Abstract: A virtual brain twin is a digital representation of an individual's brain, which is constructed from the individual's own data and informs decision making through personalized simulations. In epilepsy, the virtual brain twinhelps diagnosis and treatment of epilepsy. The patient-specific data include anatomical data such as MRI, PET and functional data such as scalp EEG, Stereo-EEG and MEG. Simulations of virtual brain twin models capture the main features of a patient's brain activity, including the range of seizure propagation patterns, interictal activity and stimulus response patterns [1]. We systematically develop the key elements of virtual brain twins and present examples of personalized brain modelling in epilepsy for diagnostic purposes, such as early-phase diagnosis for likelihood of epilepsy [2], and later-phase diagnosis for estimating epileptogenic networks [3], and therapeutic interventions, such as surgical resection and stimulation.
[1] Jirsa, V., Wang, H., Triebkorn, P., Hashemi, M., Jha, J., Gonzalez-Martinez, J., Guye, M., Makhalova, J., & Bartolomei, F. (2023). Personalised virtual brain models in epilepsy. *The Lancet Neurology*. https://doi.org/10.1016/S1474-4422(23)00008-X
[2] Tait, L., Staniaszek, L. E., Galizia, E., Martin-Lopez, D., Walker, M. C., Azeez, A. A. A., Meiklejohn, K., Allen, D., Price, C., Georgiou, S., Bagary, M., Khalsa, S., Manfredonia, F., Tittensor, P., Lawthom, C., Shankar, R., Terry, J. R., & Woldman, W. (2023). Estimating the likelihood of epilepsy from clinically non-contributory EEG using computational analysis: A retrospective, multi-site case-control study. *MedRxiv*.
[3] Wang, H. E., Woodman, M., Triebkorn, P., Lemarechal, J.-D., Jha, J., Dollomaja, B., Vattikonda, A. N., Sip, V., Medina Villalon, S., Hashemi, M., Guye, M., Makhalova, J., Bartolomei, F., & Jirsa, V. (2023). Delineating epileptogenic networks using brain imaging data

and personalized modeling in drug-resistant epilepsy. *Science Translational Medicine*, *15*(680). https://doi.org/10.1126/scitranslmed.abp8982

Disclosures: **H. Wang:** None. **P. Triebkorn:** None. **J. Makhalova:** None. **J. Lemarechal:** None. **B. Dollomaja:** None. **A. Williamson:** None. **D. Depannemaecker:** None. **J. Terry:** None. **F. Bartolomei:** None. **V. Jirsa:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.02/XX16

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

Support: Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012)
Digital Europe Grant TEF-Health # 101100700
Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907, Human Brain Project SGA3 945539, EOSC VirtualBrainCloud 826421, AISN 101057655, EBRAINS-PREP 101079717
H2020 European Innovation Council PHRASE 101058240
H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Foundation Charité
Johanna Quandt Excellence Initiative
German Research Foundation SFB 1436 (project ID 425899996), SFB 1315 (project ID 327654276), SFB 936 (project ID 178316478), SFB-TRR 295 (project ID 424778381), SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1

Title: Simulated hyperexcitation derived from amyloid PET is associated with tumor necrosis factor levels in CSF in Alzheimer’s Disease

Authors: *M. DA COSTA ZEMSCH[1,2], L. STEFANOVSKI[1,2], L. MARTIN[1,2], P. RITTER[1,2]; [1]Neurol. with Exptl. Neurol., Charité Berlin, Berlin, Germany; [2]Berlin Inst. of Hlth., Berlin, Germany

Abstract: Neuroinflammation is an emerging factor in the pathogenesis of Alzheimer's disease (AD), and recent evidence even supports its role in disease progression. Tumor necrosis factor-alpha (TNF-α) has been implicated in various neuroinflammatory responses in AD. Furthermore,

evidence indicates that a higher amyloid beta (Abeta) burden leads to regional disturbed inhibition, causing hyperexcitation (Liu, Q. et al., 2013, Ren, S.-Q., et al., 2018). As AD progresses, the hyperexcitation decreases (Busche et al., 2019), likely due to the progressive neurodegenerative processes and synaptic dysfunction associated with the disease. This generates a shift from hyperexcitation in mild cognitive impairment (MCI) to hypoexcitation in AD. We aim to explore this relationship between hyperexcitability, related to cognitive impairment in AD, and the levels of TNF-α in cerebrospinal fluid (CSF) and hope to highlight new potential treatment directions. We used The Virtual Brain (TVB; www.thevirtualbrain.org), a neuroinformatics platform for simulating brain network dynamics with empirical connectivity from patient scans. Based on individual Abeta positron emission tomography (PET), we simulate the individual neural activity based on a healthy connectome to investigate hyperexcitation (Stefanovski et al., 2019). Our study uses data from 100 ADNI (http://adni.loni.usc.edu) patients (57 male, age 65-85/43 female, age 55-89). We examined both regional and group differences (including healthy control, MCI, and AD participants) and explored correlations between the simulation results and TNF-α levels. Our results reveal a correlation between CSF TNF-α levels and in silico hyperexcitation. MCI patients display the highest correlation, consistent with the observed decrease in hyperexcitation as AD progresses. Additionally, we observe distinct spatial patterns of the correlation among different patient groups, highlighting the regional specificity of the potential TNF-α-related hyperexcitability. In conclusion, our study provides evidence linking hyperexcitability in MCI simulations to neuroinflammation. The correlation between TNF-α levels and hyperexcitation supports the potential involvement of TNF-α in the cognitive decline of AD. Understanding this relationship contributes to our comprehension of AD pathophysiology and provides a basis for developing new therapeutic approaches targeting TNF-α-mediated neuroinflammation.

Disclosures: M. da Costa Zemsch: None. **L. Stefanovski:** None. **L. Martin:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.03/XX17

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

Support: Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
Digital Europe Grant TEF-Health # 101100700
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907
H2020 Research and Innovation Action Grant Human Brain Project SGA3 945539
H2020 Research and Innovation Action Grant EOSC VirtualBrainCloud 826421

H2020 Research and Innovation Action Grant AISN 101057655, H2020 Research Infrastructures Grant EBRAINS-PREP 101079717
H2020 European Innovation Council PHRASE 101058240, H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Research Council Grant ERC BrainModes 683049, JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité, Johanna Quandt Excellence Initiative, German Research Foundation SFB 1436 (project ID 425899996)
German Research Foundation SFB 1315 (project ID 327654276), German Research Foundation SFB 936 (project ID 178316478)
German Research Foundation SFB-TRR 295 (project ID 424778381)
German Research Foundation SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1

Title: The Virtual Brain Ontology - a systematic knowledge framework and code generation toolbox for brain simulation

Authors: *L. MARTIN[1], L. STEFANOVSKI[1], K. BUELAU[1], C. HUETTL[1], R. A. SCHMITT[1], R. K. PAI[1,2], D. PERDIKIS[1], L. DOMIDE[3], P. RITTER[1,2];
[1]Berlin Inst. of Hlth. at Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany; [3]Codemart, Cluj-Napoca, Romania

Abstract: The simulation of brain activity based on dynamical systems theory is increasingly important for advancing diagnostic and therapeutic approaches. Brain network models (BNMs) as formulated with the neuroinformatics platform The Virtual Brain (TVB, www.thevirtualbrain.org) provide a detailed mathematical framework of whole-brain dynamics by deploying coupled neural mass models (NMMs). However, limited reproducibility and comparability of simulation results across studies and computational models are currently observed due to variations in model description, parameter nomenclature, and programmatic implementation.To systematize existing knowledge in the domain of large-scale brain modeling, we developed a human- and machine-readable knowledge representation system - The Virtual Brain Ontology (TVB-O), which semantically formalizes the complete mathematical framework of TVB. It enables the detailed annotation of each model component and their relations to each other with a newly developed unifying metadata schema. TVB-O is compatible with the Low Entropy Model Specification (LEMS, Cannon et al. 2014), by providing the possibility to convert models defined in the ontology into a generalized LEMS schema. It therefore complements RateML (van der Vlag et al. 2022), a code generator based on LEMS that succinctly formulates executable BNMs in Python or CUDA code. We present an automated pipeline for comparing established NMMs by exploring parameters based on their underlying biophysical phenomena annotated in TVB-O. Testing the effects of comparable parameters on model dynamics are demonstrated by automated bifurcation analyses and single-node simulations responding to an external stimulus.TVB-O contains over 700 defined classes defining all components of a BNM. Currently, 28 NMMs are supported. Simulation results obtained from the generated code execution are identical to manual implementations using the TVB Python library. Further, TVB-O streamlines the recreation of dynamical regimes in different NMMs by suggesting parameters with similar biophysical characteristics. TVB-O

provides an extensive knowledge framework alongside a programmatic pipeline for building BNMs with TVB, including automated code generation for bifurcation analyses and simulations, as well as a standardized metadata schema and storage for published model configurations. It can be used throughout the experimental process, from model creation and dynamical explorations to an accessible and reproducible reporting of simulation results. TVB-O will be available as a standalone ontology and will also be integrated as a Python package into the TVB library.

Disclosures: **L. Martin:** None. **L. Stefanovski:** None. **K. Buelau:** None. **C. Huettl:** None. **R.A. Schmitt:** None. **R.K. Pai:** None. **D. Perdikis:** None. **L. Domide:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.04/XX18

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

Support: Virtual Research Environment at the Charité Berlin - a node of EBRAINS Health Data Cloud
Digital Europe Grant TEF-Health # 101100700
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907
H2020 Research and Innovation Action Grant Human Brain Project SGA3 945539
H2020 Research and Innovation Action Grant EOSC VirtualrainCloud 826421
H2020 Research and Innovation Action Grant AlSN 101057655, H2020 Research Infrastructures Grant EBRAINS-PREP 101079717
H2020 European Innovation Council PHRASE 101058240, H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Research Council Grant ERC BrainModes 683049, JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité, Johanna Quandt Excellence Initiative, German Research Foundation SFB 1436 (project ID 425899996)
German Research Foundation SFB 1315 (project ID 327654276), German Research Foundation SFB 936 (project ID 178316478)
German Research Foundation SFB-TRR 295 (project ID 424778381)
German Research Foundation SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1

Title: Integrating biological pathways into whole-brain simulation: an interdisciplinary approach

Authors: *L. STEFANOVSKI[1], L. MARTIN[1], K. BUELAU[1], J. THEM[1], L. DEGER[1], M. PILLE[1], C. HUETTL[1], R. A. SCHMITT[1], M. DA COSTA ZEMSCH[1], C. LANGFORD[1], J. PALMER[1], J. STASINSKI[1], R. K. PAI[1,2], M. SACKS[1], K. DHINDSA[1], D. PERDIKIS[1], H. TAHER[1], J. M. MEIER[1], M. SCHIRNER[1], P. RITTER[1,2];
[1]Berlin Insititute of Hlth. at Charité - Universitätsmedizin Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci. Berlin, Berlin, Germany

Abstract: Introduction: While knowledge in neuroscience is increasing every day, this informational plethora is not merely comprehensible to human beings. Meanwhile, the method of multi-scale brain simulation is an emerging technology aiming to translate knowledge from dynamic systems theory to clinical applications. In addition, the latest developments in information science and large data repositories aim to make this knowledge programmatically accessible. We present the tools and the interdisciplinary methodological framework to link these technological advances altogether, resulting in a proof-of-concept for brain models covering neural mechanisms from genes to large-scale networks. **Methods**: We employ the neuroinformatics platform The Virtual Brain (TVB), allowing computational modeling of realistic brain activity in health and disease. We extended TVB by systematizing the mathematical knowledge linked to biological concepts in the unique hierarchical representation of the TVB-Ontology (TVB-O). Further, we developed the TVB adapter of semantics (TVBase), a text-mining-based technology allowing the creation of brain maps based on knowledge conveyed in thousands of scientific publications. In a clinical use case of Alzheimer's Disease, we demonstrate how to use multimodal information from databases, ontologies, and the literature to construct potential cause-and-effects models of the disease. **Results**: First, we show how relevance maps of TVBase can reconstruct patterns of empirical data of various modalities from electrophysiology and neuroimaging. Further, we introduce novel and unique data types: relevance networks of the brain, describing the co-occurrence of brain regions in the literature; and 'Virtuomics', providing spatial maps derived from literature mining of standardized gene names. The systematic mapping of pathways in Alzheimer's Disease suggests a mechanism of impaired NMDAergic excitation as a potential treatment target. We show how to construct a model of NMDAergic transmission in the Jansen-Rit model using TVB-O. Finally, we demonstrate that this model can reproduce the coincidence of both excitatory and inhibitory downstream effects, a known phenomenon in NMDA antagonism. **Discussion**: We extend the simulation framework of TVB by a unique ontology and an adapter of semantic knowledge, and we suggest a new paradigm of biological and mechanism-inferred modeling. The extraction of relevance maps from biomedical literature enables the construction of complex models, including scales ranging from multi-omics to large-scale network effects, as they are crucial for multifactorial phenomena such as neurodegeneration.

Disclosures: L. Stefanovski: None. **L. Martin:** None. **K. Buelau:** None. **J. Them:** None. **L. Deger:** None. **M. Pille:** None. **C. Huettl:** None. **R.A. Schmitt:** None. **M. Da Costa Zemsch:** None. **C. Langford:** None. **J. Palmer:** None. **J. Stasinski:** None. **R.K. Pai:** None. **M. Sacks:** None. **K. Dhindsa:** None. **D. Perdikis:** None. **H. Taher:** None. **J.M. Meier:** None. **M. Schirner:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.05/XX19

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

Support: Digital Europe Grant TEF-Health # 101100700
H2020 Research and Innovation Action Grant (Human Brain Project SGA2 785907, Human Brain Project SGA3 945539, EOSC VirtualBrainCloud 826421, AISN 101057655)
H2020 Research Infrastructures Grant (EBRAINS-PREP 101079717, EBRAIN-Health 101058516)
H2020 European Innovation Council PHRASE 101058240
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité, Johanna Quandt Excellence Initiative
German Research Foundation (SFB 1436 (425899996), SFB 1315 (327654276), SFB 936 (178316478), SFB-TRR 295 (424778381), SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1)
German Research Foundation (178316478, project C1, 178316478, project C2)
This work was supported by the Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud

Title: Lesion Aware automated Processing Pipeline (LeAPP) for multimodal neuroimaging data of stroke patients

Authors: *P. BEY[1,2], K. DHINDSA[1,2], A. KASHYAP[1,2], M. SCHIRNER[1,2,3,4,5], J. FELDHEIM[6], R. SCHULZ[6], M. BÖNSTRUP[7], G. THOMALLA[6], B. CHENG[6], C. GERLOFF[6], P. RITTER[1,2,3,4,5];
[1]Berlin Inst. of Hlth. at Charité, [2]Dept. of Neurol. with Exptl. Neurology, Brain Simulation Section, Charité, Universitätsmedizin Berlin, Berlin, Germany; [3]Bernstein Focus State Dependencies of Learning and Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [4]Einstein Ctr. for Neurosci. Berlin, Berlin, Germany; [5]Einstein Ctr. Digital Future, Berlin, Germany; [6]Klinik und Poliklinik für Neurologie, Kopf- und Neurozentrum, Univ. Med. Ctr., Hamburg-Eppendorf, Germany; [7]Klinik und Poliklinik für Neurologie, Universitätsklinikum Leipzig, Leipzig, Germany

Abstract: The underlying mechanisms of recovery after ischemic stroke are not well understood. Personalized brain simulations can provide insights but require accurate processing of structural and functional magnetic resonance images (MRI). Stroke lesions present a challenge for image processing pipelines. We advanced existing frameworks to reliably facilitate individual TheVirtualBrain (TVB) network models for stroke patients. The new “Lesion Aware automated Processing Pipeline (LeAPP)” extends the Human Connectome Project (HCP) minimal processing pipeline. It incorporates additional processing and correction steps, has been validated

and made available as container workflow. Thus, we created to our knowledge the first comprehensive automated pipeline for processing stroke MRI data. A total of 36 stroke patients (18 female, 65.7 years) and 15 healthy controls (7 female, 69.2 years) were processed across four timepoints (3-5, 30-40, 85-95, and 340-380 days post stroke onset) when available. The integrated correction measures include cost function masking during image registration to reduce lesion impact, and enantiomorphic normalization to enable full brain segmentation and parcellation using contralesional hemisphere signal to replace the damaged tissue. Additional processing steps were implemented to adjust for limitations in data quality and available MRI protocols due to the clinical context of data acquisition, such as single-phase encoding direction in diffusion imaging or reduced field-of-view for functional MRI. To foster reproducibility and allow for easy application to new data and system independent deployment (e.g., for high performance computing environments) the framework was implemented into a software container. For validation we transplanted lesions from patient to control data to generate a dataset of 82 artificial stroke brains. We then compared the performance of the standard HCP and LeAPP pipeline in generating the ground truth anatomical parcellation of cortical (HCP-MMP1 atlas) and subcortical (Fischl atlas) regions. Agreement was measured using dice score, center-of-gravity distance and volume differences. Connectomics-based measures included difference in clustering, node strength and centrality between ground truth and each pipeline respectively. Differences in global node strength were significantly lower for LeAPP. Further LeAPP showed higher agreement and smaller differences across local measures for both lesion-affected and lesion-unaffected regions-of-interest (ROIs). LeAPP is a robust and automated pipeline facilitating novel and reproducible stroke MRI based research.

Disclosures: P. Bey: None. **K. Dhindsa:** None. **A. Kashyap:** None. **M. Schirner:** None. **J. Feldheim:** None. **R. Schulz:** None. **M. Bönstrup:** None. **G. Thomalla:** None. **B. Cheng:** None. **C. Gerloff:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.06/XX20

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

Support: NSERC Postdoctoral Fellowships Program
CIHR Projects Grant PJT-168980

Title: Cognitive sparing in healthy aging: A structural connectivity perspective

Authors: *J. NEUDORF, K. SHEN, A. R. MCINTOSH;
Inst. for Neurosci. and Neurotechnology, Simon Fraser Univ., Burnaby, BC, Canada

Abstract: Functional complexity research has shown changes in healthy aging, whereby there is more complexity locally with lower complexity at global network scales. These changes may be

adaptive as they were associated with better cognitive ability (Heisz et al., 2015). We now investigate whether there are corresponding structural connectivity (SC) differences in healthy aging that are also adaptive, preserving cognitive function with age.
SC was calculated from the Cambridge Centre for Aging and Neuroscience cohort diffusion-weighted imaging data, using probabilistic tractography and an atlas with 220 regions (cortical, Schaefer et al., 2017; subcortical, Tian et al., 2020). Graph theory regional efficiency measures were also calculated based on SC. The Cattell (1971) fluid intelligence score was used to measure cognitive function. Multivariate PLS analysis (McIntosh & Lobaugh, 2004) was used to identify latent variables (LVs), each containing weights that describe the relationship of all connections with behaviour. We report only the most reliable PLS weights as determined by bootstrap resampling.
A PLS analysis of SC identified two significant LVs. Of particular interest, one LV was positively correlated with both age and fluid intelligence, indicating cognitive sparing with age (more streamlines with positive weights related to higher fluid intelligence in older age).
The largest positive weights for the cognitive sparing LV were primarily intrahemispheric (81.0%), and primarily in the LH (54.8%), with minimal alterations to interhemispheric connections (19.0%). The largest negative weights for LV2 were sparse, and included mostly intrahemispheric connections (80%; 35% LH and 45% RH) with only four interhemispheric connections (20%). A PLS analysis of SC regional efficiency measures will also be discussed.
These results demonstrate that SC also has a nuanced relationship with aging and cognitive ability, with a subnetwork of SC contributing to the preservation of fluid intelligence with age. This cognitive sparing network is predominantly intrahemispheric, consistent with past research demonstrating that older adults have increased functional local complexity associated with spared cognitive function. This research will be beneficial for informing the accuracy of our upcoming TVB (The Virtual Brain) Healthy Aging Model, to ensure that beneficial and not only detrimental alterations in SC are accounted for.

Disclosures: **J. Neudorf:** None. **K. Shen:** None. **A.R. McIntosh:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.07/XX21

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

Support: Alzheimer's Disease Neuroimaging Initiative (ADNI) (National Institutes of Health Grant U01 AG024904) and DOD ADNI (Department of Defense award number W81XWH-12-2-0012)
Digital Europe Grant TEF-Health # 101100700
Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907, Human Brain Project SGA3 945539, EOSC VirtualBrainCloud

826421, AISN 101057655, EBRAINS-PREP 101079717
H2020 European Innovation Council PHRASE 101058240
H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Foundation Charité
Johanna Quandt Excellence Initiative
German Research Foundation SFB 1436 (project ID 425899996), SFB 1315 (project ID 327654276), SFB 936 (project ID 178316478), SFB-TRR 295 (project ID 424778381), SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1

Title: Whole-brain simulation of TMS in Alzheimer's disease shows that cortical geometry can explain differences in evoked potential responses

Authors: *A. NEGI[1,2,3,4], L. STEFANOVSKI[1,2], M. SCHIRNER[1,2,3,5,6], P. RITTER[1,2,3,5,6]; [1]Charite Universtätsmedizin Berlin, Berlin, Germany; [2]Berlin Inst. of Hlth. (BIH) at Charité – Universitätsmedizin Berlin, Berlin, Germany; [3]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [4]Technische Univ. Berlin, Berlin, Germany; [5]Einstein Ctr. for Neurosci. Berlin, Berlin, Germany; [6]Einstein Ctr. Digital Future, Berlin, Germany

Abstract: Non-pharmacological interventions in Alzheimer's Disease (AD) have gained interest due to the limited effectiveness of current pharmacological treatments (Wang et al. 2020). Non-invasive brain stimulation techniques such as Transcranial Magnetic Stimulation (TMS) have shown promising initial results in improving cognitive function (Nardone et al. 2014). TMS-evoked potentials (TEP) up to 300 ms post-stimulation have been shown to differ significantly in amplitude and are used for diagnosing and evaluating AD progression (Nardone et al. 2021). To gain insight into the underpinnings of the observed TEP differences, we build virtual brains using The Virtual Brain framework (Ritter et al. 2013; Sanz Leon et al. 2013). Each virtual brain incorporates individual cortical geometry and connectivity to simulate the differential effects of TMS. We utilize individualized TMS electric field modeling and whole-brain modeling to simulate five AD, five Mild Cognitive Impairment (MCI), and five Healthy Control (HC) virtual brains. Data from the Alzheimer's Disease Neuroimaging Initiative is used. It is hypothesized that brain network modeling using individualized structural connectivity (SC) and cortical, skull, and scalp geometry will be able to reproduce empirically shown differences in the amplitude of the P30 component (positive peak after 30ms after onset) of TEPs between AD and HC (Julkunen et al. 2008). Our model reproduced TEPs and replicated larger P30 amplitudes in AD than in HC, consistent with empirical findings (Ferreri et al. 2016). Simulated P30 amplitudes were significantly higher in AD than in MCI. Next, we aimed to identify the causes of the observed differences in simulated P30 amplitudes and performed global parameter explorations with various simulation settings. Our analysis indicated that differences in cortical geometry arising from atrophy primarily influenced these variations in P30 amplitudes; reflected as widened sulci and reduced gray matter (GM) volumes in the individual head models. Our results suggest that P30 responses are an indirect measure of cortical atrophy in AD. Our findings reveal that P30 does not have direct causal associations with altered SC or hyperexcitability (Ferreri et al. 2016). Moreover, the simulated P30 amplitudes in AD were mildly correlated($r=0.53$) with the decline in cognitive function as measured by MMSE scores (also reported in Bagattini et al.

2019). Further examination is required to explain the TEP behavior within the MCI group wherein GM reductions were not prominent. This study paves the way for in silico explorations of TMS interventions in AD.

Disclosures: **A. Negi:** None. **L. Stefanovski:** None. **M. Schirner:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.08/XX22

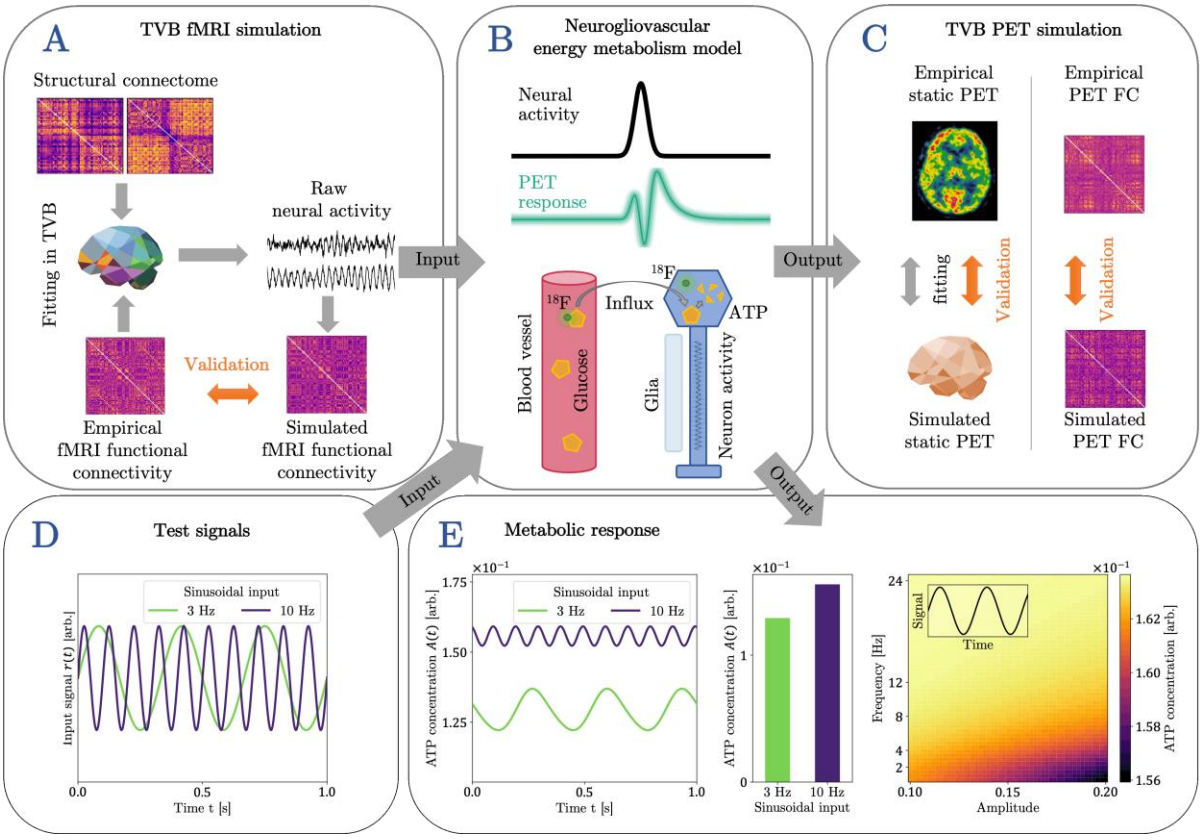
Topic: I.06. Computation, Modeling, and Simulation

Support: Virtual Research Environment at the Charité Berlin – a node of EBRAINS Health Data Cloud
Digital Europe Grant TEF-Health # 101100700
H2020 Research and Innovation Action Grant Human Brain Project SGA2 785907, SGA3 945539
H2020 Research and Innovation Action Grant EOSC VirtualBrainCloud 826421
H2020 Research and Innovation Action Grant AISN 101057655
H2020 Research Infrastructures Grant EBRAINS-PREP 101079717
H2020 Research Infrastructures Grant EBRAIN-Health 101058516
H2020 European Innovation Council PHRASE 101058240
H2020 European Research Council Grant ERC BrainModes 683049
JPND ERA PerMed PatternCog 2522FSB904
Berlin Institute of Health & Foundation Charité
Johanna Quandt Excellence Initiative
German Research Foundation SFB 1436 (project ID 425899996), SFB 1315 (project ID 327654276), SFB 936 (project ID 178316478)
German Research Foundation SFB-TRR 295 (project ID 424778381)
German Research Foundation SPP Computational Connectomics RI 2073/6-1, RI 2073/10-2, RI 2073/9-1

Title: Decoding the relation of neural oscillations and energy metabolism: a neurogliovascular model of FDG-PET in The Virtual Brain

Authors: *H. TAHER[1,2], L. STEFANOVSKI[1,2], L. MARTIN[1,2], M. MÜTING[1,2], M. SCHIRNER[1,2,3,4,5], S. JAMADAR[6], G. F. EGAN[6], P. RITTER[1,2,3,4,5];
[1]Berlin Inst. of Hlth. at Charité Universitätsmedizin Berlin, Berlin, Germany; [2]Dept. of Neurol. with Exptl. Neurol., Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Bernstein Focus State Dependencies of Learning and Bernstein Ctr. for Computat. Neurosci., Berlin, Germany; [4]Einstein Ctr. for Neurosci. Berlin, Berlin, Germany; [5]Einstein Ctr. Digital Future, Berlin, Germany; [6]Monash Univ., Melbourne, Australia

Abstract: Neural oscillations are a key element of brain dynamics and can characterize pathologies, such as neurodegeneration and encephalopathy, which alter the mechanisms underlying the oscillations. Spectral changes also occur during reduced consciousness and sleep. In this study, we link spectral properties of neural oscillations to the cerebral glucose metabolism assessed through positron emission tomography using [18F]fluorodeoxyglucose (FDG-PET) in a whole-brain computational model. Our approach is based on The Virtual Brain (TVB, www.thevirtualbrain.org) and an existing neurogliovascular (NGV) model, that captures the impact of neural activity on adenosine triphosphate (ATP) concentrations, hence on glucose levels and the FDG-PET signal. We optimize and validate the model using a dataset of simultaneous resting-state fMRI and functional FDG-PET recordings of healthy subjects. The model yields insights into cerebral metabolic rates and their relationship to frequencies of neural activity. Low frequency (2 Hz - 4 Hz), but high amplitude input, results in lower metabolic rates, as opposed to fast (8 Hz - 12 Hz), but low amplitude input. These predictions are in line with observed reductions in FDG-PET signals during non-REM sleep, associated with high amplitude δ-band activity compared to awake state, associated with low amplitude α-band activity. While the different amplitudes of α- and δ-band activity contribute to this effect, they turn out to not be crucial, as our modeling results suggest. The phenomenon is primarily mediated through a frequency sensitive response of the NGV system to neuronal activity, which leads to an elevated ATP supply in presence of fast oscillations. Whole-brain simulation approaches comprise methods to generate signals of neuroimaging modalities such as fMRI. We propose the FDG-PET model as an addition to these pipelines, bringing them closer towards clinical applications. Since PET is readily available in routine clinical settings, such as the diagnostic evaluation of neurodegenerative diseases, this extension is particularly valuable.



Disclosures: **H. Taher:** None. **L. Stefanovski:** None. **L. Martin:** None. **M. Müting:** None. **M. Schirner:** None. **S. Jamadar:** None. **G.F. Egan:** None. **P. Ritter:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.09/XX23

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

Support: NSF CBET-2123862
NSF EFMA-2223811
Teledyne FLIR

Title: A neuromorphic algorithm for rapid continual learning

Authors: ***R. MOYAL**[1], J. FOREST[1], M. EINHORN[1], A. BORTHAKUR[2], T. A. CLELAND[1]; [1]Cornell Univ., Ithaca, NY; [2]IIT Hyderabad, Sangareddy, India

Abstract: In natural environments, learning is typically rapid and incremental. The mammalian olfactory system, with its recurrent circuitry and signal conditioning capabilities, is particularly efficient at detecting and separating overlapping input in the presence of background interference. It learns and generalizes hierarchical categories over time, such that novel samples are incorporated into (but do not overwrite) the existing representational landscape. We designed a spiking neural network (SNN) model that leverages several algorithmic principles employed by the olfactory bulb: (1) periodic inhibitory signals are used to segment activity into packets and impose a spike-phase code; (2) heterogeneous firing thresholds allow the network to accommodate a wide dynamic range of inputs; (3) a neurogenesis-like mechanism in the inhibitory granule cell layer allows for the continual modification of learned categories, preventing catastrophic forgetting; (4) weight updating is achieved with local spike timing dependent plasticity rules. We tested the model in few-shot learning scenarios using real-world chemosensory data as well as synthetically generated data. In doing so, we utilized our PyTorch-based SNN modeling framework, Sapicore, to systematically investigate the effects of various connectivity and hyperparameter configurations on the network's pattern separation capabilities. Our model yielded superior classification performance when trained with degraded data from a gas sensor array or with artificial data points drawn from overlapping distributions. Our work paves the way for generalized biomimetic SNN algorithms suited for deployment on neuromorphic hardware and edge devices.

Disclosures: **R. Moyal:** None. **J. Forest:** None. **M. Einhorn:** None. **A. Borthakur:** Other; pending patent application. **T.A. Cleland:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teledyne FLIR. Other; pending patent application.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.10/XX24

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

Support: NSF CBET-2123862
NSF EFMA-2223811
Teledyne FLIR

Title: Input regularization mechanisms of the olfactory bulb glomerular layer

Authors: *J. FOREST[1], K. R. MAMA[2], R. MOYAL[1], M. EINHORN[1], A. BORTHAKUR[3], T. A. CLELAND[1];
[1]Cornell Univ., Ithaca, NY; [2]Wu Tsai Neurosciences Institute, Stanford Univ., Stanford, CA; [3]IIT Hyderabad, Sangareddy, India

Abstract: Biological and artificial neural networks require statistically predictable inputs for optimal performance. Constraining the distributions of input activation enables the coordinated computational elements of a functional circuit (i.e., neurons, synapses and network motifs) to operate within their effective response ranges. Peripheral sensory systems, however, are necessarily exposed to relatively unconstrained input variance. Consequently, they must transform and regularize these signal patterns --while preserving their information content-- before communicating them to downstream regions.
In the olfactory system, this function is governed substantially by glomerular layer circuitry within the olfactory bulb. Previous physiological and computational studies have separately characterized bulbar mechanisms that capture and compress broad-ranging variance, regulate contrast, normalize activity, and statistically regularize input distributions. Here, we unite these mechanisms in a common signal conditioning framework using our spiking neural network modeling framework, Sapicore. Specifically, our signal conditioning layer implements (1) global intensity normalization based on nonspecific lateral projections by superficial short axon cells; (2) non-topographical contrast enhancement based on feedforward inhibition and regulated by neuromodulation; and (3) statistical input regularization based upon heterogeneous duplication within columns, arising from heterogeneity in the properties of co-columnar (sibling) mitral cells. The result is a signal conditioning layer that adapts to broad unregulated patterns of external stimulation and transforms raw sensory input into a statistically reliable format with minimal loss of information. We quantify and analyze the concerted properties of this complex circuit, and show that heterogeneity in neuronal and synaptic properties can be a crucial contributor to the function of natural systems embedded in unregulated environments.

Disclosures: **J. Forest:** None. **K.R. Mama:** None. **R. Moyal:** None. **M. Einhorn:** None. **A. Borthakur:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); pending patent application. **T.A. Cleland:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Teledyne FLIR, pending patent application.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.11/XX25

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

Support: National Science Foundation Science Technology Center Award CCF-1231216
Office of Naval Research Multidisciplinary University Research Initiative (ONR MURI) N00014-13-1-0333
Understanding Human Cognition Award Grant No. 220020469
Collaboration on the Global Brain Grant No. 543061
Sloan Foundation Fellowship FG-2018-10963
NSF RI 1703161
NSF CAREER Award 1844724
NSF CAREER Award 2047191
NSF grant 2121009
DARPA Machine Common Sense program

Title: Modeling and evaluating how the brain makes physical predictions

Authors: *H. WANG[1], F. BINDER[1], R. VENKATESH[2], K. JEDOUI[2], J. TENENBAUM[3], D. YAMINS[2], J. FAN[1], K. SMITH[3];
[1]UCSD, La Jolla, CA; [2]Stanford Univ., Stanford, CA; [3]MIT, Cambridge, MA

Abstract: From judging whether a cup will fall off from the table to catching a baseball in a game, humans make fast and reliable physical predictions every day. What is the nature of the anatomical and functional constraints that leads the brain to make such physical inferences? Research in cognitive neuroscience suggests that these capabilities are supported by noisy, explicitly 3D object-centric models that use approximately accurate physical principles - called the “intuitive physics engine” (IPE). Recently there has been a growing body of work in machine learning that suggests that physical simulators can be learned from observations of physical events, and these models can serve as neurally mappable hypotheses for the brain’s simulator. Here we compare human physical predictions on stimuli generated from the Physion benchmark (Bear et al, NeurIPS 2021) with a structured model of the IPE (Battaglia et al, PNAS 2013; Allen et al, PNAS 2020) and state-of-the-art deep learning models of dynamics (RPIN: Qi et al, ICLR 2021; FitVid: Babaeizadeh et al, ICLR 2022; R3M: Nayebi et al, arXiv 2023). Participants (n=50) and models were shown 450ms of a scene, and asked to predict whether a target object would contact a goal zone if the scene continued to unfold. Across 150 different scenes, we found that human performance was significantly above chance (proportion correct=0.85, t=59.96, p~0), and highly reliable (split-half reliability=0.95, 95% CI: [0.93, 0.96]). A model that explicitly modeled noisy physics - including uncertainty over perception (object positions and orientations), physical properties (object masses) and the physical dynamics (how collisions

resolve) - achieved human-level accuracy (proportion correct=0.87) and critically, correlated well with human predictions (r=0.94). In contrast, all deep learning models failed to achieve this level of performance in terms of both accuracy (RPIN=0.66, FitVid=0.62, R3M=0.70), and correlations with human predictions (RPIN=0.58, FitVid=0.37, R3M=0.54). These findings suggest that current learned models of physics do not fully capture the cognitive and neural processes underlying human physical predictions. Further work is needed to understand what parts of the IPE have been learned by these models and what architectural constraints (e.g., object-centric representations, notions of uncertainty) need to be built into future models.

Disclosures: **H. Wang:** None. **F. Binder:** None. **R. Venkatesh:** None. **K. Jedoui:** None. **J. Tenenbaum:** None. **D. Yamins:** None. **J. Fan:** None. **K. Smith:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.12/Web Only

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

Support: NIH/NEI grant EY 13055

Title: Comprehensive Neuroanalytic Model of ERG Kinetics

Authors: *C. TYLER;
Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Introduction. The electroretinogram (ERG) is a powerful non-invasive assay of the functional integrity of the human retina, providing measures of the retinal receptor potential and the bipolar cell function, together with signals attributable to the inner plexiform layer of the amacrine retinal ganglion cells. The ERG is the summed response from all levels of the retinal processing of light, and exhibits several profound nonlinearities in the underlying processing pathways. An accurate model of the ERG is important both for understanding the multifold processes of light transduction to ecologically useful signals by the retina, but also its diagnostic capabilities for the identification of the array of retinal diseases. Methods. The present neuroanalytic approach to modeling the human rod ERG is elaborated from the same general principles as the kinetic serial/parallel model of the dark-adapted flash response Hood & Birch (1992), but incorporates the more recent understanding of the early nonlinear stages of ERG a-wave generation developed by Robson & Frishman (2014). It is a serial-process model of the retinal processing kinetics, with additive parallel readout from each processing stage to form the overall electrical signal of the ERG. Our approach is further extended from the brief flash response to account for the light-adapted On/Off step response, the first such model to do so, by incorporating separate half-wave rectifying generators for the On and Off bipolar responses. Results. As a result of the respective nonlinear component structure, this new model provides a substantially better match than previous models of rod responses in six different waveform

features of the canonical ERG flash intensity series, together with the compound nonlinearities of the On/Off ERG step responses to white light.Conclusion. The provision of an accurate model of the On and Off pathways of the retina represents the first dynamic model of the full ERG kinetics, providing a significant step towards more accurate quantification of retinal processing deficits than is available from the standard peak statistics of the ERG flash responses.

Disclosures: C. Tyler: None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.13/XX26

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

Support: Start-up funds from Johns Hopkins University (SPM, HA)

Title: Using an artificial neural network estimator to quantify synergistic information in high-dimensional datasets

Authors: *X. FAN[1], H. ADWANIKAR[3], S. P. MYSORE[2];
[2]Johns Hopkins Univ., [1]Johns Hopkins Univ., Baltimore, MD; [3]John Hopkins Univ. Sch. of Medici Training Program In Neurosci., Baltimore, MD

Abstract: Synergy is the phenomenon of multiple variables being able to collectively encode information that is not available in any single variable. Estimating synergistic information (SI) of high-dimensional systems remains an open problem despite its importance for understanding complex systems. This is partly due to the combinatorial explosion of substructures in multivariate mutual information (MI) with increasing data dimensions, which has led to the perception of the problem as unsolvable beyond six dimensions. Here, we propose a new estimation method that circumvents this problem by measuring the loss of information that results from the purposeful destruction of synergy in the data. Our method involves two repeating steps: first, we train a mutual information neural estimator (MINE) using a multi-task learning approach to gauge the current mutual information (MI); second, we calculate the MI gradients in relation to the data and scramble the data based on these gradients to reduce synergy. This method makes no explicit assumptions to the underlying data distribution, and is able to capture any SI as long as the MI in that data is learnable by artificial neural networks, thereby guaranteeing its wide applicability to various datasets. We validated our method on synthetic high-dimensional datasets, including a dataset that imitates neurons' synchronous firing, with known theoretical values derived under partial information decomposition (PID) framework. We then applied this method to real-world datasets and quantified multiple synergistic relationships suggested by previous research. Overall, our work reveals a new information-theoretic approach for better characterization of high-dimensional complex systems through an exploration of synergistic relationships within them, which are inherent in real-world systems.

Disclosures: **X. Fan:** None. **H. Adwanikar:** None. **S.P. Mysore:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.14/XX27

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

Support: Human Brain Project (HBP SGA 3 / 1.15)
The "Frontières de l'Innovation en Recherche et Éducation" (FIRE) Doctoral School

Title: Efficient approximations for probabilistic synaptic connectivity in large-scale network models

Authors: *M. CALICE, J. BALLBÉ, L. J. GRAHAM;
Ctr. Giovanni Borelli - CNRS UMR 9010, Univ. Paris Cité, Paris, France

Abstract: Large-scale network models of the nervous system aim to reproduce collective dynamics and function with a bottom-up approach based on explicit cellular nodes. One challenge is to balance granularity against model construction cost, in particular for parameter searching. For large numbers of neurons, there is a non-negligible computational burden for specifying the precise connections between nodes. Local connectivity for these models is typically defined by a probability function *F(S,T)* on a candidate pre-synaptic (source *S*) and post-synaptic (target *T*) pair of neurons (e.g. Gaussian function of distance), specified by experimental data or theoretical considerations. Brute force methods for defining the connectivity of a circuit typically apply this function to all possible pairs of source-target candidates, and thus are $O(N^2)$, where N is the number of implicated cells. To mitigate this scaling we propose a forward model approach for choosing synaptic connections, applied to mouse cerebral cortex, based on the expected number of connections rather than the probability *per se*: 1) All cells are binned according to their location on a regular grid *B* on the cortical plane with a characteristic dimension *X*. 2) Given a source cell *S*, the target cell density and an approximate value of *F()* as a function of *S* and each grid element B_{ij}, we obtain the expected number of connections M_{ij} from *S* to targets within B_{ij} ($M_{ij} = F(S,B_{ij})$ *x [#cells in* B_{ij}*]*) for a total of $M = \Sigma M_{ij}$ connections. 3) The explicit assignment of connections from *S* proceeds by iterating over *B*, choosing at random M_{ij} cells from those previously assigned to B_{ij}. The algorithm is thus of order $O(MxN)$: For a typical sub-region (e.g. primary visual area layer II/III), $N \sim 10^5$, and for a typical cortical neuron, $M \sim 10^3$, this represents a speed increase of $\sim 10^2$ over the brute force method. The precision and efficiency of the algorithm scales inversely with *X*. In the limit as *X* (approximately) goes to zero, the procedure mimics the brute force approach. There are two sources of artifacts as *X* increases: a) The approximation *F()* of *F(S,T)* over B_{ij}, and b) the geometric dis-congruence between the grid and the shape of the probability function, e.g. for a circularly symmetric *F(S,T)* the mapping between an annulus centered on *S* and the underlying

geometry of *B.* In this context *X* may be interpreted as a granularity parameter. We demonstrate the correspondence between *M(X)* and the theoretical number of connections obtained by direct integration of *F(S,T)* over the cortical plane, and the dynamical signatures of these artifacts obtained by simulations of excitatory and inhibitory networks with biophysically mimetic cell models and densities.

Disclosures: **M. Calice:** None. **J. Ballbé:** None. **L.J. Graham:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.15/XX28

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

Support: AFOSR YIP award FA9550-23-0173

Title: Impact of Neurogenesis-inspired Neuronal Age on Artificial Spiking Neural Network Training

Authors: *J. A. KILGORE, G. C. ADAM;
Electrical and Computer Engin., The George Washington Univ., Washington, DC

Abstract: The hippocampus, a core brain region for learning and memory, hosts a unique phenomenon in the healthy adult mammalian brain: the addition of new neurons via neurogenesis in its front-end region, called the dentate gyrus. While this phenomenon and its role in robust pattern separation is under study in the neuroscience community, it could be critical for designing bio-inspired artificial neural networks with superior performance in continual learning. In this study, we take the first steps to investigate the impact of neuronal age and its firing behavior on network performance to inform future neurogenesis-inspired network learning. Using experimental patch clamp recordings from the literature, we develop Izhikevich models to match the spiking behavior seen in young, middle, and mature-aged DG granule cells. These models are then used to build feed-forward spiking neural network variants. Network structures with a 100-neuron hidden layer containing a 95-to-5 ratio of middle-to-young or mature-to-young aged neurons are built respectively inspired by the typical percentage of neurogenesis in the mammalian brain. Three network variants with a hidden layer composed entirely of either young, middle, or mature-aged neurons serve as a benchmark. The input to the hidden layer is purely excitatory, transforming the Fashion MNIST dataset to spike times corresponding to 784 input neurons. The output layer aggregates the spikes received from the hidden layer into 10 leaky integrator output neurons, corresponding to the classes in the dataset. These networks are trained using surrogate gradient backpropagation through time, which we have adapted to Izhikevich model neurons. While not bio-realistic, this method is chosen in this context to provide mathematically robust training for a globally minimum loss solution. The results show that the higher threshold voltage of the middle and mature-aged neurons causes the

network to train slower, mirroring the lower synaptic plasticity in aging biological neural networks. For example, the 100%-mature variant has a 5.7% lower overall average accuracy at epoch 30 compared to the 100%-young variant. Moreover, the 95% mature:5% young network variant outperforms its 100%-mature benchmark showing the potential importance of neurogenesis in supporting higher network performance. These results will form the basis for incorporating neurogenesis-based neuron models in larger studies of bio-realistic neuromorphic systems. Further work will explore more sophisticated Izhikevich neurogenesis models as a function of age and benchmarking biologically realistic local learning rules against surrogate gradient training results.

Disclosures: **J.A. Kilgore:** None. **G.C. Adam:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.16/XX29

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

Support: MOST110-2320-B-A49A-502-MY3 from National Science and Technology Council, Taiwan

Title: A deep learning model to characterize neuron-astrocyte interaction linking cognitive deficit in diseased mouse brains

Authors: Y.-J. HUANG[1], K.-P. WU[2], *Y.-H. LEE[1];
[1]Physiol., [2]Inst. of Biomed. Informatics, Natl. Yang Ming Chiao Tung Univ., Taipei, Taiwan

Abstract: Neuron-astrocyte interaction plays a crucial role for glutamate homeostasis, neuronal excitation and synaptic function. Perineuronal astrocytic processes (PAPs) provide functional and structural support to neurons that astrocytic glutamate transporter (GLT1) keep extracellular glutamate concentrations to regulate neuron activation. Our previous study indicated that cognitive impairment was found in mouse disease model such as chronic kidney disease (CKD) accompanied with reduction of $GLT1^+$ PAPs signals. We hypothesis that the geometry and expression of $GLT1^+$ PAPs signals can be served as an indicator to predict the pathological condition in neuron-astrocyte interaction. However, the examination of functional roles on $GLT1^+$ PAPs for neuron-astrocyte interaction remains challenged. In this work, we perform a deep learning (DL) model which can predict cognitive function in mice from brain fluorescent images of astrocytic GLT1 and neuron dendritic marker MAP2. The datasets contained mouse hippocampus immunofluorescent confocal images and the discrimination index (DI) of novel object recognition test collected from the CKD mouse model and the sham-operated mice. The group of “cognitive impairment” was labeled according to the DI between 0.4 and 0.6. An automated preprocessing workflow for image segmentation and quantitative analysis of fluorescent images was performed and the labelled datasets including training dataset and

validation dataset were used to train the deep learning model. A confusion matrix and analyzed feature importance were performed that developed DL model achieved disease prediction through classification of diseases by distinguishing the features of neuron-astrocyte interaction. The trained DL model perform 90% accuracy on new dataset that have not been learned. In conclusion, this work provides an insight into the behavior alteration and computational prediction of the disturbance of neuron-astrocyte interaction in brain diseases.

Disclosures: **Y. Huang:** None. **K. Wu:** None. **Y. Lee:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.17/XX30

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

Support: ARO-MURI grant W911NF2110312

Title: Multi-scale modelling of neural-astrocyte network dynamics for context-dependent computation

Authors: *L. GONG[1], T. PAPOUIN[2], S. CHING[1];
[1]Electrical and Systems Engin., [2]Neurosci., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Neural computation proceeds on multiple spatial and temporal scales, though the mechanisms that mediate this heterogeneity in scales remain enigmatic. While much focus in theoretical neuroscience has been directed at the architecture and dynamics of neurons and neuronal networks, a significant portion of the brain is constituted by non-neuronal cells such as astrocytes, a type of glia. The role of astrocytes in impacting neuronal computation remains debated, in part because of the disparate spatial and temporal scales upon which these cells act relative to neurons. Here, we introduce a model of neural-astrocyte interaction, with the goal of understanding how astrocytes may enhance the functional expressiveness of neurons. We propose a novel two-layer hypernetwork framework which takes inspiration from biological structures of the tripartite synapse but generalizes it to embody a fuller repertoire of mechanisms by which astrocytes may propagate contextual information to neurons. By leveraging commonly-used rate-based recurrent neural network model, Hebbian learning for synapses, and an astrocytic model built upon first-order ordinary differential equations, we integrate the dynamics of neurons, synapses, and astrocytes into a single network model. Importantly, the proposed model not only describes the feedback structure between neurons and astrocytes, but also their differential spatial and temporal scales. We find that not only does the presence of astrocytes significantly expands the network dynamics that can be enacted by neurons, but that this dynamical range may enable context-dependent learning. To explore this point, we used the neural-astrocyte model within a reinforcement learning algorithmic setup designed to solve multi-armed bandits, i.e., sequential value-based decision-making problems. When the context of

the task (i.e., underlying reward probabilities) fluctuates, the neural-astrocyte network model is able to adapt and learn with performance that exceeds conventional bandit algorithms, including artificial intelligent ones designed for context-dependence. More importantly, theoretical analysis of the model and trained networks show that the neural-astrocyte hyperstructure enables key mechanisms that are instrumental in achieving adaptation to dynamic decision-making environments. These findings generate new theory for how astrocytes may impact neural computation, particularly for context-dependent functions.

Disclosures: L. Gong: None. **T. Papouin:** None. **S. Ching:** None.

Poster

PSTR511. Network Computations: Theory and Modeling II

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR511.18/XX31

Topic: I.07. Data Analysis and Statistics

Support: NIMH R01MH11559
NIGMS P01GM118269
The JPB Foundation

Title: A hierarchical hidden semi-Markov model for characterizing multi-region dynamics during decreased states of arousal

Authors: *J. CORREA MENENDEZ[1,2,3], Z. CHEN[5,6], E. K. MILLER[1,2], E. N. BROWN[1,2,3,7,4]; [2]The Picower Inst. for Learning and Memory, [3]Inst. for Data, Systems and Society, [4]Inst. for Med. Engin. and Sci., [1]MIT, Cambridge, MA; [5]Dept. of Neurosci. and Physiol., [6]Dept. of Psychiatry, New York Univ. Sch. of Med., New York City, NY; [7]Dept. of Anesthesia, Critical Care and Pain Med., Harvard Med. Sch., Boston, MA

Abstract: During decreased states of arousal such as sleep and unconsciousness, the cortex exhibits periodic fluctuations in population neural spiking activity. These fluctuations manifest as transitions between states of high and low activity, referred to as Up/Down states. For instance, during propofol-mediated unconsciousness, multi-region cortical spike trains exhibit coupled Up/Down dynamics. Hidden Markov models (HMMs) have been used in the neuroscience literature for inferring Up and Down states from neural spiking activity. However, a statistical inference framework for combining information across subjects and sessions to derive population and subject-level model parameters is lacking. Here we develop a hierarchical hidden semi-Markov model where multi-region dynamics are modeled as probabilistic functions of a coupled hidden semi-Markov process. We use a Farlie-Gumbel Morgenstern copula with Weibull marginals to obtain joint distributions of state durations across region pairs. We introduce random effects to pool information across datasets and obtain population and session-level parameter estimates. Our approach provides a framework for goodness-of-fit assessment and parameter uncertainty quantification at each level of the data hierarchy. We showcase the

accuracy of our model in simulation and apply our model to characterize Up/Down states from neural activity recorded from 1. a non-human primate under propofol-maintained unconsciousness, 2. several rodents during sleep. Our analyses show the flexibility of our statistical inference framework for characterizing Up/Down dynamics from multi-level spike train data.

Disclosures: J. Correa Menendez: None. **Z. Chen:** None. **E.K. Miller:** None. **E.N. Brown:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.01/XX32

Topic: I.07. Data Analysis and Statistics

Support: R01 DC 019124
R01 DC 014701

Title: Scoring rodent digging behavior with Annolid

Authors: *J. FANG, C. YANG, T. A. CLELAND;
Cornell Univ., Ithaca, NY

Abstract: The automated identification of complex behaviors in animal studies is a crucial yet challenging task. We present a novel method for detecting 'digging' activity in mice using Annolid, a deep learning-based behavior analysis package based on instance segmentation (https://cplab.science/annolid). Notably, digging is defined by directed motion over time rather than by static pose or position, presenting a challenge for behavior classification strategies based on the scoring of individual frames. Here, we analyzed video recordings of freely behaving mice trained to dig in dishes of sand and captured from a top-down perspective. Our analysis strategy was twofold. First, “signature frames” were identified in which a characteristic pose combined with a specific background texture were together strongly predictive of digging behavior. In this research, specifically, masks were generated that included the mouse’s head plus some surrounding area in front of it. Frames in which the head was lowered and oriented downward atop a background of sand, the digging substrate, were labeled as positive instances. In contrast, frames that may appear similar at a glance - such as those showing the head lowered but not above the sand, or those depicting an upward gaze while above the sand - but did not represent digging behavior were classified as negative instances. We then used these labeled frames to train an instance segmentation model in Annolid, and applied that model to score all video frames. Second, we applied a post-processing thresholding/gap-filling transformation to translate the resulting frame-based labels into continuous behavioral epochs. We then assessed these model-generated interpretations of digging behavior against human-scored assessments. Using this strategy, the model demonstrated robust performance across multiple videos of different

animals based on a training set of fewer than 150 frames. Accuracy was measured using the area under the Receiver Operating Characteristic curve (AUROC) metric. Our current performance of AUROC = 0.98 confirms the potential of this approach to classify digging behavior at a high level of consistency compared to human scorers. This research underscores the flexibility of deep learning-based analysis methods applied to behavioral neuroscience; by automating the identification of complex behaviors such as 'digging', we can significantly enhance the efficiency of animal behavior analysis.

Disclosures: **J. Fang:** None. **C. Yang:** None. **T.A. Cleland:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.02/XX33

Topic: I.07. Data Analysis and Statistics

Support: R01 DC 019124
R01 DC 014701

Title: Annolid: Annotate, Segment, and Track Anything You Need

Authors: ***C. YANG**, J. FOREST, M. EINHORN, T. A. CLELAND;
Cornell Univ., Ithaca, NY

Abstract: Annolid is a deep learning-based software package for labeling and segmenting research targets in video files, with a focus on animal behavior analysis. Based on instance segmentation models rather than pose estimation, Annolid leverages overfitting to achieve robust tracking of multiple identified animals even in environments where animals may be partially or entirely obscured by environmental features or by one another. This instance-based strategy also enables body part tracking and the recognition and classification of diverse, specialized behavioral measurements such as freezing, pup huddling, or urine deposition. Annolid's instance segmentation strategy is a natural fit for a broad range of behavior analysis applications. Using a graphical user interface, users first outline (mask), label, and annotate objects of interest on selected frames; this can be done manually or with the aid of Meta AI's Segment Anything Model, a new addition to Annolid. Labeling and annotation strategies are highly flexible; labeled elements can include polygons, instance objects, object parts, semantic segmentations, bounding boxes (bbox), keypoints, and event segments, including event start and end times for the delimiting and scoring of ongoing behaviors such as digging. Following labeling and annotation, Annolid manages the training of segmentation and tracking models via deep learning. The training strategy for these models depends on user-defined criteria and research requirements; for example, they can be overfitted to optimize the identification and separate tracking of many interacting animals, or generalized so that labeled behaviors can be recognized more robustly in newly added videos. Model training can be performed locally or by utilizing online high-

performance computing resources such as Google Colab. Models also can be iteratively tuned and updated with a human-in-the-loop strategy enabling direct edits of candidate masks and labels generated by Annolid. Finally, in addition to direct behavior classification by a model, behaviors of interest can be identified or filtered through post-processing analyses, computing outcomes based on combined factors such as nose location or body mask overlap. Annolid also is compatible with our manual video scoring package, Glitter2. Both packages are open-source and freely available: https://cplab.science/annolid, https://cplab.science/glitter2.

Disclosures: C. Yang: None. **J. Forest:** None. **M. Einhorn:** None. **T.A. Cleland:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.03/XX34

Topic: I.07. Data Analysis and Statistics

Support: NIH R01 Grant MH120073
NIH R01 Grant MH060013
NIH R01 Grant MH052090
Office of Naval Research MURI N00014-19-1-2571
Office of Naval Research MURI N00014-16-1-2832

Title: BU-CVKit: Extendable computer vision framework for behavioral neuroscience

Authors: *M. PATEL[1], L. C. CARSTENSEN[2], Y. GU[1], S. MALMBERG[2], M. BETKE[1], M. E. HASSELMO[2];
[1]Computer Sci., [2]Psychological & Brain Sci., Boston Univ., Boston, MA

Abstract: Computer Vision (CV) has become an integral part of interdisciplinary neuroscience research. It facilitates accurate tracking of animals, which in turn allows for complex behavioral analysis. Combining these methods with neural recordings enables researchers to study the neural basis of behavior and map neural circuits. However, there is a significant research bottleneck due to the lack of a framework that eases the reuse and abstraction of state-of-the-art computer vision methods for neuroscientists. We present here BU-CVKit, a computer vision framework that allows the creation of research pipelines with chainable modules where each module performs a specific computational task. These pipelines can be interpreted as a black box that performs semantically meaningful computations on the input data, such as analyzing spatial occupancy, rearing frequency, or generating firing rate plots. In addition to the provided modules, the framework supports plugins for extending its computational capabilities. We also provide **Mu**ltiview **Seq**uential **Pose** (MuSeqPose) Kit, a user interface for the pose estimation and tracking packages included in our framework. It automatically scans for installed plugins and programmatically generates an interface based on the metadata provided. In addition, it supports standard pose estimation features such as pose and behavioral annotations, 3D reconstruction,

reprojection, and camera calibration. Finally, we show several examples of behavioral neuroscience pipelines created through the DeepLabCut and OptiPose plugins created for our framework. These pipelines include 3D tracking, ray tracing, viewpoint reconstruction, immobility analysis, rearing detection, heading analysis, and occupancy analysis. By pairing these behavioral results with electrophysiology or calcium imaging data, we showcase our cell analysis pipelines and provide examples that enable other labs to analyze behavioral and neural data easily.

Disclosures: **M. Patel:** None. **L.C. Carstensen:** None. **Y. Gu:** None. **S. Malmberg:** None. **M. Betke:** None. **M.E. Hasselmo:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.04/XX35

Topic: I.07. Data Analysis and Statistics

Support: NSF 1707398
Gatsby Charitable Foundation GAT3708
Simons Foundation 543023
NIH U19NS123716
NIH K99NS128075
Wellcome Trust
German National Academy of Sciences Leopoldina
Netherlands Organisation for Scientific Research (VI.Veni.212.184)
NSF IOS-2115007
NIH NS075023
Irma T Hirschl Trust

Title: Lightning Pose: improved animal pose estimation via semi-supervised learning, Bayesian ensembling, and cloud-native open source tools

Authors: ***M. WHITEWAY**[1], D. BIDERMAN[1], C. L. HURWITZ[1], N. GREENSPAN[1], R. S. LEE[4], A. VISHNUBHOTLA[1], M. SCHARTNER[5], J. M. HUNTENBURG[6], A. KHANAL[7], G. MEIJER[5], J. NOEL[8], A. PAN-VAZQUEZ[9], K. SOCHA[10], A. E. URAI[11], I. LABORATORY[12], R. WARREN[2], D. NOONE[1], F. PEDRAJA[1], J. P. CUNNINGHAM[3], N. SAWTELL[1], L. PANINSKI[3];
[2]Neurosci., [3]Statistics, [1]Columbia Univ., New York, NY; [4]Lightning.ai, New York, NY; [5]Champalimaud Fndn., Lisbon, Portugal; [6]MPI for Biol. Cybernetics, Tubingen, Germany; [7]Univ. of California Los Angeles, Los Angeles, CA; [8]New York Univ., New York, NY; [9]Princeton Univ., Princeton, NJ; [10]Univ. Col. London, London, United Kingdom; [11]Cognitive Psychology, Leiden Univ., Univ. of Leiden, Leiden, Netherlands; [12]Intl. Brain Lab., Lisbon, Portugal

Abstract: Pose estimation algorithms are shedding new light on animal behavior and intelligence. Most existing models are only trained with labeled frames (supervised learning). Although effective in many cases, the fully supervised approach requires extensive image labeling, struggles to generalize to new videos, and produces noisy outputs that hinder downstream analyses. We address each of these limitations with a semi-supervised approach that leverages the spatiotemporal statistics of unlabeled videos in two different ways. First, we introduce unsupervised training objectives that penalize the network whenever its predictions violate smoothness of physical motion, multiple-view geometry, or depart from a low-dimensional subspace of plausible body configurations. Second, we design a new network architecture that predicts pose for a given frame using temporal context from surrounding unlabeled frames. These context frames help resolve brief occlusions or ambiguities between nearby and similar-looking body parts. The resulting pose estimation networks achieve better performance with fewer labels, generalize better to unseen videos, and provide smoother and more reliable pose trajectories for downstream analysis; for example, these improved pose trajectories exhibit stronger correlations with neural activity. We also propose a Bayesian post-processing approach based on deep ensembling and Kalman smoothing that further improves tracking accuracy and robustness. We demonstrate our results on a range of datasets, including head-fixed mice running on a treadmill, freely swimming fish, and head-fixed mice data from the International Brain Lab. In addition, we release a deep learning package that adheres to industry best practices, supporting easy model development and accelerated training and prediction. Our package is accompanied by a cloud application that allows users to annotate data, train networks, and predict new videos at scale, directly from the browser.

Disclosures: **M. Whiteway:** None. **D. Biderman:** None. **C.L. Hurwitz:** None. **N. Greenspan:** None. **R.S. Lee:** None. **A. Vishnubhotla:** None. **M. Schartner:** None. **J.M. Huntenburg:** None. **A. Khanal:** None. **G. Meijer:** None. **J. Noel:** None. **A. Pan-Vazquez:** None. **K. Socha:** None. **A.E. Urai:** None. **I. Laboratory:** None. **R. Warren:** None. **D. Noone:** None. **F. Pedraja:** None. **J.P. Cunningham:** None. **N. Sawtell:** None. **L. Paninski:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.05/XX36

Topic: I.07. Data Analysis and Statistics

Support: This work was supported by JSPS KAKENHI Grant Numbers 19H05467 to M.T.
This work was supported by JSPS KAKENHI Grant Numbers 22H05157 and 22K19480 to K.I.
This work was supported by JSPS KAKENHI Grant Numbers 22K07325 to T.K.
AMED Brain/MINDS Grant Number JP22dm0207077 to M.T.

Title: An AI system for quantification of common marmoset natural behaviors using markerless 3D pose estimation

Authors: *X. ZHAO[1], T. KANEKO[1,2], J. MATSUMOTO[3,4], W. LU[1], L. UENO[1], T. OISHI[1], K. IKENAKA[5], K. BABA[5], H. NISHIJO[3,4], H. MOCHIZUKI[5], K.-I. INOUE[1], M. TAKADA[1]; [1]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Aichi, Japan; [2]Div. of Behavioral Development, Dept. of Syst. Neurosci., Natl. Inst. for Physiological Sciences, Natl. Inst. of Natural Sci., Aichi, Japan; [3]Dept. of Syst. Emotional Sci., Fac. of Medicine, Univ. of Toyama, Toyama, Japan; [4]Res. Ctr. for Idling Brain Sci., Univ. of Toyama, Toyama, Japan; [5]Dept. of Neurol., Osaka Univ. Grad. Sch. of Med., Osaka, Japan

Abstract: In recent decades, ecological and ethological studies have revealed a continuity of socio-ecological traits across primates including humans. This emphasizes that nonhuman primates (NHPs) constitute excellent animal models in behavioral neuroscience. However, most studies on NHPs have so far been conducted in well-controlled laboratory setups under constraints of head and body movements. While these experimental conditions allow efficient and robust behavioral evaluation, they cause a dilemma on the limitation of behavioral repertoires that could be tested. Here we challenged to overcome this situation by introducing a state-of-the-art artificial intelligence (AI) system to estimate three-dimensional (3D) poses of multiple common marmosets under freely moving conditions. We developed an end-to-end analytic pipeline that was fully optimized for quantification of marmoset natural behaviors. Employing this novel system, we showed the utility of our methodology by demonstrating differential contributions of male and female marmosets to parenting behavior. Since adult marmosets are known as cooperative breeders and share their food with infant marmosets, the infants are able to not only satisfy their nutritional needs, but also obtain an opportunity of learning about diet. Our analytic pipeline could successfully detect food-sharing events and clarify that such events occurred more frequently in fathers than in mothers, which was consistent with the findings in their natural habitats. Furthermore, we explored an advantage of our system for longitudinal evaluation of symptomatic behaviors in a marmoset model of Parkinson's disease. The automated and high-throughput nature of our system enabled us to detect motor symptoms that progressively developed over a year. In summary, we established the analytic framework that permitted an objective and large-scale quantification of marmoset natural behaviors and defined a potential to open a new avenue in behavioral neuroscience using NHPs.

Disclosures: **X. Zhao:** None. **T. Kaneko:** None. **J. Matsumoto:** None. **W. Lu:** None. **L. Ueno:** None. **T. Oishi:** None. **K. Ikenaka:** None. **K. Baba:** None. **H. Nishijo:** None. **H. Mochizuki:** None. **K. Inoue:** None. **M. Takada:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.06/XX37

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01MH129641

Title: Assessing multiple cognitive constructs of the research domain criteria (RDoC) with a novel multi-task unity software suite in nonhuman primates

Authors: *A. NEUMANN[1], M. WATSON[1], N. TRACZEWSKI[1], S. DHUNGANA[1], K. BANAIE BOROUJENI[3,1], X. WEN[1,4], T. WOMELSDORF[1,2,4];
[1]Psychology, [2]Biomed. Engin., Vanderbilt Univ., Nashville, TN; [3]Princeton Univ., Princeton, NJ; [4]Vanderbilt Brain Inst., Nashville, TN

Abstract: Nonhuman primates (NHPs) are key animal models for understanding the cell and circuit mechanisms of higher cognitive functions. Among the higher cognitive functions particularly important for understanding neuropsychiatric diseases in humans are attentional control, effort control, value-based learning from gains and losses, working memory and long-term relational memory. Systematically assessing these functions in NHPs requires (1) determining suitable consensus tasks measuring each function, (2) implementing these tasks in a diagnostic tool that is readily-available and easy to use across multiple laboratories, and (3) identifying a multi-task protocol that assesses multiple cognitive, motivational and memory functions in the same experimental sessions. Here we delineate an integrated solution addressing these challenges.

For assessing multiple cognitive and motivational constructs in NHPs we developed a multi-task assessment suite using the Unity video game creation platform. This Multi-Task Suite for Unified Experiment (M-USE) is optimized to allow an experimenter to create experimental sessions with multiple different tasks. Tasks can be presented in multiple configurations such as: an experimenter determined order, random, or subjects can choose the tasks themselves. The suite handles keyboard, mouse, touchscreen, joystick, and eye tracker input. Calibration of gaze and touch are integrated tasks that can be interleaved flexibly in a session. M-USE is compatible with single- and multi-display setups and is playable via a web-browser useful for testing human subjects.

M-USE has pre-configured tasks assessing attentional control, effort control, value-based learning from gains and losses, visuo-spatial problem solving, working memory and long-term relational memory. Rhesus monkeys are trained on the tasks using touchscreen kiosk stations. NHPs perform multiple tasks at varying difficulty levels and durations per session. Performance levels remain high and stable for multiple weeks even for complex tasks involving intra- and extra-dimensional attentional set shifting, visual search, and sequence learning.

Taken together, NHPs engaging with multiple tasks per session in M-USE reflects a novel paradigm for efficiently assessing multiple RDoC constructs. M-USE provides a versatile tool that addresses major challenges by (1) offering pre-configured tasks assessing constructs of the NIMH Research Domain Criteria, (2) proposing a standard diagnostic assessment protocol usable in animals and humans, and (3) by being freely online-available to foster easy adaptability in different research contexts.

Disclosures: A. Neumann: None. **M. Watson:** None. **N. Traczewski:** None. **S. Dhungana:** None. **K. Banaie Boroujeni:** None. **X. Wen:** None. **T. Womelsdorf:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.07/XX38

Topic: I.07. Data Analysis and Statistics

Support: NSF 1948181

Title: The OpenBehavior Project: A database and dissemination platform for open-source tools for behavioral neuroscience research

Authors: K. CHAVEZ LOPEZ[1], J. A. PALMER[1], S. R. WHITE[1], L. M. AMARANTE[2], J. FRIE[3], A. BANDROWSKI[4], J. KHOKHAR[3], A. V. KRAVITZ[5], ***M. LAUBACH**[1]; [1]Neurosci., American Univ., Washington, DC; [2]Allen Inst., Seattle, WA; [3]Univ. of Western Ontario, London, ON, Canada; [4]SciCrunch, La Jolla, CA; [5]Psychiatry, Washington Univ., St. Louis, MO

Abstract: The OpenBehavior Project promotes the use of open-source tools for behavioral neuroscience research. Since 2016, the project has disseminated information on more than 250 research tools on a weekly basis through blog posts to our website and through social media. Over the past 3 years, we have (i) created a database of all tools featured on openbehavior.com and issued Research Resource IDentifiers (RRIDs) that facilitate the citation and tracking of the tools in research publications; (ii) created a repository of raw video recordings of animals performing behavioral tasks that are commonly used in neuroscience research, organized a series of community conversations on video analysis tools, and written a commentary on setting video methods in a lab and best practices for the use of video methods; (iii) developed in-person and virtual training workshops on Arduino-based microcontrollers, including a workshop run immediately prior to this year's SfN meeting; (iv) created a repository of validated open-source designs for 3D printed objects used in neuroscience research. We hope that these efforts continue to stimulate development and innovation, as well as more widespread use of the powerful and cutting-edge methods that have emerged from the open-source neuroscience community. Going forward, we are planning to launch new community conversations and expanded in-person and virtual workshops on the use of microcontrollers and 3D printing methods and expand our efforts to collect designs for circuits, Arduino programs, and 3D designs. We also will launch a new effort to collect data and data analysis code and models for common behavioral tasks used in neuroscience, and provide examples of programming these tasks using the open-source Bonsai platform. We would welcome community input on these new initiatives.

Disclosures: K. Chavez Lopez: None. **J.A. Palmer:** None. **S.R. White:** None. **L.M. Amarante:** None. **J. Frie:** None. **A. Bandrowski:** None. **J. Khokhar:** None. **A.V. Kravitz:** None. **M. Laubach:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.08/XX39

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 2R44 MH125238

Title: Comparative Analysis of Virtual Reality Narration and Traditional Textbook Instruction in Learning and Retention of Biological Structure and Physiological Concepts

Authors: B. BARRAGAN[1], J. OSBORNE[1], M. MOREHEAD[1], N. SPENCER[1], R. DEDRICK[2], C. HOWARD KIRBY[2], ***G. SPIROU**[3];
[1]IstoVisio, Inc., Morgantown, WV; [2]Col. of Educ., [3]Med. Engin., Univ. of South Florida, Tampa, FL

Abstract: The increasing popularity of virtual reality (VR) in education necessitates continuous evaluation and improvement of educational products and embedded active learning activities. In this study, we examined the relative effectiveness of a VR environment in student learning and knowledge retention compared to traditional textbook instruction. A cohort of 31 12th-grade students, divided equally into two groups based on demographic factors such as gender and learning ability, participated in the study.Students were chosen from year-long neuroscience course which involved learning to use syGlass VR software. We chose a subject matter unrelated to the course, cardiac anatomy and physiology, but which used clinical scans involved similar structure/function relationships in a single lesson. Group One (n = 15) received their lesson through traditional textbook instruction using printed information as reference. Group Two (n = 16) experienced the same material word-for-word through a VR narrations, where the structure and functional principles were presented in 3D. Following the instruction and during the same class period, both groups underwent an assessment (Test #1) to measure immediate knowledge retention. Unbeknownst to the students, a second test (Test #2) was administered one week later to evaluate long-term retention without study or review time.Learning was similar between the two groups (Textbook Mean = 10.7, SD = 2.3; VR Mean = 10.4, SD = 3.7). However, students in the VR group exhibited significantly higher retention rates compared to the textbook group (Textbook Mean = 6.4, SD = 3.3; VR Mean = 10.1, SD = 3.3; $p < 0.05$; t-test). In other surveys, the VR environment was perceived as safer and more immersive, enabling focused concentration on the material due to its three-dimensional nature. Distractions such as student conversations and cell phone messages were reduced, enhancing the learning experience.Our findings support the notion that learning in VR narration facilitates retention of concepts that relate biological structure to function. These findings highlight the potential of VR as an effective educational tool, capable of enhancing knowledge acquisition and retention in various academic settings. We plan to continue these studies with larger student cohorts and across graded complexity of structure and function.

Disclosures: **B. Barragan:** A. Employment/Salary (full or part-time):; IstoVisio, Inc. **J. Osborne:** A. Employment/Salary (full or part-time):; IstoVisio, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IstoVisio, Inc. **M. Morehead:** A. Employment/Salary (full or part-

time):; IstoVisio, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IstoVisio, Inc. **N. Spencer:** A. Employment/Salary (full or part-time):; IstoVisio, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IstoVisio, Inc.. **R. Dedrick:** None. **C. Howard Kirby:** None. **G. Spirou:** A. Employment/Salary (full or part-time):; IstoVisio, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IstoVisio, Inc..

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.09/XX40

Topic: I.07. Data Analysis and Statistics

Title: A framework to quantify innate defensive responses with high accuracy

Authors: *S. GARG[1,2,3], G. PINO[1], C. ACUNA[1];
[1]Heidelberg Univ., Heidelberg, Germany; [2]Biol. Sci. and Bioengineering, Indian Inst. of Technol. (IIT) Kanpur, Kanpur, India; [3]Biol. Sci., Univ. of California San Diego, San Diego, CA

Abstract: There has been a rise in the usage of machine-learning approaches to track animal behavior with high spatial and temporal resolution. However, there is a lack of toolkits to integrate and process the coordinate datasets in a user-friendly manner. Here, we introduce Fear-Mouse Tracker (FMT), a simple and open-source MATLAB-based pipeline to extract and quantitatively analyze Deeplabcut-derived coordinates of mice presented with threatening stimuli that commonly trigger innate defensive responses. This framework allows for unbiased quantitative estimations of stretch-attend posture (SAP) observed during risk assessment behaviors, as well as for measurements of the timing and extent of freezing and escape responses that follow the presentation of threatening stimuli, such as exposure to a natural predator, a predator odor, or sweeping and looming stimuli resembling predator approaches. FMT is specially designed for users not very experienced in using programming languages, thus making it more accessible to a broader audience. As proof of principle, we use FMT-based analysis to obtain a quantitative estimation of defensive behaviors when the activity of chemically-defined subsets of dorsal periaqueductal grey neurons is manipulated using chemo- or optogenetic approaches.

Disclosures: S. Garg: None. **G. Pino:** None. **C. Acuna:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.10/XX41

Topic: I.07. Data Analysis and Statistics

Support: NIH DP2 MH126375
NIH R01 MH126035
Simons 876115SPI
Klingenstein Fund
New York Stem Cell Foundation NYSCF-R-NI69
Salk Collaboration Grant

Title: Multi-animal 3D pose tracking using SLEAP

Authors: S. AFSHAR[1], S. N. OLINE[2], L. MAREE[1], A. FALKNER[2], *T. PEREIRA[1];
[1]Salk Inst. for Biol. Studies, La Jolla, CA; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Accurately measuring animal movement is vital in understanding the neural basis of complex natural behaviors such as those underlying social interactions. Modern pose estimation tools like SLEAP (Pereira et al, 2022) have enabled high accuracy and robust quantification of movement in this setting by leveraging deep learning to track landmarks across multiple animals in 2D. While powerful, these approaches do not capture 3D kinematics and can suffer from occlusions when capturing videos from a single viewpoint. Extending 2D multi-animal pose tracking to 3D is non-trivial owing to the challenge of associating animal poses consistently across multiple views-an essential step to recovering 3D poses which is not present in the single-animal setting. Furthermore, a systematic study of 3D vs 2D representations of animal behavior has not yet been conducted to determine when this approach should be employed, or optimal strategies for implementing 3D multi-animal motion capture systems.To address these gaps, we developed SLEAP-Anipose (github.com/talmolab/sleap-anipose), an open-source tool to facilitate multi-animal pose tracking by integrating 2D multi-animal pose tracking (SLEAP) with multi-view triangulation (Anipose; Karaschuk et al, 2021). This tool serves as an integration layer, facilitating data conversion and ease-of-use with high-level command line interfaces. In addition, we have developed a multi-view GUI extension to SLEAP for annotation of ground truth 3D data. To further aid in automating annotation, we also developed a new algorithm for automated alignment of animal identities across views through reprojection consistency-based track reassignment.We used SLEAP-Anipose to build a large-scale dataset of freely moving, unrestrained mice in a variety of environmental conditions in the lab. This dataset spans 11.7 million frames (1.95 million timepoints, or 18 hours) across 74 sessions and 8 camera views. We tracked 15 landmarks along the body of the animals, and manually proofread identity switches across the entire dataset. The dataset is representative of a wide variety of behavioral conditions, including sessions with 1 to 4 animals and varying degrees of environmental enrichment, both of which induce challenging and frequent occlusions.We use this dataset to compare 3D vs 2D representations when used as inputs to tools for behavior segmentation (e.g., motif detection). These analyses provide evidence to inform the use of multi- vs single-camera behavioral monitoring hardware for capturing specific behaviors of interest, as well as characterizing the trade-offs in increasing camera count and positioning.

Disclosures: **S. Afshar:** None. **S.N. Oline:** None. **L. Maree:** None. **A. Falkner:** None. **T. Pereira:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.11/XX42

Topic: I.07. Data Analysis and Statistics

Support: 1RF1AG062234-01

Title: Machine learning approach reveals prominent behavioral alterations and cognitive dysfunction in a humanized Alzheimer model

Authors: *S. R. MILLER[1], K. LUXEM[2], N. KALISS[3], Y. QIU[6], P. NAMBIAR[1], C. CAI[3], K. SHEN[7], T. SAITO[8], T. C. SAIDO[9], A. PICO[4], R. THOMAS[5], S. REMY[10], J. J. PALOP[11]; [1]Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]LIN - Magdeburg, Magdeburg, Germany; [4]Gladstone Bioinformatics Core, [3]Gladstone Inst., San Francisco, CA; [5]Gladstone Bioinformatics Core, Gladstone Inst., San Franciscl, CA; [6]Neurosci., UNC - Chapel Hill Curriclum In Neurobio., Chapel Hill, NC; [7]Northwestern Univ., Chicago, IL; [8]Brain Sci. Inst, RIKEN, Wako, Japan; [9]RIKEN Brain Sci. Inst., Saitama, Japan; [10]Univ. of Bonn, Bonn, Germany; [11]Gladstone Inst. & UCSF, South San Francisco, CA

Abstract: Neurological disorders are characterized by behavioral manifestations, but our understanding of the behavioral changes induced by these diseases remains incomplete and primarily relies on conventional behavioral assessments that focus on specific domains. Although newly humanized App knock-in (KI) models of Alzheimer's disease (AD) more accurately mimic disease mechanisms compared to transgenic overexpression models, they do not consistently exhibit behavioral alterations in standard tests, despite the presence of severe AD-related neuropathological changes. To overcome this limitation in AD modeling, we have developed a machine learning platform (ethoML), based upon the tools DeepLabCut and VAME, to explore the hypothesis that deconstructing complete sequences of spontaneous mouse behavior into fundamental behavioral units (motifs) better captures the brain dysfunction induced by AD in App-KI mice. Indeed, we found that humanized $App^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ mice have robust impairments in spontaneous behavior evidenced by prominent alterations in motif use and motif transitions consistent with cognitive dysfunction, including blunted novelty response, impaired habituation and sensitization, and disorganized behavioral sequences. Our results demonstrate that humanized mice exhibit substantial impairments in spontaneous behavior. Therefore, we conclude that the ethoML platform offers a direct and unbiased means of assessing AD-related mechanisms underlying brain dysfunction.

Disclosures: **S.R. Miller:** None. **K. Luxem:** None. **N. Kaliss:** None. **Y. Qiu:** None. **P. Nambiar:** None. **C. Cai:** None. **K. Shen:** None. **T. Saito:** None. **T.C. Saido:** None. **A. Pico:** None. **R. Thomas:** None. **S. Remy:** None. **J.J. Palop:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.12/XX43

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

Title: PAWS: a next-generation tool for pain assessment in rodents

Authors: *S. OGUNDARE, J. BURDGE, A. JHUMKA, W. MAYISENI, W. FOSTER, I. ABDUS-SABOOR;
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Understanding how individuals interact with their pain on a reflexive and affective level may lend crucial insights into how to deliver truly personalized treatments for pain disorders. Furthermore, the tools to measure pain are robust, yet many lack the resolution to dissect out the behavioral underpinnings of paw withdrawal at the sub-second timescale. This timescale offers access to rapid shaking and guarding behaviors in mice, which are often inaccessible to cameras recording at low frame rates. Here, we showcase the latest iteration of our custom pipeline: Pain Assessment at Withdrawal Speeds (PAWS). Using markerless tracking of the mouse hindpaw, we reliably track the withdrawal of a paw elicited by a given stimulus. Using PAWS, multiple kinematic metrics (features) are abstracted from this original trajectory, giving an indication of the severity of pain. Using the ARM (Automated Rodent Mechanostimulator) designed in our lab to apply innocuous or painful stimuli with a high degree of precision and consistency, we examine how the reflexive and affective features of withdrawal differ with varying stimuli.

Disclosures: **S. Ogundare:** None. **J. Burdge:** None. **A. Jhumka:** None. **W. Mayiseni:** None. **W. Foster:** None. **I. Abdus-Saboor:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.13

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

Support: NIH RO1 (5R01MH129732-02, A-CM)

Title: Reveals-an open source multi camera gui for rodent behavior acquisition

Authors: *M. SHA[1], R. PHADKE[2], A. WETZEL[3], L. FOURNIER[1], A. CRUZ-MARTIN[1]; [1]Neurobio., [2]Mol. and Cell. Biol. and Biochem., [3]Dept. of Biomed. Engin., Boston Univ., Boston, MA

Abstract: Understanding the rich behavioral data generated by disease mouse models is essential for deciphering brain function because it allows us to unravel the complex interplay between genetics, neural circuitry, and behavior, providing invaluable insights into the underlying mechanisms of brain disorders. However, the current landscape lacks effective, affordable, and accessible methods for acquiring such data, especially when employing multiple cameras simultaneously. To resolve this, we have developed REVEALS (**R**odent B**E**ha**V**ior Multi-cam**E**r**A L**aboratory Acqui**S**ition), a new Graphical User Interface (GUI) for acquiring rodent behavioral data using USB3 FLIR cameras. REVEALS is designed to allow for user-friendly control of simultaneous recording from multiple cameras while streamlining the data acquisition process, enabling researchers to efficiently collect and analyze large datasets of rodent behavior. This software package is based on a stand-alone, open source framework freely available for researchers to use and modify according to their specific research needs. Here we describe the technical details of the GUI implementation, including the camera control software, the video recording functionality, and the synchronization mechanisms used to align the different camera feeds. We validate results demonstrating the stability, reliability and accuracy of the GUI for capturing and analyzing rodent behavior using DeepLabCut pose estimation in an object interaction assay. REVEALS can be incorporated into existing DeepLabCut and Moseq pipelines to capture and analyze complex rodent behavior. In summary, REVEALS provides an easy-to-use interface for the collection of behavioral data from multiple perspectives that, combined with deep learning algorithms, will allow for the discovery and characterization of previously unknown behavioral phenotypes to better understand the function of the healthy and diseased brain.

Disclosures: M. Sha: None. **R. Phadke:** None. **A. Wetzel:** None. **L. Fournier:** None. **A. Cruz-Martin:** A. Employment/Salary (full or part-time):; Center for Systems Neuroscience, Boston University, Boston, MA, USA, Department of Pharmacology and Experimental Therapeutics, Boston University, Boston, MA, USA, The Center for Network Systems Biology, Boston University, Boston, MA, USA.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.14/XX44

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

Support: 1R15 AG060461-(01)

Title: Application of Novel Machine Learning Algorithms on Freely Exploring Juvenile Rats Complements Previous Findings

Authors: *D. GONZALEZ[1], J. H. PEEL[2], J. R. CRESSMAN[3], T. DUMAS[3];
[1]George Mason Univ. Interdisciplinary Neurosci. Phd Program, Alexandria, VA; [2]Physics and Astronomy, [3]George Mason Univ., Fairfax, VA

Abstract: Semi-synchronous activity of multiple neurons in the hippocampus produces oscillatory network behaviors observed in local field potentials (LFPs). Well characterized oscillations occur in varying frequency bands including theta (4-12 Hz), slow gamma (30-60 Hz), fast gamma (65-100 Hz), and sharp wave ripples (SWRs, 140-200 Hz). Changes in power and event rate in these frequency ranges have been associated with changes in cognitive states in awake animals engaging in spatial navigation. For example, slow gamma power increases during periods of immobility prior to movement to a known goal location whereas fast gamma power increases during movement. Traditional frequency analyses, such as Fourier transformations or Morelet wavelets, decompose signals as a series of sinusoids. However, neuronal signals are inherently nonlinear and quasi-periodic, meaning that these analyses may fail to capture the full nature of these signals. To address this, we applied Diffusion mapped Delay Coordinates (DMDC), which has been applied successfully to model nonlinear and dynamical physical systems, but yet to biological signals. DMDC operates on the assumption that a dynamic attractor underlies each temporal measurement and that the distribution density of measurements determines the manifold to return measurements of Dimension and Volume about this measured manifold. Dimension reflects the number of higher dimensional projection states needed to resolve the original attractor and Volume represents the length of the attractor period. When applied to LFP signals collected from hippocampal area CA1 in juvenile rats freely exploring a Y-maze, DMDC reported effects of developmental stage on slow and fast gamma Dimension, but not Volume. However, There was a significant interaction effect of age and drug on theta Volume. Dimension and Volume were not impacted by the delivery of a positive allosteric modulator of AMPA receptors, location in the Y-maze, or alternation behavior. Thus, DMDC outcomes partially corroborate prior analyses of these LFPs using more traditional approaches but also reveal novel effects.

Disclosures: D. Gonzalez: None. **J.H. Peel:** None. **J.R. Cressman:** None. **T. Dumas:** None.

Poster

PSTR512. Software Tools: Behavior

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR512.15/XX45

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

Support: NSF Grant No. DGE1745016 and DGE2140739

Title: Arm-drive: assistive robotic manipulation and driving using electromyography for intuitive gesture-based teleoperation

Authors: *J. YANG[1], Z. ERICKSON[2], D. J. WEBER[3];
[1]Biomed. Engin., [2]Robotics Inst., [3]Mechanical Engin. and Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Millions of Americans report some form of paralysis, making it difficult for them to perform tasks of daily living. For people with tetraplegia, the neural drive to muscles is highly impaired, but we and others have demonstrated that volitional myoelectric activity can still be detected using high-density electromyography (HDEMG). In this study, neural network-based classification of hand-gestures using a wearable HDEMG interface is used to teleoperate a mobile manipulator robot to perform activities of daily living. The model is trained in a 10-class gesture classification model using training data from each human study session. We performed a study with 13 able-bodied human subjects that were instructed to perform a series of hand gestures while 64 channels of HDEMG were recorded from the right forearm. Training data is collected through a series of animated prompts and audio cues. Following training, the participants were instructed to operate a mobile robotic manipulator using gestures that were mapped to specific degrees of freedom on the robot. Participants remained seated in a chair throughout the experiment and completed a survey at the end to rate their comfort and ease of use of using this new interface to control a robot to perform assistive tasks. Using a fully-connected neural network, we are able to achieve an average intrasubject classifier test accuracy of 99.27%, along with an average test accuracy of 96.00% when evaluating subsequent test datasets collected after an average of 67.5 minutes. We also tested the interface on 4 different assistive robot tasks, including feeding, meal preparation, blanket manipulation, and lightbulb turning and found participants were able to finish all tasks within 15 minutes; all participants agreed that the time in which they were able to accomplish tasks is reasonable. This study demonstrates the capabilities of this interface in controlling a mobile manipulator through real-time decoding of HDEMG recorded from forearm muscles in able-bodied subjects. This work will be extended to participants with tetraplegia to further develop this interface for controlling assistive telerobots.

Disclosures: J. Yang: None. **Z. Erickson:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BIONIC POWER INC, IOTA BIOSCIENCES, INC., NEURONOFF, INC., NEUROONE, INC., REACH NEURO, INC..

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.01/XX46

Topic: I.08. Methods to Modulate Neural Activity

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) PGS D

Title: Optical interrogation of cAMP function for hippocampal neural dynamics in freely-behaving mice during learning and memory

Authors: *J. RAI[1], H. LI[1], M. ZHEN[1], K.-I. OKAMOTO[2];
[2]Lunenfeld-Tanenbaum Res. Inst., [1]Lunenfeld-Tanenbaum Res. Inst., Toronto, ON, Canada

Abstract: The spatiotemporal organization of hippocampal circuit activity is critical for hippocampus-dependent learning and memory. However, the molecular mechanisms underlying the coordination of hippocampal neural activity and their task-related dynamics remain unclear. Here, we introduce our strategy to study the role of cAMP (adenosine 3′,5′-cyclic monophosphate) in hippocampal neural dynamics underlying learning and memory in freely-behaving animals by combination of fiber-based micro-endoscopic calcium imaging and optogenetic cAMP manipulation *in vivo*. cAMP is a ubiquitous second messenger in various intracellular signaling pathways that mediate sensory transduction and neuromodulation and serve for certain types of synaptic plasticity. We have previously reported the spatiotemporal function of cAMP to rapidly enhance synaptic strength and neuronal depolarization in murine hippocampal slices (Luyben et al., 2020), suggesting that cAMP may have roles not only at the synapse-level but may also serve to regulate neuronal activity at the circuit-level. However, its spatiotemporal role to regulate neural activity dynamics during hippocampus-dependent learning and memory remains obscure. To directly interrogate cAMP function for hippocampal neural activity in freely behaving mice, we virally co-expressed fluorescent calcium sensors with light-sensitive enzymes PAC (Photoactivatable Adenylyl Cyclase) and/or Dr-HsPDE4 (Phosphodiesterase 4) in hippocampal CA1 pyramidal neurons, then implanted a micro-endoscopic GRIN (gradient index) lens probe in the mouse brain. Using our custom 3D-printed headmount implant, we coupled the GRIN lens probe to a wirelessly controlled fiber optic-LED on the animal's head for simultaneous *in vivo* calcium imaging and light-dependent cAMP manipulation in the brain of a freely behaving animal. We optically recorded activity of CA1 pyramidal neurons with or without optogenetic cAMP manipulation in the same freely-behaving animals during memory tests and revealed that the spatiotemporal activation of cAMP signal is critical for hippocampus-dependent memory. We will discuss our computational approaches to characterize the effect of cAMP perturbation on population-level activity dynamics that correlate with murine memory behaviors.

Disclosures: **J. Rai:** None. **H. Li:** None. **M. Zhen:** None. **K. Okamoto:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.02/XX47

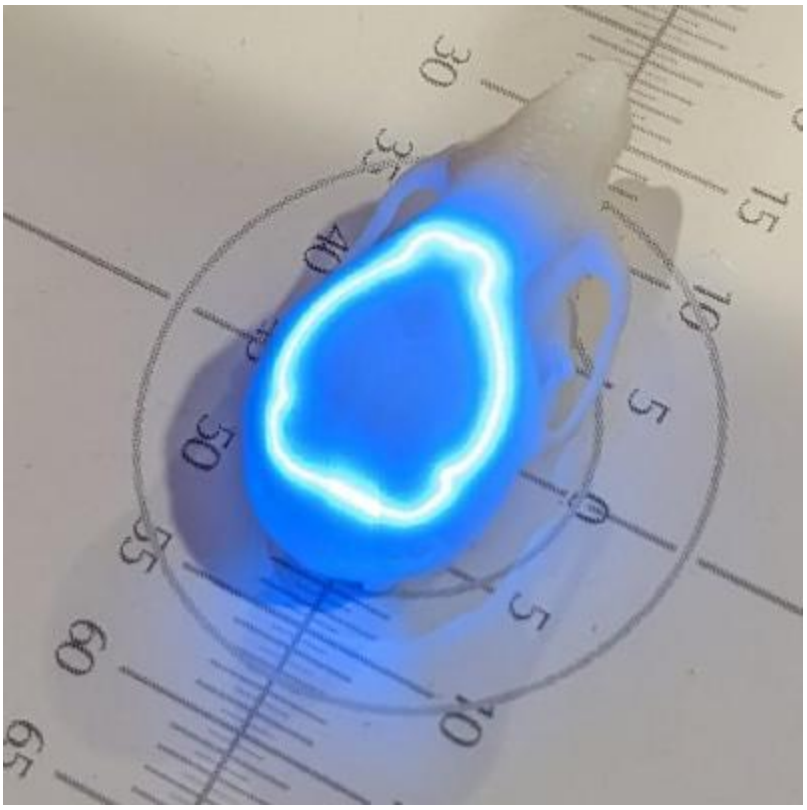
Topic: I.08. Methods to Modulate Neural Activity

Support: Wellcome Trust 219627/Z/19/Z
Gatsby Foundation GAT3755
Wellcome Trust 217211/Z/19/Z
Wellcome Trust 217211/Z/19/Z

Title: Zapit: A random-access optogenetics platform for head-fixed mouse behavior

Authors: *R. A. A. CAMPBELL[1], M. SKRęTOWSKA[4], M. LOHSE[2], O. M. GAULD[2], P. VINCENT[2], Q. PAJOT-MORIC[2], M. VÉLEZ-FORT[2], S. WEILER[2], P. WINDMILL[2], S. TOWNSEND[2], T. D. MRSIC-FLOGEL[3], C. A. DUAN[2], A. AKRAMI[2], T. W. MARGRIE[2]; [2]Sainsbury Wellcome Ctr., [1]Univ. Col. London, London, United Kingdom; [3]Sainsbury Wellcome Ctr., Univ. Col. London, London,, United Kingdom; [4]Univ. Of Cambridge, Cambridge, United Kingdom

Abstract: Causally linking neural activity to behavior requires perturbations of defined neural circuits with high spatiotemporal precision. In mice this is often achieved using optogenetics, with light being delivered via implanted optical fibres. Whilst fibres allow access to deep brain areas, they can not be easily repositioned so only a small number of stimulus locations can be tested in a single animal. Here we present "Zapit", a complete scanner-based photo-stimulation solution for the dorsal brain surface of head-fixed mice. The system directs a focused laser spot to stereotaxically defined locations through the exposed skull of mice expressing channelrhodopsin or other light-activated proteins. The scanners are fast: multiple spots can be stimulated in very close succession (<2 ms) within a single behavioral trial.
We tested Zapit in a visual change detection task. Mice indicated their responses by licking a spout. We expressed channelrhodopsin in GABAergic interneurons using the VGAT promoter: photoactivating a cortical region with blue light thus led to localised inhibition of excitatory neurons in that area. We found that inhibition of highly specific brain regions impaired task performance, with different effects being elicited by stimulation points separated by under 1 mm.Zapit is open source and comprises both a hardware design and well-documented software. An easy to use GUI is used for calibration and stimulus configuration. A closely associated API is used for control during experiments. The core software is written in MATLAB for Windows but experiments can be run via a different programing language. We have built a shared memory Python bridge to the MATLAB API that can be run on the same PC. Furthermore, Zapit's TCP/IP server allows cross-platform control via any programming language from any PC on the same network. Stimulus triggering can be done via TTL to ensure latency considerations never come into play. This poster demonstrates the power, utility, and accuracy of Zapit using a range of experimental approaches. Zapit is available at https://github.com/Zapit-Optostim.



Disclosures: **R.A.A. Campbell:** None. **M. Skrętowska:** None. **M. Lohse:** None. **O.M. Gauld:** None. **P. Vincent:** None. **Q. Pajot-Moric:** None. **M. Vélez-Fort:** None. **S. Weiler:** None. **P. Windmill:** None. **S. Townsend:** None. **T.D. Mrsic-Flogel:** None. **C.A. Duan:** None. **A. Akrami:** None. **T.W. Margrie:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.03/XX48

Topic: I.08. Methods to Modulate Neural Activity

Title: A Reconfigurable, Spatially Resolved Fiber-Optic Interface for Neural Stimulation

Authors: *S. YANG[1], K. YANG[2], Q. CHEVY[2], A. KEPECS[2], S. HU[1];
[1]Dept. of Biomed. Engin., [2]Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Fiber-optic technology has revolutionized neuroscience through its simplicity, affordability, high throughput, and compatibility with optogenetics and cell-type-specific targeting. Consequently, fiber-optic neural interfaces have been pivotal in deconstructing complex neural circuits, identifying behavioral and cognitive process-associated pathways, and

cell types. Despite these breakthroughs, current fiber-optic neural interfaces face significant challenges including limited accessible volume and poor reconfigurability, which restrict comprehensive control over stimulation in expansive and layered brain regions. Existing modifications, such as tapered fibers and multi-fiber devices, only partially mitigate those limitations. In this work, we utilized laser 3D microfabrication technology to engineer a novel fiber-optic interface with reconfigurable light emission capability both longitudinally and radially, allowing for depth and angular-resolved stimulation. Our design consists of a single 160μm-diameter fiber-optic implant featuring 1200 independently addressable emitters spread over a 2.5mm span and encompassing 360 degrees around the fiber. Each emitter covers an area of 150×20 μm^2, with a total coverage of 2500×500 μm^2 per fiber. We packaged this device with a standard fiber ferrule and utilized a butt-to-butt connectorization, adhering to common fiber optogenetics practices in neuroscience labs. We demonstrated the efficacy of this technology by integrating the fiber with a silicon electrode array, recording light-evoked neural activity across mouse cortical layers in vivo. We detected localized, light-evoked spike activity with a longitudinal resolution of 200 μm, selectively confined to one or the other side of the fiber when stimulating in different directions. To evaluate the device in behaving mice, we implanted it in the superior colliculus and deployed spatially-varying light patterns. Delivering light to different layers and in different radial directions through the same fiber produced divergent behavioral responses. Our findings demonstrate the potential of this laser-engineered fiber-optic interface as a versatile tool for spatially reconfigurable neural stimulations in freely-behaving animals through single fibers.

Disclosures: **S. Yang:** None. **K. Yang:** None. **Q. Chevy:** None. **A. Kepecs:** None. **S. Hu:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.04/XX49

Topic: I.08. Methods to Modulate Neural Activity

Support: the National Natural Science Foundation of China (32071040 to BL, 82071241 and 81871048 to LH)
Guangdong Basic and Applied Basic Research Foundation (2023B1515040019 to BL)
Guangdong Project (2017GC010590 to BL)

Title: An optogenetic toolbox for tunable control of endogenous ion channels

Authors: *L. CHEN, Z. LUO, Z. ZHANG, P. WANG, Y. LI, S. ZHAO, X. ZHANG, L. HUANG, B. LI;
Dr. Sun Yat-Sen Univ., Guangzhou, China

Abstract: Ion channels mediate ions flow through the membrane and play a key role in neuronal signaling transduction. States of ion channels change dynamically under different physiological and pathological conditions. However, fast and genetically encoded tools to regulate endogenous ion channels are lacking. Here, we developed two optogenetic tools for the BK channel: BK-OCIC (Optical Control of Ion channel Conformation), an allosteric modulator created by fusing light-sensitive LOV2 domain with BK channel-specific binding peptide, and BK-lumi, a light-sensitive toxin created by fusing BK channel blocker iberiotoxin with LOV2 domain. BK-OCIC significantly amplified BK current following blue light illumination, whereas BK-lumi specifically blocked BK channels upon expression and unblocked them in a light-dependent manner. Both BK-OCIC and BK-lumi significantly reduced the firing rate of pyramidal neurons after blue light illumination. Furthermore, dendritic light illumination led to a greater reduction in neuronal firing rate than somatic illumination, suggesting that BK channels in different subcellular compartments might have diverse capacities to regulate neuronal excitability. Finally, we found our tools could optically control mice locomotion in the open field test without affecting motor ability in the rotarod test. Collectively, we demonstrate that our optogenetic tools can modulate the activity of endogenous BK channels, offering a powerful approach to investigate the function of endogenous ion channels.

Disclosures: L. Chen: None. **Z. Luo:** None. **Z. Zhang:** None. **P. Wang:** None. **Y. Li:** None. **S. Zhao:** None. **X. Zhang:** None. **L. Huang:** None. **B. Li:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.05/XX50

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Intramural Funding

Title: Optogenetic Tagging of the Oculomotor Pathway in Non-Human Primates

Authors: *X. YU[1], A. GOPAL P A[2], K.-I. INOUE[3], M. TAKADA[4], O. HIKOSAKA[5]; [2]Lab. Of Sensorimotor Res., [1]NIH, Bethesda, MD; [3]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama,Aichi, Japan; [4]Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Inuyama, Aichi, Japan; [5]Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: Pathway selective optogenetics provides a potent method for manipulating neuronal responses based on specific projections within the brain. Despite its growing application in non-human primate research, the use of retrograde viral strategies to tag neurons that provides inputs to an area of interest is not validated. In this study, we employed retrograde tagging to selectively target neuronal populations that directly project to the superior colliculus (SC) in the oculomotor pathway of the rhesus macaque. It is well known that SC receives inputs from various cortical

and subcortical sources. The retrograde viral tagging allows us to dissect the specific functional contributions of these inputs in generating saccadic eye movements. Here we aim to identify the specific functional contributions of direct projections from the Frontal Eye Fields (FEF) and the Basal Ganglia (BG) to the SC. We confirmed the successful retrograde tagging of input neurons by stimulating FEF with a blue laser (470nm) which resulted in immediate neuronal activation. Notably, local stimulation of the labeled FEF neurons elicited saccades into the contralateral visual field, with latencies comparable to saccades induced by electrical stimulation. This provides compelling evidence that FEF neurons which directly project to SC are causally involved in saccade generation. Subsequently, we explored the nature of information these opto-tagged FEF neurons transmit to SC using multiple behavioral tasks. Our results demonstrated that the opto-tagged neurons encompassed the visual, visual-motor, and motor subtypes in FEF. Additionally, they exhibited value-related responses in two types of value tasks 1-Location Task where value is associated with specific locations 2- Object Task where value is associated with object identity. These findings suggest that the direct frontal-colliculus pathway conveys visual, motor, and value-related information. These results underscore the importance of retrograde viral strategies in dissecting the functional roles of the intricate oculomotor circuitry that underly complex eye movement behaviors in non-human primates. Now, we are in the process of extending this approach to study the contribution of BG (Nigro-collicular pathway) in the generation of saccades.

Disclosures: **X. Yu:** None. **A. Gopal P A:** None. **K. Inoue:** None. **M. Takada:** None. **O. Hikosaka:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.06/XX51

Topic: I.08. Methods to Modulate Neural Activity

Support: IHU FOReSIGHT - P-ALLOP3-IHU-000
National Institute of Health - NIH 1UF1NS107574–01
e Institut Carnot Voir et Entendre - P-SPAM00-ANR-000
Axa research funding
Grant WASCO - DIM-Elicit
European Research Council - HOLOVIS - ERC2019-ADG-885090

Title: Ultrafast light targeting for high-throughput precise control of neuronal networks

Authors: *G. FAINI, D. TANESE, C. MOLINIER, C. TELLIEZ, M. HAMDANI, F. BLOT, C. TOURAIN, V. DES SARS, F. DEL BENE, B. FORGET, E. RONZITTI, V. EMILIANI;
Inst. De La Vision, Paris, France

Abstract: The brain encodes and processes information through complex patterns of synchronous and asynchronous neuronal activations. Two-photon single-cell resolution optogenetics based on holographic light-targeting approaches enables the generation of precise spatiotemporal activity patterns, opening the way for the investigation of the neural codes for perception. Nevertheless, current approaches for cell-resolved photostimulation are limited in the resolution for tuning the relative spiking time of distinct cells and typically require high illumination intensities and dedicated powerful laser sources. To overcome these limitations and expand the capabilities of single-cell optogenetics, we introduced in this study a novel optical configuration capable of ultrafast light targeting (FLiT), based on switching temporally focused beams between holograms at kHz rates. We demonstrated two new illumination methods, which we named *hybrid*- and *cyclic*-FLiT, and achieved sub-millisecond control of sequential neuronal activation and high throughput multicell illumination, both *in vitro* and *in vivo*, while minimizing light-induced thermal rises, respectively (Faini et al., Nature comm., 2023). Moreover, the lower power requirement under the *cyclic*-FLiT configuration presents some advantages when working with small living animals such as zebrafish, reducing thermal effects upon multicell targeted illumination. Finally, we present a scheme where we took advantage of efficient FLiT photostimulation to perform all-optical experiments using a conventional Ti:sapphire single laser source, achieving simultaneous two-photon calcium imaging and photostimulation of multiple targets. The presented approaches will be important for experiments that require rapid and precise cell stimulation with defined spatio-temporal activity patterns and optical control of large neuronal populations.

Disclosures: G. Faini: None. **D. Tanese:** None. **C. Molinier:** None. **C. Telliez:** None. **M. Hamdani:** None. **F. Blot:** None. **C. Tourain:** None. **V. des Sars:** None. **F. Del Bene:** None. **B. Forget:** None. **E. Ronzitti:** None. **V. Emiliani:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.07/XX52

Topic: I.08. Methods to Modulate Neural Activity

Support: NIDA Grant DA000069
NIH NINDS 1R21NS123487
P51-OD011132

Title: Chrmera: non-invasive tracking of opsin expression for translational neuronal circuit studies

Authors: *O. SOLÍS CASTREJÓN[1], M. A. BOEHM[1], J. BONAVENTURA[5], X. HU[6], T. WICHMANN[6], H. JEDEMA[2], P. MIRANDA[3], C. RICHIE[4], Y. APONTE[3], C. W. BRADBERRY[2], A. GALVAN[6], M. MICHAELIDES[1];
[2]Preclinical Pharmacol. Section, [3]Neuronal Circuits and Behavior Section, [4]Genet. Engin. and

Viral Vector Core, [1]Natl. Inst. on Drug Abuse, BALTIMORE, MD; [5]Inst. de Neurociències, Univ. de Barcelona, Barcelona, Spain; [6]Neurol., Emory Univ., Atlanta, GA

Abstract: Channelrhodopsins (ChR) are powerful tools for dissecting neural circuit connectivity. However, tracking ChR expression in vivo remains challenging, limiting their use in studying neuronal circuits and for developing therapies. To address this, we developed ChRmERa, a fused protein of the potent and red-shifted ChR (ChRmine) with the estrogen receptor ligand binding domain for non-invasive tracking using positron emission tomography (PET) and the radiopharmaceutical [18F]-fluoroestradiol (FES). To characterize the electrophysiological properties of ChRmERa, we injected either AAV2/5-nEF DIO ChRmERa or AAV2/5-nEF DIO ChRmine into the lateral hypothalamus of *Vglut2*^{Cre}-cre mice and 3 weeks later performed whole-cell recordings. We found that ChRmERa shows efficient light-gated ion channel activity that was comparable to ChRmine indicating that ChRmERa retains its functional properties as an opsin. Next to non-invasively visualize the expression of ChRmERa, we performed PET imaging with [18F]-FES in rats and squirrel monkeys before and after (rats: 5 weeks; squirrel monkey: 5 months) the injection of AAV2/5-nEF hSyn ChRmERa in the motor cortex. Our PET imaging data showed specific binding of [18F]-FES to ChRmERa at the site of the injection. Expression of ChRmERa in rats was confirmed using immunohistochemistry. To extend its translational use, we tracked the expression of ChRmERa in two female rhesus macaques that were injected with AAV2/5-nEF hSyn ChRmERa in the putamen. We performed PET scans before and after the viral injection (6, 12, 24 weeks), and were able to longitudinally track the expression of ChRmERa in these macaques. We further investigated the functional activation of ChRmERa by performing single unit recordings in awake monkeys. We found light-gated responses in ~50% of putamen cells. Our findings demonstrate that ChRmERa can be tracked longitudinally and in a non-invasive manner across rodents and nonhuman primates. Ongoing studies are aimed at addressing the behavioral efficacy of light-evoked ChRmERa activation in rodents and non-human primates.

Disclosures: O. Solís Castrejón: None. **M.A. Boehm:** None. **J. Bonaventura:** None. **X. Hu:** None. **T. Wichmann:** None. **H. Jedema:** None. **P. Miranda:** None. **C. Richie:** None. **Y. Aponte:** None. **C.W. Bradberry:** None. **A. Galvan:** None. **M. Michaelides:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.08/XX53

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant NS047384
NIH grant NS122316

Title: An optogenetic tool for cell type-specific inhibition of eIF4E-dependent translation in the mammalian brain

Authors: *J. M. ALAPIN[1], M. MOHAMED[1], M. M. OLIVEIRA[1], P. SHRESTHA[1], H. KHALED[1], A. G. VOROBYEVA[1], H. L. BOWLING[1], A. WOOLLEY[2], E. KLANN[1];
[1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]Chem., Univ. of Toronto, Toronto, ON, Canada

Abstract: An optogenetic tool for cell type-specific inhibition of eIF4E-dependent translation in the mammalian brain
The protein kinase mechanistic target of rapamycin complex 1 (mTORC1) is one of the primary triggers for initiating cap-dependent translation. Amongst its functions, mTORC1 phosphorylates eIF4E-binding proteins (4E-BPs), which prevents them from binding to eIF4E and thereby enables translation initiation. mTORC1 signaling is required for multiple forms of protein synthesis-dependent synaptic plasticity and various forms of long-term memory (LTM), including associative threat memory. However, the approaches used thus far to target mTORC1 and its effectors, such as pharmacological inhibitors or genetic knockouts, lack fine spatial and temporal control. The development of a conditional and inducible eIF4E knockdown mouse line partially solved the issue of spatial control, but still lacked optimal temporal control to study memory consolidation resolution (Shrestha et al. Nat. Neurosci. 23: 281-292; Shrestha et al. Nature 586: 407-411). Here, we have designed a novel optogenetic tool (Opto4E-BP) for cell type-specific, light-dependent regulation of eIF4E in the brain. We show that light-activation of Opto4E-BP decreases protein synthesis in HEK cells and primary mouse neurons. *In situ*, light-activation of Opto4E-BP in excitatory neurons decreased protein synthesis in acute amygdala slices. Finally, light activation of Opto4E-BP in principal excitatory neurons in the lateral amygdala (LA) of mice after training blocked the consolidation of LTM. The development of this novel optogenetic tool to modulate eIF4E-dependent translation with spatiotemporal precision will permit future studies to unravel the complex relationship between protein synthesis and the consolidation of LTM.

Disclosures: J.M. Alapin: None. **M. Mohamed:** None. **M.M. Oliveira:** None. **P. Shrestha:** None. **H. Khaled:** None. **A.G. Vorobyeva:** None. **H.L. Bowling:** None. **A. Woolley:** None. **E. Klann:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.09/XX54

Topic: I.08. Methods to Modulate Neural Activity

Support: HHMI Gilliam Felllow

Title: Parapinopsin: a photoswitchable GPCR for two-photon optogenetics

Authors: *B. J. BROWN[1], B. A. COPITS[2], R. W. GEREAU, IV[3];
[1]Anesthesiol., Washington Univ. (WUSTL), University City, MO; [2]Pain Center, Anestesiology,

Washington Univ. in St. Louis Sch. of Med., St louis, MO; [3]Anesthesiol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: The development of optogenetic tools has greatly advanced the capability of researchers to understand higher-order functions of the nervous system by allowing for a way to precisely regulate activity within neurons. Optogenetics makes use of light-activated ion channels, ion pumps, or G-Protein coupled receptor (GPCR-based) proteins called opsins to excite or inhibit neurons. Paired with advances in microscopy and genetics, optogenetics can be used to control specific populations of cells in a time-locked, spatially specific manner. The experiments possible with optogenetics depend largely on the capabilities and limitations of the opsin used; thus, there have been continuous efforts to design and employ new opsins with a variety of characteristics (such as speed, trafficking, and wavelength sensitivity) to allow for a greater variety of optogenetic manipulations. An example of this has been the more recent developments of two-photon (2P) activated optogenetic tools, the focus of this study. Despite the key role optogenetics has played within neuroscience, the number of tools available for neuronal excitation greatly outnumber those for neuronal inhibition. Most inhibitory opsins available are ionotropic and are restricted by off-target effects, poor performance at synaptic terminals, and require constant light to maintain their effect. Inhibitory GPCR-based systems are effective at synaptic inhibition, but few of these optogenetic tools are available; to our knowledge, none of these have been implemented as tools for multi-photon optogenetics. These limitations exclude inhibitory optogenetics from many of the advantages of 2P microscopy such as greater depth penetration and increased axial resolution. In 2021, we (Copits et. al. 2021) along with Mahn et. al. (2021) published studies addressing some limitations of current inhibitory optogenetic tools. Our publication identified parapinopsin (PPO) as a bistable GPCR-coupled opsin that rapidly and reversibly inhibits synaptic terminals. Our publication included preliminary data suggesting that PPO could be utilized in studies where 2P activation is favorable over single-photon (1P) activation; we now have new data supporting PPO as a 2P-activated inhibitory opsin. Building on our last publication, we recently discovered that 2P-acticated PPO can couple to G-protein Inwardly Rectifying K+ channels (GIRK channels), which can allow for GPCR-based inhibition in neurons via. We will present: 1) a characterization of GIRK current responses to 2P activation of PPO and 2) an exploration of the capabilities of 2P-activated PPO (2P-PPO) in intact neurons.

Disclosures: **B.J. Brown:** None. **B.A. Copits:** None. **R.W. Gereau:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.10/XX55

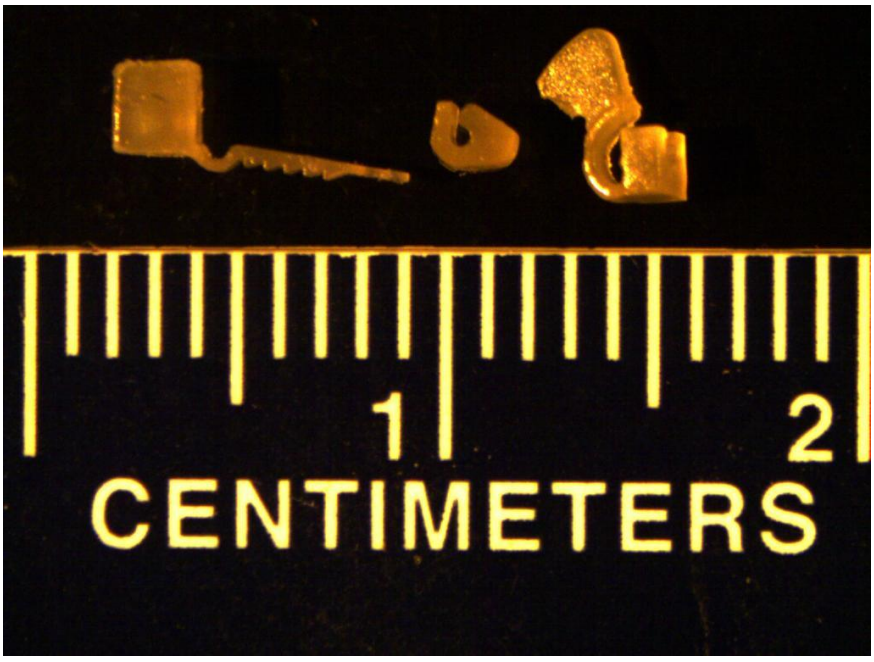
Topic: I.08. Methods to Modulate Neural Activity

Support: NIH – National Institute of Neurologic Disorders and Stroke (R21NS124313)

Title: 3d printing optogenetic interfaces

Authors: *T. R. CURRIE[1], R. R. MCLEOD[2], R. F. WEIR[1], A. K. FONTAINE[1];
[1]Bioengineering, Univ. of Colorado Denver, Aurora, CO; [2]Electrical, Computer, & Energy Engin., Univ. of Colorado Boulder, Boulder, CO

Abstract: Optogenetically modulating the peripheral nervous system is a promising experimental tool and therapeutic for a variety of diseases (post-traumatic stress disorder (PTSD), chronic inflammation, etc.) because it allows the targeting of specific pathways. Traditional, electrical stimulation is non-specific, causing off-target effects which are complications in patient populations (i.e., vagus nerve stimulation (VNS)). The geometry needed for an optogenetic neuromodulation interface can vary widely depending on which part of the nervous system stimulation is being applied. 3D printing provides the flexibility for rapid prototyping of the interface and enables unique geometries to be easily manufactured. Currently, there is no commercial solution which provides the necessary resolution for these tiny interfaces in mice with material that is soft, tough, and biocompatible. Using a custom-research digital light processing (DLP) printer, a polyethylene glycol diacrylate (PEGDA) resin formulation is being adapted to be able to create neuromodulation interfaces, or nerve cuffs, for mice that are easily manufactured, tough, soft, and non-cytotoxic. PEGDA proportion, PEGDA molecular weights/PEGDA composite molecular weights, and pentaerythritol tetrakis(3-mercaptopripionate) (PETMP) proportions are altered in the resin to observe the pattern of their effects to create a suitable nerve interface. This data is used to tune the resin to match the elastic modulus of nerve tissue while also providing a structurally sound, implantable nerve cuff. A variety of nerve cuff designs are currently being explored and printed (image 1). The passive nerve cuff design has been prototyped to include an optical fiber for optical stimulation as proof of concept (not pictured). This technology will open the design space allowing for novel neuromodulation sites to be easily and quickly implemented for optogenetic stimulation.
Image 1: 3D Printed Neuromodulation Interfaces, or Nerve Cuffs. Left: Self-Securing Optical Probe; Center: Passive Nerve Cuff; Right: Pull-Through Nerve Cuff.



Disclosures: **T.R. Currie:** None. **R.R. McLeod:** None. **R.F. Weir:** None. **A.K. Fontaine:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.11/XX56

Topic: I.08. Methods to Modulate Neural Activity

Support: NeuroNex-1707352

Title: Improved bioluminescent-optogenetic tools for manipulation of neural circuits

Authors: ***A. SLAVIERO**[1], E. C. IKEFUAMA[1], M. PRAKASH[2], A. BJOREFELDT[4], N. GORANTLA[1], J. SIMKINS[5], M. TREE[1], L. M. BARNETT[6], G. G. LAMBERT[6], N. C. SHANER[7], U. HOCHGESCHWENDER[3];
[2]Central Michigan Univ., [1]Central Michigan Univ., Mount Pleasant, MI; [3]Central Michigan Univ., Central Michigan Univ., MT Pleasant, MI; [4]Central Miichigan Univ., Mount Pleasant, MI; [5]Central Michigan Univ., Central Michigan Univ. Undergraduate Program In Neurosci., Mount Pleasant, MI; [6]Univ. of California-San Diego, La Jolla, CA; [7]UCSD, Univ. of California San Diego, La Jolla, CA

Abstract: Bioluminescent optogenetic (BL-OG) tools employ biological light generated by luciferase enzymes oxidizing their small molecule substrate, luciferin, to activate light sensing molecules. When combined with ion-moving optogenetic elements, these luciferase-opsin fusions, Luminopsins (LMOs), allow activating and inhibiting neural activity in the brain of behaving animals in a bimodal fashion: Opsins can be activated by light from a physical source or by applying the luciferin, thus expanding optogenetic tools to include a chemogenetic component (Medendorp 2021). Various luciferases have been tethered to opsins, and these LMOs have been applied for excitation and inhibition of targeted neuronal populations in vivo (Berglund 2016, Yu 2019, Park 2020, Berglund 2020, Zenchak 2020, Petersen 2022, Ikefuama 2022). To expand the utility of LMOs we generated a series of combinations of light emitters and light sensors and tested their efficacy for modifying membrane potential in whole-cell patch recordings in HEK cells and in multi electrode arrays (MEAs) in primary neurons. Light emitters were variants of *Gaussia*, *Renilla*, and *Oplophorus* luciferases, either alone or in combination with fluorescent proteins to leverage Förster resonance energy transfer (FRET) for bright light emitters. Light sensors resulting in depolarization or hyperpolarization were native or molecularly evolved channelrhodopsins or pumps, recently described super-sensitive channelrhodopsins, blue- and red-light sensors, and light-sensing G-protein coupled receptors. We also tested different configurations of LMOs, with the luciferase tethered to the opsin via the N-terminus, C-terminus, or both. LMOs were analyzed to gauge effects of bioluminescent response (luciferin application) relative to optogenetic response (LED stimulation). Whole cell voltage patch clamp of HEK cells allowed direct comparison of LED induced photocurrent to the

current elicited by substrate application to the same cell. This comparison is quantified through coupling efficiency, the fraction of the maximum photocurrent capable of being produced by biolight. Coupling efficiencies of the initial LMOs 1 (wildtype GLuc-ChR2), 2 (wildtype GLuc-VChR1), and 3 (sbGLuc-VChR1) were 0.1%, 1.2%, and 11%, respectively. Coupling efficiencies of the improved versions are substantially higher (>50%). This set of novel LMOs expands the toolbox for bimodal control of neural activities in the brain.

Disclosures: **A. Slaviero:** None. **E.C. Ikefuama:** None. **M. Prakash:** None. **A. Bjorefeldt:** None. **N. Gorantla:** None. **J. Simkins:** None. **M. Tree:** None. **L.M. Barnett:** None. **G.G. Lambert:** None. **N.C. Shaner:** None. **U. Hochgeschwender:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.12/XX57

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH – National Institute of Neurologic Disorders and Stroke (R21NS124313)

Title: Optogenetic vagus nerve stimulation with nerve cuff for inflammation attenuation in mice

Authors: *K. S. MIRANDETTE, R. F. WEIR, A. K. FONTAINE;
Bioengineering, Univ. of Colorado Denver - Anschutz Med. Campus, Aurora, CO

Abstract: Vagus nerve stimulation (VNS) is a therapeutic technique clinically approved to treat depression, stroke recovery, migraines, and epilepsy. Recently, it has been shown to have anti-inflammatory effects and is being studied for the treatment of chronic inflammatory diseases such as Crohn's disease and rheumatoid arthritis. Current VNS methods utilize electrode-based systems, which are particularly non-specific, activating off-target pathways that lead to complications and poor pathway and mechanism mapping. Optogenetic methods can be used to stimulate cells in a highly controlled manner without off-target spillover. Our novel device capitalizes on these optogenetic methods for VNS to provide specific, targeted stimulation for the study and regulation of VNS-mediated inflammatory diseases. The spiral nerve cuff consists of two layers of MDX4-4159 silicone, manufactured to naturally curl, with an embedded µLED. The spiral design allows for ease of implantation without the need for suturing. Preliminary tests were performed in ChAT-ChR2 mice with the cuff placed on the vagus nerve in the cervical region. Upon optical stimulation, a significant reduction in heart rate was recorded, with recovery to normal levels post-stimulation. This confirmed the activation of cholinergic fibers in the vagus nerve, validating our device as a method for VNS with targeted cell types. The success of the validation testing leads the way to further experimentation with our device to determine the functional effects of optogenetic VNS stimulation on inflammatory markers in models of systemic inflammation.

Figure 1: Silicone Spiral Nerve Cuff with Embedded µLED.

Disclosures: **K.S. Mirandette:** None. **R.F. Weir:** None. **A.K. Fontaine:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.13/XX58

Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH Grant ZIAMH002797
NIMH Grant ZIAMH002971
BRAIN Initiative Grant U19 NS107464-01

Title: Holographic perturbation analysis of neuronal avalanches in the cortex of the awake mouse

Authors: *T. L. RIBEIRO, A. VAKILI, B. GIFFORD, V. SINFUEGO, Y. BIBINEYSHVILI, D. PLENZ;
NIH, Bethesda, MD

Abstract: Neuronal synchronization in cortex has recently been shown at the cellular level to organize as scale-invariant parabolic avalanches, both during ongoing as well as sensory-stimulus evoked activity (Ribeiro, Capek et al., 2023). To further understand the contribution of local groups of neurons in the formation of parabolic avalanches, we combined a low-repetition, high-power laser (Carbide/Orpheus; LightConversion) and a high-resolution spatial light modulator (MeadowLark) to achieve successful 2-photon (2P) excitation of individual neurons expressing the opsin ChrimsonR in the awake mouse. Simultaneously, we imaged layer II/III pyramidal neurons of widefield-identified primary visual cortex (V1) expressing the GECI GCaMP7s/8s using a Resonant-Galvo pathway and a high-repetition, low-power laser (Discovery NX; Coherent). The activity of 150 - 300 neurons was recorded using 2P imaging in a ~450 μm x 450 μm area of V1 (100 - 200 μm depth) at ~45 Hz framerate while holographically stimulating (100 ms; <10 mW) single/groups of pyramidal neurons co-expressing the opsin (~150 trials). Images were denoised using a machine-learning-based algorithm and denoised calcium traces were then deconvolved for spike extraction. To achieve highest stimulation efficacy at lowest laser power while minimizing neuronal damage, we tested the point-spread function (PSF) of three different holographic beam profiles: 1) top-hat pattern for larger 10-μm spots (LS); 2) 2-μm diffraction-limited spot (DL); and 3) 4 diffraction-limited spots in a diamond pattern (4DL). To evaluate the system's performance, we scanned fluorescent beads (3 μm) embedded in a gel using a raster motion which allowed us to reconstruct the PSF of those holographic beam profiles. Our results demonstrate that the 4DL pattern exhibits the highest precision in *in vitro* stimulation, effectively reducing aberration and speckle noise observed in the top-hat pattern and minimizing off-target effects. During stimulation of the target cell(s), a subset of non-stimulated cells responded significantly to the perturbation (> 92% baseline spike count). Holographically-triggered avalanches were power-law distributed in size and duration. Avalanche shapes were analyzed with respect to stimulation intensity, target group size, and cut-offs in avalanche size distributions. Our results identify the perturbation parameters within the local network that give rise to the scale-free parabolic avalanches in the awake animal.

Disclosures: T.L. Ribeiro: None. **A. Vakili:** None. **B. Gifford:** None. **V. Sinfuego:** None. **Y. Bibineyshvili:** None. **D. Plenz:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.14/XX59

Topic: I.08. Methods to Modulate Neural Activity

Title: A new approach to preclinical pain assessment and analgesic screening - 'Optical von-Frey' for the determination of nociceptive threshold and response

Authors: *J. A. IREDALE[1], A. J. PEARL[2], R. J. CALLISTER[1], C. V. DAYAS[1], E. E. MANNING[1], B. A. GRAHAM[1];
[1]Univ. of Newcastle, Callaghan, Australia; [2]The Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia

Abstract: Despite decades of intensive preclinical research, new pain treatments have not been forthcoming. While there are many potential explanations for this failure, questions have been raised about the validity and predictive capacity of current preclinical nociceptive assessment and analgesic screening tools, suggesting a need for the development of new tools. Nociceptive thresholding, targeting purely nociceptive pathways without the confound of other sensory modalities or the induction of tissue damage, holds promise as one such new approach. By building on existing methods for optogenetic activation of peripheral nociceptors, in combination with a thresholding approach similar to that used in von-Frey testing for mechanical thresholding, here we present a new approach to preclinical nociceptive assessment. Transient receptor potential cation channel subfamily V member 1 (TRPV1) is an ion channel selectively expressed peripherally in nociceptors. By crossing TRPV1-Cre mice with mice expressing channelrhodopsin-2 (ChR2), TRPV1-ChR2 mice enable peripheral optogenetic activation of nociceptors via an external illumination system (473nm LED positioned under the hindpaw). Using a simplified up/down approach, adapted from von-Frey testing methods, nociceptive thresholds were assessed at baseline and following subcutaneous administration of the established analgesic morphine. Baseline nociceptive threshold, latency and response duration were comparable between animals and stable for each animal across multiple trials ($p=1$). Following administration of morphine (10mg/kg), nociceptive threshold was significantly increased compared to both baseline (baseline:0.80±0.12mW, morphine:2.75±0.20mW, $p<0.01$) and vehicle (saline:0.84±0.18mW, $p<0.001$). This indicates a significant increase in nociceptive threshold with morphine, demonstrating the effects of an analgesic on the model. Lastly, confirming the effects seen in our model were dependent on optogenetic activation of nociceptors, two control models were tested, firstly an alternative animal strain lacking the necessary cre-promoter to drive ChR2 expression and secondly using a different wavelength of light (532 nm) outside the peak absorption/activation of ChR2, both of which showed no response to optical stimuli exposure. Overall, these studies established a novel system for selective optogenetic activation of nociceptors, allowing characterization of behavioural responses to a pure nociceptive input. Thus, this offers an alternative assessment of nociceptive threshold and makes for a useful addition to the preclinical nociceptive and analgesic assessment toolkit.

Disclosures: J.A. Iredale: None. **A.J. Pearl:** None. **R.J. Callister:** None. **C.V. Dayas:** None. **E.E. Manning:** None. **B.A. Graham:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.15/XX60

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH NINDS Grant R01NS118188

Title: Drumbeat Optogenetics: Improving the firing frequency of channelrhodopsin-2

Authors: *T. A. WELTON[1], A. FONTAINE[1,2], J. H. CALDWELL[3], S. LITTICH[1], R. WEIR[1,2], D. RESTREPO[3], E. A. GIBSON[1];
[1]Bioengineering, Univ. of Colorado Denver | Anschutz Med. Campus, Aurora, CO; [2]Rocky Mountain Regional VA Med. Ctr., Aurora, CO; [3]Cell & Developmental Biol., Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: The use of optogenetics has allowed neuroscientists to study neurons and neuronal ensembles with incredible selectivity and temporal precision. Although the catalog of available opsins continues to grow, Channelrhopsin-2 (ChR2) is still one of most reliable opsins for depolarizing a variety of cells in response to light. Unfortunately, the utility of ChR2 is limited by its slow kinetics which makes exciting action potentials at frequencies ≥ 40 Hz difficult. In this work, we investigate an opsin stimulation scheme called "Drumbeat Optogenetics" where optical stimulation occurs at two separate locations asynchronously in time. Theoretically, this "drumbeat" allows the opsin at one location to recover slightly while stimulation occurs at the other. Here, we tested whether drumbeat optogenetics could be used to improve the firing frequency of a peripheral nerve relative to optogenetic stimulation of a single opsin population. Loose patch clamp was used to record compound action potentials (CAPs) from the sciatic nerve of ChAT-ChR2-YFP mice *ex-vivo* in response to traditional one-photon optogenetic stimulation, drumbeat one-photon optogenetic stimulation, and electrical stimulation. Our preliminary results show that the drumbeat scheme does generate CAPs with slightly higher currents than traditional optogenetic stimulation at lower frequencies (~20 Hz); however, this effect appears to be lost at higher stimulation frequencies (~40 Hz) where it would be the most helpful. Unsurprisingly, optogenetic CAPs have much smaller currents than electrically induced CAPs because ChR2 is only expressed in cholinergic neurons and only activated in a subset of the cholinergic neurons that are adequately exposed to light. Overall, this work represents progress towards a better understanding of how optogenetic stimulation generates signal in the peripheral nervous system at different stimulation frequencies while also exploring an innovative stimulation strategy to attempt to bypass the inherently limiting kinetics of ChR2.

Disclosures: T.A. Welton: None. **A. Fontaine:** None. **J.H. Caldwell:** None. **S. Littich:** None. **R. Weir:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder of Point Designs, llc. **D. Restrepo:** None. **E.A. Gibson:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.16/XX61

Topic: I.08. Methods to Modulate Neural Activity

Support: Walter and Berry Fellowship

Title: Structural basis and neuroscience applications for selectivity and kinetic variants of potassium-selective channelrhodopsins

Authors: *Y. KIM[1], S. TAJIMA[3], P. WANG[1], Y. JO[1], E. F. X. BYRNE[1], K. KISHI[3], C. RAMAKRISHNAN[1], M. INOUE[1], H. KATO[3], K. DEISSEROTH[2];
[1]Stanford Univ., Palo Alto, CA; [2]Stanford Univ., Stanford, CA; [3]Univ. of Tokyo, Japan, Japan

Abstract: The KCR channelrhodopsins are a recently discovered class of light-gated ion channels that selectively allow the passage of K+ ions. Their potential as inhibitory optogenetic tools has garnered significant attention. However, the mechanism behind their K+ selectivity remains a fundamental mystery, as they lack structural similarity to known K+ channels, which typically employ a four-fold symmetric conduction pathway. In this study, we present three cryo-electron microscopy structures of two KCRs, HcKCR1 and HcKCR2, derived from Hyphochytrium catenoides, alongside a structurally guided variant exhibiting enhanced K+ selectivity. Through a comprehensive approach encompassing structural, electrophysiological, computational, spectroscopic, and biochemical analyses, we uncover a novel mechanism for achieving K+ selectivity. Unlike canonical K+ channels, which feature a symmetrical filter for both selectivity and dehydration, we observe that three residues at the extracellular vestibule of each KCR monomer form a flexible asymmetric selectivity gate, distinct from a narrow dehydration pathway extending to the intracellular side. Additionally, we create- and test for neuroscience application- KCR variants with increased or decreased K+ selectivity, prolonged kinetics (up to 1000 seconds off-kinetics and photocurrents up to 3 nA), and shifted action spectra (blue-shifted by ~20 nm, compatible to be used with red-shifted opsins like ChRmine), revealing experimental advantages for optogenetic inhibition and excitation both in vitro and in vivo. Notably, the variants called Kali (K+-selectivity-Augmented Light-gated Ion-channel) display significantly hyperpolarized reversal potentials (HcKCR1-WT: -71 mV ± 3, Kali-1: -82 ± 4 mV, HcKCR2-WT: -63.2 ± 2, Kali-2: -70 ± 1.5 mV) and evoke similarly large photocurrents (KCR1-WT: 4.4 ± 1.5 nA, Kali-1: 3.5 ± 1 nA), making it highly advantageous for optogenetic inhibition. Our findings not only unveil an unprecedented mechanism for robust K+ selectivity in ion channels but also provide a framework for the development of next-generation optogenetic tools.

Disclosures: Y. Kim: None. **S. Tajima:** None. **P. Wang:** None. **Y. Jo:** None. **E.F.X. Byrne:** None. **K. Kishi:** None. **C. Ramakrishnan:** None. **M. Inoue:** None. **H. Kato:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.17/XX62

Topic: I.08. Methods to Modulate Neural Activity

Support: HHMI
NIH Grant R37MH075957

Title: All-optical physiology resolves cell-type-specific synaptic transmission and plasticity at single-cell resolution during behavior

Authors: *L. FAN[1], D. KIM[5], C. WENARDY[5], C. RAMAKRISHNAN[5], B. DUDOK[2], J. H. JENNINGS[3], H. TIAN[6], P. WANG[5], S. RANDLES[5], Y. SUN[3], E. THADHANI[5], Y. KIM[1], S. QUIRIN[3], L. GIOCOMO[3], I. SOLTESZ[4], A. COHEN[7], K. DEISSEROTH[5];
[1]Stanford Univ., Palo Alto, CA; [2]Neurosurg., [4]Sch. of Med., [3]Stanford Univ., Stanford, CA; [5]Stanford, Stanford, CA; [6]Dept. of Chem. and Chem. Biol., [7]Harvard Univ., Cambridge, MA

Abstract: Learning has been associated with modifications of synaptic and circuit properties, but the precise changes storing information in mammals have remained largely unclear. We combined genetically targeted voltage imaging with targeted optogenetic activation and silencing of pre- and post-synaptic neurons to study the mechanisms underlying hippocampal behavioral timescale plasticity. In mice navigating a virtual-reality environment, targeted optogenetic activation of individual CA1 cells at specific places induced stable representations of these places in the targeted cells (n = 32 cells, 8 mice). Optical elicitation, recording, and modulation of synaptic transmission in behaving mice revealed that activity in presynaptic CA2/3 cells was required for the induction of plasticity in CA1 and, furthermore, that during induction of these place fields in single CA1 cells, synaptic input from CA2/3 onto these same cells was potentiated (n = 17 cells, 5 mice). We further optically resolved inhibitory postsynaptic potentials in CA1 from a specific subtype of inhibitory neurons, cholecystokinin-expressing inhibitory neurons (n = 29 cells, 3 mice). These results reveal synaptic implementation of hippocampal behavioral timescale plasticity and define a methodology to resolve synaptic plasticity during learning and memory in behaving mammals.

Disclosures: L. Fan: None. **D. Kim:** None. **C. Wenardy:** None. **C. Ramakrishnan:** None. **B. Dudok:** None. **J.H. Jennings:** None. **H. Tian:** None. **P. Wang:** None. **S. Randles:** None. **Y. Sun:** None. **E. Thadhani:** None. **Y. Kim:** None. **S. Quirin:** None. **L. Giocomo:** None. **I. Soltesz:** None. **A. Cohen:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.18/XX63

Topic: I.08. Methods to Modulate Neural Activity

Support: Keck Foundation
NIH5T32GM12007
HHMI

Title: Microbial opsin-based tools for light-controlled membrane fusion events targeted to individual neurons

Authors: *J. DOU, K. LOH, C. KADUR, M. INOUE, L. FAN, M. LO, C. RAMAKRISHNAN, K. DEISSEROTH;
Stanford Univ., Stanford, CA

Abstract: Membrane-defined compartments serve as fundamental elements of information processing in biological systems - including individual cells, projections, and synapses in the nervous system. Here we design and test an optogenetic approach, using microbial opsins in a novel way, to perform light-controlled membrane fusion with single-cell and subcellular resolution. This approach consists of a light-driven outward proton pump and pH-dependent fusogen that together transduce photons into targeted membrane fusion events via a pH signal. We first demonstrated in HEK293 cells that using this method, targeted light (initially over a 20x20 μm^2 area) can robustly induce membrane fusion at specific contact sites between two cells. By varying the proton pump, the fusogen, and a designed interaction domain, we tested 28 different combinations; the optimal combination attained a mean success rate of detectable fusion in 45.3±12% of targeted cells (4 biological replicates, with 50, 38, 38, and 35 single cells in each test). We then showed that light-targeted membrane fusion can be induced at subcellular regions in primary hippocampal neurons *in vitro*, and *in vivo* with an optical fiber targeting the long-range projection from hippocampal CA3 to contralateral CA1 in rodent brains (8 mice each in control and light stimulation groups respectively; p= 0.0133). In a mouse stimulated with 20 mW/mm^2 560nm laser light for 30 seconds, we observed fusion events between cells each expressing a single genetic component of the two-component system, thus achieving intersectional specificity in *trans*, centered at the middle of the light-targeted site *in vivo* (with 39 fused cells in the central 20 μm brain slice). This novel microbial opsin-based approach allows optical manipulation of targeted membrane structures, including the fundamental topology delimiting neural information-processing elements, that can be readily integrated with widely-used and state-of-the-art optogenetic technology.

Disclosures: **J. Dou:** None. **K. Loh:** None. **C. Kadur:** None. **M. Inoue:** None. **L. Fan:** None. **M. Lo:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.19/XX64

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant K08 MH131888

Title: Spontaneous Activity Adaptively Biases Visual Perceptual Decision Making

Authors: *A. MITRA[1], J. KOCHALKA[2], S. QUIRIN[3], C. RAMAKRISHNAN[2], C. KADUR[2], S. DRUCKMANN[3], K. DEISSEROTH[4];
[1]Stanford Univ. Sch. of Med., Palo Alto, CA; [2]Stanford Univ., Palo Alto, CA; [3]Stanford Univ., Stanford, CA; [4]Stanford, Stanford, CA

Abstract: The mammalian cortex is spontaneously active even in the absence of external stimuli. Initially dismissed as neural noise, spontaneous cortical activity is now thought to be potentially responsible for the dramatic trial-by-trial variability in sensory-evoked brain activity. However, it remains unknown whether spontaneous activity usefully shapes perceptual decisions by forming and implementing contextual predictions about the environment. To explore this question, we trained mice (N = 16) in a novel two-alternative forced choice (2AFC) visual detection task where we recorded both perceptual choice and a metric of response confidence. Applying wide-field Ca^{2+} imaging in mice expressing GCaMP8m under a CaMKII promoter, we found that spontaneous neural activity in retrosplenial (RSP) and visual (VIS) cortices prior to visual stimulus presentations shaped perceptual choice (N = 5). To characterize the content of this neural activity, we further applied two-photon microscopy to RSP and VIS while mice performed the 2AFC visual detection task (N = 11). We found that spontaneous fluctuations in population coding of the visual stimulus, prior to visual stimulus presentation, adaptively biased the perceptual choice made by mice. Finally, to test the role of spontaneous signaling from the RSP to VIS in predictive perceptual decisions, we optogenetically inhibited excitatory projection neurons between these brain regions using a potassium-conducting channelrhodopsin (HcKCR1). We found that experimental (N = 6) but not control (N = 5) animals illustrated a marked shift in bias as a function of optogenetic inhibition. Taken together, our findings offer novel evidence that ongoing spontaneous cortical activity can shift network states to predictively bias perceptual decisions.

Disclosures: A. Mitra: None. **J. Kochalka:** None. **S. Quirin:** None. **C. Ramakrishnan:** None. **C. Kadur:** None. **S. Druckmann:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.20/XX65

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U19 NS118284

Title: Brain-wide neural dynamics in dynamic foraging

Authors: *Y. JO, T. X. LIU, D. J. O'SHEA, J. SHI, T.-N. C. SHENOY, K. A. PATRON, D. KIM, C. RAMAKRISHNAN, K. V. SHENOY, D. SUSSILLO, K. DEISSEROTH;
Stanford Univ., Stanford, CA

Abstract: Building, maintaining, and updating models of the world represent fundamental computations essential to the survival of animals. Past interaction with the environment, such as the history of choice and reward, may shape the subjective value of presently available actions and guide future decision-making. Here we investigated the brain-wide neural dynamics implementing such computations in the mouse brain. We modeled the dynamic environment using a head-fixed dynamic foraging task (i.e., a two-armed bandit task) in which mice made sequential two-alternative choices, and experienced stochastic outcomes, across trials. The underlying reward probabilities, unobserved by the subject, remained constant for tens of consecutive trials before changing to new values. Upon training, the animals learned to dynamically match their choice preferences to their experience of rewarded choices over a time window of several minutes. This behavioral strategy could be described by a classic Q-learning model, which infers trial-by-trial estimates of each choice's reward probability ("action values"). Using multi-Neuropixels extracellular electrophysiology, we surveyed spiking activity across the brain to identify the neural substrates for the action value computation (>10,000 single units from 59 insertions in 32 sessions). We identified single neurons for which activity correlated with action values in multiple brain regions. At the population level, graded activity encoding action values evolved into more discrete activity predicting the upcoming decision during the preparatory period in each trial. Notably, the retrosplenial cortex showed particularly high action value encoding and temporally stable representation. Behavioral performance degradation upon optogenetic inhibition of the retrosplenial cortex was more potent during action value maintenance (p=0.003, N=9 sessions) than during action value update (p=0.177, N=6 sessions). Interestingly, the opposite pattern was observed when inhibiting a subregion of the prefrontal cortex (p=0.166, N=6 sessions for maintenance; p=0.004, N=5 sessions for update), suggesting potentially distinct roles in the action value computation. Collectively, these results provide a brain-wide picture of neural dynamics and causal influences in dynamic foraging, an important paradigm to study long-timescale computations in the brain.

Disclosures: **Y. Jo:** None. **T.X. Liu:** None. **D.J. O'Shea:** None. **J. Shi:** None. **T.C. Shenoy:** None. **K.A. Patron:** None. **D. Kim:** None. **C. Ramakrishnan:** None. **K.V. Shenoy:** None. **D. Sussillo:** A. Employment/Salary (full or part-time):; Meta Reality Labs. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.21/XX66

Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH R37MH075957
HHMI

Title: Two-photon optogenetics using engineered potassium-selective channelrhodopsins

Authors: *Y. WANG[1], A. DRINNENBERG[2], Y. JO[2], Y. KIM[2], M. INOUE[2], E. F. BYRNE[2], C. RAMAKRISHNAN[2], S. QUIRIN[2], H. E. KATO[3], K. DEISSEROTH[2];
[1]Bioengineering, STANFORD, Palo Alto, CA; [2]Stanford Univ., Stanford, CA; [3]Univ. of Tokyo, Tokyo, Japan

Abstract: We recently solved the structure of a wildtype potassium-selective channelrhodopsin (*Hc*KCR1-WT) and performed structure-guided re-design to engineer Kali (for K+-selectivity-Augmented Light-gated Ion-channel) mutants with significantly improved performance for *in vivo* applications. We first compared the efficacy of Kali-1 vs *Np*HR and *Gt*ACR1 by performing acute Neuropixels recordings of retrospenial cortex neurons in awake mice. Kali-1 drove robust inhibition (norm. firing rate: 0.21±0.03, n=133 neurons) at low illumination powers (<0.5 mW). In contrast, *Gt*ACR1 drove incomplete inhibition (norm. firing rate: 0.67±0.1, n=16 neurons) and *Np*HR required high-intensity continuous illumination (10 mW) to drive sustained inhibition (norm. firing rate: 0.18±0.03, n=17 neurons). We then compared the utility of Kali-1 vs. *Hc*KCR1-WT and *Gt*ACR1 under targeted two-photon stimulation and found that Kali-1 photocurrents are ten-fold higher (Kali-1: 1.2±0.2 nA, *Gt*ACR1: 0.15±0.1 nA) and with ten-fold less cross-talk compared to *Gt*ACR1 (and also compared favorably to *Hc*KCR1-WT). To develop these new variants for reading and inhibition of neural activity with cellular specificity and sub-second resolution *in vivo*, we next designed tools to enable healthy co-expression of Kali-1 and jGCaMP8m, which allowed us to perform all-optical experiments in the visual cortex in awake behaving mice. Drifting gratings were presented to acquire visually evoked responses in V1 neurons that co-express Kali-1 and jGCaMP8m. In half of these trials, visually responsive neurons were photo-inhibited using holographic stimulation (7.8 mW per cell, 6 ms exposure, 32 Hz, 2 Mhz rep. rate at 1035 nm) during drifting grating presentation. This revealed that the majority of targeted neurons (65%) were significantly inhibited (visual response without inhibition: 0.85 ± 0.2 ΔF/F, with: 0.33 ± 0.1 ΔF/F). In 40% of these neurons, inhibition drove GCaMP fluorescence to baseline or under baseline levels. In summary, our structure-guided variants enable large-scale all-optical recording and inhibition experiments to be conducted without trading off cross-talk and potency, thus providing a significant opportunity for the rapidly expanding field of 2P optogenetics.

Disclosures: Y. Wang: None. **A. Drinnenberg:** None. **Y. Jo:** None. **Y. Kim:** None. **M. Inoue:** None. **E.F. Byrne:** None. **C. Ramakrishnan:** None. **S. Quirin:** None. **H.E. Kato:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.22/XX67

Topic: I.08. Methods to Modulate Neural Activity

Support: BBRF Young Investigator Award

Title: All optical interrogation of primary visual cortex interneuron circuit dynamics during mouse visual behavior

Authors: *J. H. MARSHEL, C. RAJA, A. CHIBUKHCHYAN, C. RAMAKRISHNAN, S. QUIRIN, K. DEISSEROTH;
Stanford Univ., Stanford, CA

Abstract: Understanding how behavioral states and visual cortical interneuron circuits together modulate visual detection and discrimination behavior requires approaches for recording and manipulating neuronal activity patterns with cellular resolution across depths of cortex in vivo. To facilitate this, we implemented a genetic approach in which vasoactive intestinal peptide-expressing (VIP) neurons, somatostatin-expressing (SST) neurons, and other neurons including putative excitatory neurons were each distinctly labeled within the same mice. All labeled neurons expressed both GCaMP6m and the excitatory, soma-targeted opsin ChRmine-Kv2.1. Using a multiplexed spatial light modulator system (MultiSLM, Marshel et al., 2019), varying numbers of VIP and/or SST cells in superficial or deep layers were holographically (SLM) stimulated during a visual discrimination behavior of varying salience and difficulty. Two photon Ca^{2+} imaging was simultaneously performed across the volume, and movement on a spherical treadmill was recorded. We found that stimulating VIP or SST cells in primary visual cortex (V1) led to strong suppression of visual discrimination behavior across mice. The most robust suppression was observed at low visual contrasts for superficial layer VIP neurons ($p<0.01$, n=10 mice), and deep layer SST neurons ($p<0.01$, n=9 mice). To account for different densities of each cell type across depth, experiment conditions were designed which varied the number of stimulated neurons proportionally with cell type or held the number of stimulated neurons of each cell type constant. Periods when the animals were moving corresponded to improved visual discrimination behavior ($p<0.01$, n=6 mice). This effect was further analyzed during trials when different types of interneurons were stimulated to investigate potential interaction or independence of behavioral and neuronal modulation. Simultaneous Ca^{2+} imaging of the stimulated neurons and surrounding populations across the volume revealed functional circuit interactions between the interneuron cell types, as well as tuned neurons (putative excitatory cells), within and across cortical layers and under different states of movement. These studies help to reveal the functional circuit impacts of specific interneurons on V1 dynamics, and on visual detection and discrimination performance, under different behavioral states.

Disclosures: J.H. Marshel: None. **C. Raja:** None. **A. Chibukhchyan:** None. **C. Ramakrishnan:** None. **S. Quirin:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.23/XX68

Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH R01MH075957
Howard Hughes Medical Institute
NSF NeuroNex program
NOMIS Foundation
Else Kröner Fresenius Foundation
Gatsby Foundation

Title: All-optical observation and control of communication between cell-types during behavior via structure-guided design of K+-selective channelrhodopsins

Authors: *M. INOUE[1], P. WANG[1], Y. KIM[1], Y. JO[1], E. F. BYRNE[1], A. DRINNENBERG[1], S. QUIRIN[1], C. RAMAKRISHNAN[1], M. K. LO[1], S. TAJIMA[2], H. E. KATO[2], K. DEISSEROTH[1]; [1]Stanford Univ., Stanford, CA; [2]Univ. of Tokyo, Meguro, Japan

Abstract: Understanding the nature and role of communication between distinct cellular populations in the brain will require simultaneous measurement and perturbation of activity within those populations during behavior. Employing all-optical molecular tools that enable the measurement and control of neural activity in a cell type-specific manner has emerged as an effective approach, but the inhibitory optogenetic tools that have been discovered and engineered have limitations for all-optical neuroscience, including one or more of: poor spectral compatibility with activity sensors, modest efficacy, and paradoxical effects when applied to presynaptic terminals. To resolve these shortcomings for all-optical neural circuit interrogation, we created novel variants of K^+-selective channelrhodpsins (KCRs) based on our cryo-EM structures. In particular, we generated *Hc*KCR variants with enhanced K+ selectivity which we term Kali-1 and Kali-2 (for K^+-selectivity-Augmented Light-gated Ion-channels). Compared to WT KCRs, Kali-1 and Kali-2 variants exhibit pronounced hyperpolarization of reversal potential, and comparably large photocurrents. Here, we integrate these new opsins with genetically-encoded Ca^{2+} indicators (blue and red GECIs) using frame-projected independent-fiber photometry to selectively silence specific neural circuit elements while simultaneously reading-out the responses of those and other circuit elements within the same network. To recruit Kali-2, 470 nm light was delivered at 0.50 mW/mm^2 in 50 Hz trains of 2 ms pulse width over 4 seconds; concurrent readout signals were collected with 6 ms pulse-width excitation light for two independent GECIs (0.025 mW/mm^2 for 385 nm, and 0.080 mW/mm^2 for 565 nm) and sampled at 50 Hz. Optically-induced inhibition of GECI signals could be readily measured ($p = 0.0008$ from $n = 4$ mice, compared to control mice not expressing Kali-2). Applying this approach to mPFC in freely-behaving mice, we measured real-time dynamics of evoked information transmission between excitatory and inhibitory neuronal populations (defining type-to-type, or T2T, signals), and both induced and measured axon-terminal inhibition using Kali-2 alongside paired recording of pre- and post-synaptic neural populations during free mouse behavior.

Disclosures: M. Inoue: None. **P. Wang:** None. **Y. Kim:** None. **Y. Jo:** None. **E.F. Byrne:** None. **A. Drinnenberg:** None. **S. Quirin:** None. **C. Ramakrishnan:** None. **M.K. Lo:** None. **S. Tajima:** None. **H.E. Kato:** None. **K. Deisseroth:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.24/XX69

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF NeuroNex
Gatsby Foundation
NIMH
NINDS

Title: Neural landscape diffusion resolves competing needs across time

Authors: *E. RICHMAN, N. TICEA, W. ALLEN, K. DEISSEROTH, L. LUO;
Stanford, Palo Alto, CA

Abstract: Animals perform flexible goal-directed behaviors to satisfy their basic physiological needs. However, little is known about how appropriate unitary behaviors are chosen under competing needs. This quandary is reflected in the story of Buridan's ass: whether an equally hungry and thirsty animal placed between equidistant food and water will be unable to choose either option and remain immobile. Here we reveal principles by which the brain resolves such competing needs across time. We developed an experimental analogue of Buridan's ass in which a hungry and thirsty mouse is given free choices between equidistant food and water. We found that mice collect need-appropriate rewards by structuring their choices into persistent epochs with stochastic transitions (n = 16 mice / 22 sessions). The most recent previous choice was more predictive of current choice than need level; indeed, the probability of repeating choices remained generally above 80% across relative need values, and for trials with relatively balanced needs, choice outcomes recurred with greater than 90% probability. These results suggested that transitions between persistent choices occur probabilistically, rather than being determined by the exact balance of needs. To directly test this persistence and stochasticity, we performed transient optogenetic stimulation of channelrhodopsin-expressing Rxfp1+ neurons in the subfornical organ (SFO), which have been found to be activated by increased osmolarity and, when directly activated by optogenetics, produce a naturalistic thirst state that is aversive and drives drinking. Thirst stimulation drove hungry mice to transition from choosing food to choosing water, but these transitions appeared stochastic on any given stimulation epoch. High-density electrophysiological recordings revealed distributed single neuron and population correlates of a persistent internal goal state guiding future choices of the animal (n = 7 mice/sessions). Neurons with significant information about future choices tended to exhibit mixed selectivity across task features and their associated timescales. We captured these phenomena with a mathematical model describing a global need state that noisily diffuses across a shifting energy landscape. Simulations based on this model predicted behavioral and neural data, including population neural dynamics before choice transitions and in response to optogenetic thirst stimulation. These results suggest a general framework for resolving competing needs across time, rooted in the emergent properties of need-dependent state persistence and noise-driven shifts between behavioral goals.

Disclosures: **E. Richman:** None. **N. Ticea:** None. **W. Allen:** None. **K. Deisseroth:** None. **L. Luo:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.25/XX70

Topic: I.04. Physiological Methods

Support: NIH 5R01NS116464
NIH T32 EB029365

Title: Smart Dura: High-density, opto-electrical artificial dura for large-scale monitoring of NHP brain.

Authors: *S. MONTALVO VARGO[1], T. BELLOIR[2], I. KIMUKIN[1], Z. AHMED[1], D. J. GRIGGS[2,3], N. STANIS[2], A. YAZDAN[2], M. CHAMANZAR[1];
[1]Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Washington, Seattle, WA; [3]Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Chronic monitoring of the brain's activity for studying neurological function and designing effective therapeutics is highly desired. Micro-electrocorticography (μECoG) recording is widely used since it provides a good compromise between the level of invasiveness and high-resolution neural recording. For stimulation, optogenetics provides excitation and inhibition of neural activity with cell specificity and high temporal resolution. Combining μECoG with optogenetics has the potential to record and stimulate large neuron populations across large areas. Developing such a platform for larger brain sizes such as non-human primates (NHP) presents challenges in design, fabrication and system level integration. Addressing these challenges with next generation devices is critical to advancing the neuroscience toolset.
The artificial dura is an elastomer-based passive device used in NHPs that replaces the native dura and provides a stable optical window to the cortex. In previous work, we demonstrated the use of a commercially available ECoG array molded in an artificial dura, named multi-modal artificial dura (MMAD), for simultaneous electrophysiology and optical access. Here we discuss the design of a novel microfabricated device that improves the current capabilities of the MMAD by providing larger optical access, higher density recording and integrated light sources into one platform. We call this device, Smart Dura, since it enables a read/write access to the brain tissue. The Smart Dura incorporates high-density recording electrodes with a high density array of light-emitting diodes (LEDs). The electrode array is microfabricated on flexible polymer substrates, with microelectrodes of 10-40 μm in diameter and spanning a large area of the NHP cortex (300mm2). The resolution of our lithographic technique enables the number of channels to 512 in each single thin-film layer. The LED array is fabricated on polyimide, a flexible substrate, using commercially available off-the-shelf LEDs and printed circuit board (PCB) manufacturing technology. The LED array is first fabricated separately and then integrated with the recording

electrode array to form the Smart Dura.
Our initial in-vivo experiments demonstrate successful recordings of spontaneous multi-unit activity as well as evoked activity in sensorimotor cortex in response to tactile and optogenetic stimulation. We have also demonstrated the ability of our electrical dura to capture neural signals correlated with behavior in a center-outreach task. We will discuss the details of our design, the microfabrication method and in-vivo validation of the Smart Dura in the cortex of macaques.

Disclosures: **S. Montalvo Vargo:** None. **T. Belloir:** None. **I. Kimukin:** None. **Z. Ahmed:** None. **D.J. Griggs:** None. **N. Stanis:** None. **A. Yazdan:** None. **M. Chamanzar:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.26/XX71

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Sloan Fellowship Grant FG-2021-16332
NIH K01 Grant NS114191-01A1

Title: Elucidating the temporal dynamics of non-invasive BioLuminescent-OptoGenetics effects in neocortex

Authors: *E. MURPHY, A. NELMS, M. GOMEZ-RAMIREZ;
Univ. of Rochester, Rochester, NY

Abstract: BioLuminescent OptoGenetics ("BL-OG") is a noninvasive chemogenetic method that enables optogenetic activation via bioluminescent light. In BL-OG, opsins are activated by bioluminescence generated by injecting a substrate (*luciferin*, e.g., Coelenterazine; *CTZ*) that is catalyzed by a photo-enzyme (*luciferase*, e.g., slow-burn Gaussia Luciferase; *sbGLuc)* that is molecularly tethered to an opsin. We previously showed that BL-OG can be used as a gain modulator by showing that increases in CTZ dosage proportionally increases bioluminescence activity. Importantly, we found that increases in bioluminescence activity were linearly correlated with enhancements in multi-unit activity, indicating that bioluminescence can be used as a proxy for population neural spiking. Although highly informative, these data were collected in experiments that directly injected CTZ into the brain. Thus, it is unclear whether similar relationships occur when the luciferin is injected noninvasively. Here, we image bioluminescent activity while injecting different concentrations of the luciferin (in this case h-Coelenterazine; h-CTZ) via the tail vein of mice. Similar to our previous findings, we observed that bioluminescence systematically increased as a function of h-CTZ concentration. Importantly, we observed that the onset time of bioluminescence was similar across h-CTZ concentrations, indicating that noninvasive BL-OG reactions modulate brain activity at the same time regardless of luciferin concentration. As expected, we found that higher concentrations of h-CTZ generated longer bioluminescence, but these activations were back to baseline in less than 20 minutes. We

conducted additional experiments to determine whether rate of injections also modulate BL-OG's temporal dynamics. Data reveal that faster rate of injections lead to higher maximum bioluminescence response, a quicker onset of the bioluminescence peak, and longer time decay of bioluminescence. Our studies provide critical information of how parameters of noninvasive luciferin delivery can lead to changes in BL-OG effects in brain activity. Our studies provide additional insight into the advantages of BL-OG over other chemogenetic methods (e.g., DREADDs), by demonstrating robust, fast, and relatively short-duration modulations in neural activity in awake-behaving animals.

Disclosures: E. Murphy: None. **A. Nelms:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR513. Optogenetics and Applications

Location: WCC Halls A-C

Time: Wednesday, November 15, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR513.27/XX72

Topic: I.08. Methods to Modulate Neural Activity

Support: Funding from Beverly and Frank MacInnis via the University of St Andrews.
Leverhulme Trust RPG-2017-231
EU Horizon 101023743
DFG RTG2591

Title: Wireless Micro-OLEDs for Deep Brain Stimulation

Authors: *J. BUTSCHER[1,2], S. HILLEBRANDT[2], J. BOOTH[1,2], A. MISCHOK[2], A. POPCZYK[2], M. GATHER[2,1];
[1]Univ. of St Andrews, St Andrews, United Kingdom; [2]Univ. of Cologne, Cologne, Germany

Abstract: Due to the strong scattering of light in tissue, efficient optogenetic stimulation deep in the brain often requires the use of implants. Compact and wirelessly powered devices are the preferred choice here to enable experiments on freely moving animals. However, traditional methods of wireless power transfer such as RF coils or IR light are unsuitable for miniaturization to microscopic scales or are hindered by significant tissue absorption. In this context, magnetoelectric laminates offer a promising solution by exploiting low-frequency magnetic fields that can penetrate centimeters into watery environments while still maintaining a compact size.
In this work, we introduce a compact wireless platform for optogenetic stimulation that utilizes organic light-emitting diodes (OLEDs) integrated directly onto a thin film magnetoelectric transducer [1]. OLEDs, commonly used as light emitters in consumer electronic displays, offer an attractive alternative to traditional inorganic LED technology for optogenetic applications. Their advantage lies in the ability to deposit them directly onto various substrates, in any desired shape and color, using low-temperature processes [2]. Notably, in our implementation, the

OLEDs add negligible weight and volume to the magnetoelectric laminate, which serves as both a substrate and a power harvester. We present an ultrasmall device with a volume of less than 0.6 mm^3 that can be implanted directly at the site of optogenetic stimulation. In addition, tuning of the magnetoelectric transducers to different resonance frequencies enables independent addressing of individual wireless OLEDs without the need for additional on-device electronics. A thin film passivation layer ensures stability of the implant under continuous operation. To demonstrate the viability of our approach, we utilize a wirelessly powered OLED to elicit behavioral responses in genetically modified Drosophila larvae.

References

[1] Butscher, J.F., Hillebrandt, S., Booth, J.H.H., Mischok, A., Popcyk, A. & Gather, M.C., Wireless OLEDs for Deep Brain Bioimplants, In Preparation (2023).
[2] Murawski, C., & Gather, M. C., Emerging Biomedical Applications of Organic Light-Emitting Diodes. Advanced Optical Materials, 2100269 (2021).

Disclosures: **J. Butscher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Cologne has filed a patent application (DE 10 2022 109 762.5) on the technology described in this paper.. **S. Hillebrandt:** None. **J. Booth:** None. **A. Mischok:** None. **A. Popczyk:** None. **M. Gather:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The University of Cologne has filed a patent application (DE 10 2022 109 762.5) on the technology described in this paper..