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Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.01/A1

Topic: A.03. Stem Cells and Reprogramming

Title: Generation of functional 3D spinal cord organoids from human pluripotent stem cells

Authors: J. WANG[1], J. CHAN[1], ***M. SCHMIDT**[1], A. EAVES[1,2], S. A. LOUIS[1], E. KNOCK[1,3]; [1]STEMCELL Technologies, Inc., Vancouver, BC, Canada; [2]Terry Fox Laboratory, BC Cancer, Vancouver, BC, Canada; [3]Simon Fraser University, Burnaby, BC, Canada

Abstract: There is no cure for motor neuron (MN) diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. Access to a reliable human MN model would be invaluable to help uncover disease mechanisms. Advanced culture models such as spinal cord organoids (SCO) contain a variety of tissue-specific cell types including MNs, glial cells, and interneurons, improving their physiological relevance. Here, we describe the STEMdiff™ Spinal Cord Organoid Differentiation Kit which generates SCOs from human pluripotent stem cells (hPSCs) at a high efficiency. A single-cell suspension of hPSCs was cultured for 6 days in AggreWell™800 plates containing organoid formation medium. The resulting organoids were replated and cultured in expansion medium from days 6 - 19, followed by differentiation medium from days 19 - 43, and STEMdiff™ Neural Organoid Basal 2 with supplement from day 43 onwards. We examined the expression of HOX genes by qPCR to determine the rostral-caudal patterning at day 19. Then, we evaluated the cell identity by qPCR and immunocytochemistry at specific days: day 19 for OLIG2+ and NKX6.1+ neural progenitor cells, day 30 for MNX1+, ISL1+, and/or FOXP1+ post-mitotic MNs, CHX10+ for interneurons, and finally day 75 for GFAP+ or MBP+ glial cells. Spontaneous electrophysiological currents were measured using the Axion microelectrode array (MEA) system throughout the culture. The activity of SCOs under the treatment of MG-132, a proteasome inhibitor that could affect TDP-43 localization in motor neurons was measured using the MEA. The SCOs expressed *HOXB4* and *OLIG2* on day 19 and *MNX1* and *ISL1* on day 30 at significantly higher levels than hPSCs (fold changes relative to hPSCs of 29103, 1568, 1105, and 3059, respectively, $p < 0.0001$, $n = 11$). Moreover, we observed a large number of MNX1+, ISL1+, and/or CHAT+ MNs and CHX10+ interneurons at day 30 and GFAP+ or MBP+ glial cells at day 75. The SCOs displayed spontaneous firing as early as 4 weeks in culture (0.7154 ± 0.1982 Hz, weighted mean firing rate mean ± SEM), which became more synchronous and active with maturation (average synchrony index of 0.0672 and 0.3252 from weeks 4 and 9, respectively). Additionally, the SCOs had more bursts when matured in BrainPhys™ than Neural Organoid Basal 2 (13.52 and 0.40 bursts per minute respectively, $p = 0.0016$, $n = 5 - 8$). We found that acute treatment of MG-132 could largely abolish the electric activity of SCOs. Taken together, STEMdiff™ Spinal Cord Organoid Differentiation Kit provides a powerful tool to generate functional hPSC-derived SCOs 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. **M. Schmidt:** A. Employment/Salary (full or part-time):; STEMCELL Technologies Inc. **A. 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

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.02/Web Only

Topic: A.03. Stem Cells and Reprogramming

Support: CONACYT Grant 181779
FODECIJAL Grant 8148

Title: Schwann-like cells and their functional participation in the myelination process in cortical neurons in vitro co-culture

Authors: *Y. GASCA MARTÍNEZ, N. J. CARRILLO-GONZÁLEZ, T. CAMPOS ORDONEZ, G. ESCOBAR CAMBEROS, G. S. REYES-GUTIERREZ, G. GUDIÑO-CABRERA;
Dept. de Biología Celular y Mol., Univ. de Guadalajara, Zapopan, Mexico

Abstract: Bone marrow mesenchymal stem cells (uBMSCs) differentiated to Schwann-like phenotype (dBMSCs) are potential candidates for cell therapy to counteract myelin damage caused by neurodegenerative diseases. The properties of Schwann-like cells include the release of trophic substances such as interleukin 10, fibroblast growth factor-2, glial cell derived neurotrophic factor, chemokines, and antioxidant molecules, which promote the proliferation and regeneration of neurons. In addition, these cells express proteins associated with the formation and maintenance of myelin that includes SOX 10, NF-kB, Pou3f, NFATc4, YY1, Egr2/Krox20, and myelin basic protein (MBP). This study aims to evaluate the capacity to myelinate of dBMSCs in co-culture with cortical neurons to promote myelin formation and maintenance. For this purpose, we used primary cultures of olfactory ensheathing cells (OECs) from the olfactory bulb, uBMSCs, and dBMSCs obtained from male Wistar rats of 8 weeks of postnatal age, as well as primary cultures of cortical neurons (CN) from embryos (E17 n=3). The co-cultures were maintained for 7 days, processed for immunocytochemistry and analyzed by fluorescence microscopy. Our data showed the establishment of co-culture of CN with OECs, uBMSCs, and dBMSCS, where the growth and guidance of neurities were observed. In addition, the expression of MBP, was observed in the three groups, this offers an experimental alternative for regeneration of myelin analysis.

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Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.03/A2

Topic: A.03. Stem Cells and Reprogramming

Title: Modulation of glial differentiation in a 3D iPSC-derived CNS model

Authors: *L. J. HARBOM, M. TERRAL, E. SCHMIDT, E. SCHWARZBACH CHAMBLISS, S. DILLON, A. MCCRIMMON, E. SPACK, J. L. CURLEY;
AxoSim, Inc., New Orleans, LA

Abstract: Progress in neurological drug discovery has been hindered by lack of effective models for drug testing. The development of human induced pluripotent stem cell- (iPSC-) derived neurons has been integral in bridging the translational gap between animal models and clinical trials. However, accurate modeling of the central nervous system is dependent upon a cellular landscape comprised of both neuronal and glial cells, including astrocytes and the oligodendrocyte lineage pathway consisting of oligodendrocyte precursor cells (OPCs), oligodendrocytes (OLs), and myelinating OLs. Astrocytes are integral in maintaining neuronal homeostasis and network function, while myelin sheaths formed by OLs enable efficient neuronal conduction. To optimize AxoSim's proprietary iPSC-derived BrainSim platform for glial differentiation, spheroids were differentiated for 12 weeks in two different media formulations, with collection points at DIV42, DIV56, DIV70, and DIV84. Transcript and protein expression for markers associated with astrocytes, OPCs, and OLs were assessed via immunohistochemistry (IHC), Western blot, and qPCR. These endpoints confirmed the presence of all three cell types within the spheroids in both test groups. Additionally, one test group was further supplemented with clemastine, a pro-myelinating compound known to target OL differentiation, for a 4-week and a 2-week treatment period starting at DIV56 and DIV70 respectively. Clemastine treatment resulted in an increase in OPC and OL markers, including myelin basic protein (MBP) at DIV70 and DIV84. This demonstrates that the OPC/OL differentiation pathway is both present and modulable in BrainSim, making it an ideal candidate for testing compounds affecting the myelination or re-myelination process, including those targeting demyelinating diseases such as multiple sclerosis.

Disclosures: L.J. Harbom: A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **M. Terral:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of

intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **E. Schmidt:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **E. Schwarzbach Chambliss:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **S. Dillon:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **A. McCrimmon:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc. **E. Spack:** 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.; AxoSim, Inc. **J.L. Curley:** A. Employment/Salary (full or part-time):; AxoSim, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AxoSim, Inc..

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.04/A3

Topic: A.03. Stem Cells and Reprogramming

Title: Comparative Analysis of iPSC-Derived Neuron and Glia Cultures on Various Substrates for Enhanced Cell Attachment and Morphological Development

Authors: A. JOHNSON, C. PERRY, J. HJELMHAUG, D. HELD, K. REMONDINI, A. MASSMAN, H. RUETH, S. HANSON, M. HENDRICKSON, *K. XU;
BrainXell Inc., Madison, WI

Abstract: In recent years, induced pluripotent stem cells (iPSCs) have emerged as a promising tool for generating a variety of cell types, including neurons and glia, for both in vitro studies and potential therapeutic applications. Ensuring optimal adhesion, preventing detachment, and facilitating maturation are crucial for the success of in vitro applications. This study investigates the effects of six plate coatings (Poly-D-Lysin (PDL), Poly-L-Lysin (PLL), Poly-L-Ornithine (PLO), Poly-DL-Ornithine (PDLO), Poly-Ethyleneimine (PEI), and dendritic polyglycerol amine (dPGA)) and six extracellular matrix proteins (Laminin 511, Fibronectin, Collagen IV, Gelatin, Vitronectin, and Cultrex) on the attachment and morphology of iPSC-derived spinal motor neurons, cortical glutamatergic neurons, cortical astrocytes, and microglia. It was concluded that using PDL plate coating with Cultrex in the seeding medium provides the most favorable conditions for iPSC-derived neurons and glia, promoting optimal attachment and neurite growth.

The findings offer valuable insights for optimizing in vitro culture conditions in neuroscience and regenerative medicine.

Disclosures: A. Johnson: None. **C. Perry:** None. **J. Hjelmhaug:** None. **D. Held:** None. **K. Remondini:** None. **A. Massman:** None. **H. Rueth:** None. **S. Hanson:** None. **M. Hendrickson:** None. **K. Xu:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.05/A4

Topic: A.03. Stem Cells and Reprogramming

Title: Efficient generation of myelinating oligodendrocytes from human induced pluripotent stem cells for Alzheimer's disease modeling

Authors: *G. SAMTANI[1], Y. YOU[1,2], M. ISHIKAWA[3], H. OKANO[3], S. IKEZU[1,2], T. IKEZU[1,2];
[1]Mayo Clin. Florida, Jacksonville, FL; [2]Pharmacol. and Exptl. Therapeut., Boston Univ. Sch. of Med., Boston, MA; [3]Physiol., Keio Univ. Sch. of Med., Kanagawa, Japan

Abstract: White-matter decline is a well-known core feature of both sporadic and familial Alzheimer’s disease (AD), yet the therapeutic potential of myelin protection and regeneration in AD is severely understudied. Oligodendrocytes (OLGs) are known beyond their myelination role to provide metabolic and trophic support to neuronal axons throughout the lifespan, with exact mechanisms still being elucidated. The differential molecular roles and early therapeutic efficacy of OLGs in various AD disease stages can be modeled using human patient-derived induced pluripotent stem cells (iPSCs). However, few protocols to date have successfully and efficiently generated human stem-cell derived OLGs for disease modeling, with caveats of extended culture periods (>150 days) and low yield of fully mature cells. Here, we evaluate the efficacy of three transcription-factor based protocols in the efficient production of functional iOLGs for myelination and axonal support. Our results indicate that the overexpression of human transcription factors OLIG2 and SOX10 via a doxycycline-inducible lentivirus transfection system yields the most efficient and rapid generation of progenitor cells (iOPCs) and iOLGs. Immunocytochemistry confirmed expression of PDGFRa, O4, OLIG2, and SOX10 in iOPCs, and OLIG2, SOX10, and PLP in mature iOLGs. iOPCs introduced onto electro-spun nanofibers showed enhanced cell maturation, with attachment and elaboration of cell processes within 3 days *in vitro* (DIV 3) and a myelination phenotype within 3 days of switching to OLG media (DIV 9-12). Further, iOPCs from two protocols were introduced into primary cortical and hippocampal neuronal cultures (DIV 12-14) and demonstrated rapid maturation into PLP+ mature OLGs within 3-7 days of co-culture and into myelinating OLGs within 13 days of co-culture, indicating beneficial cross-talk between neurons and OPCs to accelerate maturation and

initiate myelination. Further, we investigate the early therapeutic potential of iOLGs via brain transplantation of iOPCs into the corpus callosum of immunodeficient neonatal mice. Preliminary results demonstrate the survival of transplanted mice beyond two months of age with no phenotypic differences and normal MBP+ expression in myelinated tracts in the brain, which can be used as a baseline for comparison to future transplantation studies of iOPCs into amyloidosis and myelin-deficient immunodeficient mice. Taken together, the strategies presented here may facilitate new studies to delineate mechanisms of endogenous myelin repair and promote remyelination to restore axonal integrity in AD models.

Disclosures: G. Samtani: None. **Y. You:** None. **M. Ishikawa:** None. **H. Okano:** None. **S. Ikezu:** None. **T. Ikezu:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.06/A5

Topic: A.03. Stem Cells and Reprogramming

Title: Generation of human pluripotent stem cell-derived astrocytes with the STEMdiff™ Astrocyte Serum-Free Culture System

Authors: J. CHAN[1], J. WANG[2], ***J. KEIL**[3], A. EAVES[4], S. A. LOUIS[2], E. KNOCK[1]; [1]STEMCELL Technologies Inc., Vancouver, BC, Canada; [2]STEMCELL Technologies Inc, Vancouver, BC, Canada; [3]STEMCELL Technologies, Inc., Oak Park, IL; [4]STEMCELL Technologies, Vancouver, BC, Canada

Abstract: Astrocytes are the most abundant cell type in the brain and play important roles in the development and homeostasis of the nervous system. All commercially available kits for generating human pluripotent stem cell (hPSC)-derived astrocytes contain serum, which may introduce variability to the functional assays that require serum-free conditions. To address this, we developed the STEMdiff™ Astrocyte Serum-Free Maturation Kit to generate functional hPSC-derived astrocytes without serum. hPSC-derived neural progenitor cells (NPCs) generated using STEMdiff™ SMADi Neural Induction Kit were differentiated in STEMdiff™ Astrocyte Differentiation Medium for three weeks, then matured in STEMdiff™ Astrocyte Serum-Free Maturation Medium (SF Maturation Medium) for a further three weeks. Cells were single-cell passaged weekly. Astrocyte identity was confirmed using RNA sequencing and immunocytochemistry for calcium-binding protein S100β and glial fibrillary acidic protein (GFAP). The autophagic activity of astrocytes were monitored after medium switch from respective maturation media to serum-free conditions mimicking media changes during functional assays. To evaluate astrocyte function, the resulting calcium fluctuations after ATP addition were visualized using the calcium ion indicator Fluo-4 AM. Additionally, these astrocytes were tri-cultured with forebrain neurons and microglia. Astrocytes maintained in SF

Maturation Medium expanded comparably to the serum-containing control (1.60 ± 0.58 and 1.68 ± 1.03-fold, respectively, mean ± SD, n = 7 cell lines) and displayed high astrocyte purity (S100β: 80.8 ± 14.6%; GFAP: 86.7 ± 10.0%, mean ± SD, n = 7 cell lines). Furthermore, astrocytes maintained in serum-free or serum-containing medium were clustered closely in a principal component analysis (PCA) plot, indicating a similar gene expression profile. Importantly, astrocytes maintained in SF Maturation Medium had significantly fewer autophagic vesicles than astrocytes maintained in serum-containing medium upon medium switch for functional assays ($p = 0.03$, n = 4 cell lines). The serum-free astrocyte culture displayed robust calcium fluctuations with a maximum normalized fold change of calcium signal ranging from 1.6 - 5.4 after ATP treatment (n = 4 cell lines). In the tri-culture model, neurons, astrocytes, and microglia can be co-cultured for a month. These data demonstrate that STEMdiff™ Astrocyte Serum-Free Maturation Kit enables the generation of functional astrocytes suitable for downstream applications, such as neuroinflammation modeling without the unintended autophagy induction upon serum-removal in functional assays.

Disclosures: J. Chan: A. Employment/Salary (full or part-time):; STEMCELL Technologies. **J. Wang:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **J. Keil:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **A. Eaves:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **S.A. Louis:** A. Employment/Salary (full or part-time):; STEMCELL Technologies. **E. Knock:** A. Employment/Salary (full or part-time):; STEMCELL Technologies.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.07/A6

Topic: A.03. Stem Cells and Reprogramming

Title: Label-free functional analysis for the characterization of iPSC-derived neural organoid development and maturation

Authors: *S. CHVATAL, D. SULLIVAN, B. STREETER, P. J. ELLINGSON, A. PASSARO, D. C. MILLARD;
Axion BioSystems, Atlanta, GA

Abstract: Over the past few decades, induced pluripotent stem cells (iPSCs) have provided an invaluable cell source for tissue engineering and regenerative medicine therapies, enabling high-throughput reproduction of complex human biology *in vitro*. Rapid advances in stem cell technology have provided alternative paths for neural drug discovery and safety screening with the widespread adoption of *in vitro* iPSC-derived neural electrophysiology models. More recently, neural organoids generated from human induced pluripotent stem cells (iPSCs) have emerged as a model of the human brain to study the complex neural network activity observed

during early brain formation. The objective of this work was to develop and validate a live-cell analysis workflow for the characterization of neural organoids *in vitro*. First, whole-vessel live-cell imaging with the Omni was used to monitor iPSC colony formation and expansion in real-time. The Omni iPSC module was used to determine iPSC colony coverage, count, area, and diameter to ensure consistent iPSC passaging and prevent spontaneous differentiation. Next, iPSC aggregates, known as embryoid bodies (EBs) were differentiated towards neural organoids. The Organoid analysis module was used to track the size, shape, and count of EBs undergoing neural differentiation. At day 50+, neural organoids were transferred to a multiwell microelectrode array (MEA) plate and allowed to attach. Impedance measurements were used to quantify the attachment of the organoids to the substrate and microelectrodes, as a measure of cell viability and electrode coverage. Broadband (1 - 5000 Hz) electrophysiological data was acquired and then separately processed for action potential detection (200 - 5000 Hz) and low frequency oscillations (1 - 50 Hz). The power spectral density was computed from the low frequency signal sampled after network burst events, and then absolute power was computed in the delta (1-4 Hz), theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), and gamma (30-50 Hz) bands. The emergence and maturation of neural organoid electrophysiological activity was tracked via these measurements of spiking activity and low frequency oscillations, coupled with the long-term monitoring of size via live-cell imaging. This new workflow combining live-cell imaging and MEA measurements supports the continued development of *in vitro* 3D models of neural function.

Disclosures: S. Chvatal: A. Employment/Salary (full or part-time):; Axion BioSystems. **D. Sullivan:** A. Employment/Salary (full or part-time):; Axion BioSystems. **B. Streeter:** A. Employment/Salary (full or part-time):; Axion BioSystems. **P.J. Ellingson:** A. Employment/Salary (full or part-time):; Axion BioSystems. **A. Passaro:** A. Employment/Salary (full or part-time):; Axion BioSystems. **D.C. Millard:** A. Employment/Salary (full or part-time):; Axion BioSystems.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.08/A7

Topic: A.03. Stem Cells and Reprogramming

Support: INPer 212250-3230-21214-03-16
2024-1-7

Title: Exploring the role of prolactin in murine pluripotency & neural differentiation: insight into early development and corticogenesis

Authors: *O. MARTÍNEZ[1], D. COLIN LAGOS[1], X. RAMÍREZ MEZA[1], D. AVILA-GONZÁLEZ[1], G. GARCÍA[1], A. MOLINA[1], W. PORTILLO[2], N. DIAZ[1];
[1]Inst. Nacional de Perinatologia, Mexico, Mexico; [2]Inst. de Neurobiologia, Queretaro, Mexico

Abstract: Prolactin (PRL), a multifunctional hormone involved in numerous physiological processes, including maternal behavior and neurogenesis, has garnered attention for its potential impact on early developmental processes. However, its exact role in early development remains poorly elucidated. This study aimed to explore the influence of PRL on murine pluripotency and its subsequent differentiation into cortical lineage cells *in vitro*. Initially, the presence of the PRL receptor (PRLr) was examined in mouse embryonic stem cells (mESCs) maintained under different culture conditions promoting distinct pluripotent states. A decline in PRLr expression was noted in cells cultured under conditions allowing both ground and primed pluripotency, potentially attributed to population heterogeneity. Subsequent analysis tracked the dynamic changes in PRLr expression over 144 hours following the removal of pluripotency maintenance signals, revealing a redistribution of the receptor from membranal/cytoplasmic to cytoplasmic/nuclear compartments. Furthermore, PRL treatment did not sustain the population of Nanog+ cells in pluripotent cells, whereas Oct4+ and Sox2+ cells remained unaffected at certain concentrations of the hormone. Interestingly, PRLr expression persisted throughout all stages of differentiation, as evidenced by co-localization with markers of neural lineage cells, including Oct4+, Nestin+, Tuj1+, and NeuN+ cells. While no significant effect on neural stem cell proliferation was observed, PRL treatment led to an increase in immature (Tuj1+) and mature (Map2+) neurons at specific concentrations. Additionally, concentrations of PRL showed differential effects on the expression of neuronal markers Tbr1 and NeuN, particularly when administered during early differentiation stages. Moreover, neurons treated with PRL exhibited enhanced dendritic complexity and increased soma distance at certain concentrations, suggesting potential morphological effects on neuronal development. In summary, these findings shed light on the roles of PRL and its receptors in murine pluripotency and neural differentiation, with potential implications for early development and corticogenesis. The study contributes to our understanding of the regulatory mechanisms governing stem cell biology and neurodevelopment.

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Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.09/A8

Topic: A.03. Stem Cells and Reprogramming

Support: Forska Utan Djurförsök, 2023-0007

Title: Brainphys media causes acute epileptiform activity in human ipsc-brain cell models

Authors: *T. LYCKENVIK[1], S. ILLES[1,2];
[1]Univ. of Gothenburg, Gothenburg, Sweden; [2]Oscillution AB, Gothenburg, Sweden

Abstract: Current cell culture media aim to replicate the physiological environment for brain cells cultured in vitro by mimicking cerebrospinal fluid composition. BrainPhys media has been introduced as it is supposed to reflect human cerebrospinal fluid ion concentration, which is not provided by classical cell culture media such as Neurobasal (1).
In this study, we measured human CSF and human serum ion concentrations from healthy human subjects and compared the ion concentrations to those in BrainPhys and other commonly used neuro culture media in the field. Our findings demonstrate that BrainPhys contains non-physiological ion concentrations, diverging from human CSF levels. Combining human iPSC brain cells with microelectrode array recordings, we uncovered that aberrant ion concentrations in BrainPhys induce acute epileptiform activity, and we identified the underlying mechanism of this aberrant activity.
These results prompt questions about the accuracy of current measurements in neuronal development and recorded activity levels, and their reflection of physiological or pathophysiological neuronal in vitro development and function.
References: (1) Bardy *et al.* Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro, 2015, PNAS

Disclosures: T. Lyckenvik: None. **S. Illes:** A. Employment/Salary (full or part-time):; Oscillution AB, Gothenbrug, Sweden.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.10/A9

Topic: A.03. Stem Cells and Reprogramming

Support: Forska Utan Djurförsök, 2023-0007

Title: How ion-concentrations regulate synchronous spiking and local field potential activity in human iPSC-brain cells

Authors: *E. ARTHURSSON[1], S. ILLES[2,3];
[1]Univ. of Gothenburg, Göteborg, Sweden; [2]Univ. of Gothenburg, Gothenburg, Sweden; [3]Oscillution AB, Gothenburg, Sweden

Abstract: Ion concentrations within the brain are not static; their changes drive the transition of neuronal network activity into different stages, which are considered important prerequisites for various brain states such as wakefulness and sleep [1]. In vitro models applied in academic neuroscience and the pharmaceutical industry utilize conditions using aCSF solutions and cell culture media with fixed ion concentrations, which, in addition, are often non-physiological (see

our SFN 2024 poster [2]). The concept of dynamic ion concentration driving neuronal network activity stages at the mesoscale as an underlying mechanism for the dynamic nature of brain states at the macroscale is currently not recapitulated in brain cell in vitro models.
We have begun implementing this concept and present how potassium, calcium, and magnesium, as the major ion species responsible for neuronal excitability and synaptic function, modulate neuronal network spiking and local field potential activity in human brain cell assemblies generated from human iPSCs. The data serve as a starting point for defining and improving the mimicry of human brain function and dysfunction in functional human iPSC brain cell in vitro models.
References: (1) Rasmusson *et al.* Interstitial ions: A key regulator of state-dependent neural activity?, 2020, Progress Neurobiology (2) Lyckenvik and Illes, BrainPhys media causes acute epileptiform activity in human iPSC-brain cell models, poster at SFN meeting 2024.

Disclosures: E. Arthursson: None. **S. Illes:** A. Employment/Salary (full or part-time):; Oscillution AB, Gothenburg, Sweden.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.11/A10

Topic: A.03. Stem Cells and Reprogramming

Title: Acceleration of human induced pluripotent stem cell differentiation into neuronal stem cells through mild thermal stimulation

Authors: *S. MI[1], H. KAWAGUCHI[2], L. YU[3];
[1]Toyo Univ., Saitama, Japan; [2]东洋大学, 群马, Japan; [3]Toyo Univ., Saitame, Japan

Abstract: Human induced pluripotent stem (hiPS) cells can differentiate into various cell types, including those relevant to neurological diseases. Thus, the pluripotency of hiPS cells holds great promise for modeling neurological disorders and developing accurate treatments. However, achieving targeted differentiation of hiPS cells into specific neuronal cell subtypes remains challenging, mainly due to the knowledge gaps regarding differentiation technologies. Concerns over low differentiation efficiency and potential tumorigenesis risks further complicate the clinical application of hiPS cells.Heat shock factor 1 (*HSF1*) induces the expression of heat shock proteins that repair protein denaturation caused by heat stimulation. Primary mouse neural stem cells cultured at 38.5°C for 4 days exhibit altered expression levels of the heat shock protein 27 (*HSP27*) alongside accelerated cell differentiation. However, the impact of mild thermal stimulation on hiPS cell differentiation has not been reported.First, we examined the effect of 4 days of mild thermal stimulation on hiPS cell viability and identified the peak HSP27expression time during the 4-day stimulation. Subsequently, we investigated the effect of HSP27 on pluripotency genes and neural stem cell markers before and after cell differentiation.

Additionally, using PAX6 (a neural stem cell marker) and βIII-tubulin (a neural cell marker), we tracked and compared the differentiation stages of hiPS cell-derived neural stem cells.After culturing at 37°C and 38.5°C, hiPS cells were differentiated at 37°C. Expression changes in the heat stimulation genes *HSF1* and *HSP27*, the pluripotency genes *Oct4*, *Sox2*, and *NANOG*, and the early neural differentiation marker gene *Sox1* were assessed following heat stimulation, with increased expression of *HSP27*, *Oct4*, and *Sox1* observed.On the 4th and 7th days of differentiation culture, we examined expression levels of *Oct4*, *Sox2*, *NANOG*, and *Sox1*again, in addition to *PAX6*, *βIII-tubulin*, and the neural progenitor cell marker gene *TBR2*. On the 4th day, heat-stimulated hiPS cells maintained higher *Sox1* expression levels. On the 7th day, significant differences in *PAX6* expression levels were observed, consistent with immunostaining results on the 7th day. These findings suggest that appropriate heat stimulation enhances hiPS cell differentiation potential, accelerating the differentiation into neural stem cells.

Disclosures: S. Mi: None. **H. Kawaguchi:** None. **L. Yu:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.12/A11

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant 5R44ES032782-03
NIH Grant 1R43GM143978-01

Title: Accelerating the use of patient-derived hiPSCs neuronal models by increasing efficiency of reprogramming and differentiation using CellRaft Technology

Authors: *S. MIHAILOVIC, L. LAND, A. STERN, J. HARTMAN, **C. SENGUPTA**; Cell Microsystems, Durham, NC

Abstract: Establishing and differentiating patient-specific hiPSCs provides unmatched potential in developing accurate neuronal disease models. Despite the potential, the protocols associated with reprogramming somatic cells and differentiating hiPSCs are highly technical and require manual manipulation, which contributes to low efficiency, limited throughout, and lack of reproducibility. We hypothesized that CellRaft® technology could overcome technical challenges in reprogramming and differentiating hiPSCs into 2D and 3D neuronal models, providing an automated solution for these manual workflows. To evaluate reprogramming efficiency, electroporated fibroblasts were seeded in either a 6-well plate or in a CellRaft® Array. The CellRaft Array provides a novel culture environment combining flask-like culture with single cell separation, enabling precise monitoring of reprogramming efficiency, viability, and clonality. Using live staining for Tra-1-60 on the CellRaft Array, we identified more than 300 pluripotent clones, and fully characterized monoclonal hiPSC cell lines more than 1 month

faster than the standard manual workflow which generated only 1 unconfirmed hiPSC colony. To optimize neural progenitor (NPC) differentiation, embryoid bodies were seeded on CellRaft Arrays in neural induction media to monitor formation of neural rosettes. Automated retrieval of CellRafts containing neural rosettes was performed using the AIR System, eliminating the need for manual dissection or rosette selection reagents. Post-isolation staining revealed 100% of CellRafts containing neural rosettes were Pax6 positive, indicating high fidelity of NPC differentiation. We also developed protocols for differentiating hiPSCs into neural organoids, including cerebral and choroid plexus. Using the CellRaft Array, segregated organoids, including clonal organoids, can be reliably imaged and analyzed over time, enabling temporal phenotypic selection of hundreds of single organoids, which is not possible using standard techniques. At desired timepoints, single organoids of interest can be retrieved from the array using the CellRaft AIR System for continued growth or downstream assays. Using software-guided selection, we have demonstrated the ability to reliably generate custom, reproducible single organoid assay plates for drug screening of normal and diseased hiPSCS. Together, these results demonstrate the ability of the CellRaft AIR Technology to provide an end-to-end solution for otherwise challenging workflows that will accelerate the utility and reproducibility of generating patient-specific hiPSCs and mature neuronal models.

Disclosures: S. Mihailovic: A. Employment/Salary (full or part-time):; Cell Microsystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cell Microsystems. **L. Land:** A. Employment/Salary (full or part-time):; Cell Microsystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cell Microsystems. **A. Stern:** A. Employment/Salary (full or part-time):; Cell Microsystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cell Microsystems. **J. Hartman:** A. Employment/Salary (full or part-time):; Cell Microsystems. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cell Microsystems. **C. Sengupta:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.13/A12

Topic: A.03. Stem Cells and Reprogramming

Support: Grant 1R21NS130319-01A1

Title: Exploring the Neurodevelopmental Consequences of POU4F1 Haploinsufficiency in Human Cellular Models

Authors: *R. HU[1], S. MARRO[2];
[1]Icahn Sch. of Med. at mount sinai, New York City, NY; [2]Icahn Sch. of Med. at mount sinai, New York, NY

Abstract: Our research has implicated heterozygous variants in POU4F1 in the etiology of a novel neurological disorder characterized by childhood onset ataxia, global developmental delay with impaired intellectual development. POU4F1 is a transcription factor within the class IV POU domain, known to play crucial roles in neurogenesis, neuronal differentiation, and survival. Despite its significance, the specific functions of POU4F1 in human neurons remain largely unexplored. Animal models, such as Pou4f1$^{-/-}$ mouse (lethal within 24 hours postnatal), and Pou4f1$^{+/-}$ mouse (no observable phenotypic changes), are unsuitable for further study. However, prior research shows that POU4F1 is vital for maintaining the identity of VGluT1^{+} neurons across brain regions. Strikingly, using human postmortem brain tissue, we've detected robust expression of POU4F1 in cortical neurons of young and adult individuals. We, therefore, set out to investigate the effect of the patient variants in the context of human glutamatergic neurons. Human neurons (iGlut) were induced by accelerating Ngn2 expression in human induced pluripotent stem cells (hiPSC). Our transcriptomic data suggest that POU4F1 haploinsufficiency in iGlut influences a transcriptional network related to neurogenesis and maintenance of neuronal identity. These findings have led us to focus on elucidating the role of patient variants in early human neurogenesis. We have developed an efficient, inducible brain organoid model derived from hiPSCs. Experiments are currently being performed to explore the effects of POU4F1 haploinsufficiency on cortical organoids that model the developing human brain. Finally, to expand the list of human pathogenic variants of POU4F1, we established a patient registry for POU4F1-related ataxia, thereby enriching the clinical characterization of this newly identified disorder. Through these approaches, our research intends to elucidate the pathophysiology and molecular mechanisms underlying POU4F1-related ataxia, providing insights into its clinical spectrum and addressing fundamental questions of human neurodevelopmental biology, and epigenetic cell identity.

Disclosures: R. Hu: None. **S. Marro:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.14/A13

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant HD103360
NIH Grant NS122316
DOD Grant W81XWH-21-1-0247

Title: Differentiation of iPSCs into parvalbumin-positive neurons

Authors: *A. CHEN, M. S. MOHAMED, E. KLANN;
New York Univ., New York, NY

Abstract: Parvalbumin-positive (PV) interneurons are fast-spiking GABAergic neurons present throughout the central nervous system. These neurons inhibit other types of neurons, such as cortical pyramidal neurons. Previous studies in animal models of autism and epilepsy have shown that diverse neural circuits have elevated excitation relative to inhibition due to PV inhibitory interneuron dysfunction. Thus, being able to generate and study these PV neurons in an in vitro model can aid in understanding the mechanisms involved in autism and epilepsy, as well as assist in drug development. Previously, small molecule differentiation was used to generate neurons from stem cells, but recently, transcription factors have been used instead, as they can produce neurons more quickly and abundantly. Recent research has shown that transcription factors Ascl1 and Dlx2 together can induce differentiation into inhibitory neurons without any PV expression, and Lhx6 alone can induce stem cell differentiation into a mixed population of inhibitory neurons with PV expression. Still, a protocol has yet to be developed to efficiently produce a large population of these neurons with a high rate of PV expression and firing pattern. We are developing a protocol to differentiate induced pluripotent stem cells (iPSCs) into PV neurons using a combination of Ascl1, Dlx2, and Lhx6 with high efficiency. Our preliminary findings indicated that after growing the neurons on astrocytes for 28 days, they are positive for neuronal markers MAP2 and NeuN and inhibitory marker GAD67. They are also negative for VGlut, an excitatory neuronal marker. Future plans include validation with other inhibitory markers, including Parvalbumin, Somatostatin, and GAT1, as well as further characterizing the firing patterns of these neurons.

Disclosures: A. Chen: None. **M.S. Mohamed:** None. **E. Klann:** None.

Poster

PSTR200: Methods for Improving Differentiation and Reprogramming of Pluripotent Stem Cells

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR200.15/A14

Topic: A.03. Stem Cells and Reprogramming

Title: High throughput assessment of barrier function using human ipsc-derived brain microvascular endothelial cells

Authors: S. HILCOVE, C. SAVIC, M. GOEDLAND, R. FIENE, *J. LIU, R. VAIDYANATHAN, C. B. CARLSON;
FUJIFILM Cell. Dynamics, Madison, WI

Abstract: *OBECTIVE / RATIONALE* The blood-brain barrier (BBB) is composed of specialized brain microvascular endothelial cells (BMEC) that serve to regulate the flow of substances into and out of the brain. BMEC have physical, transport, and metabolic properties that are regulated

by other vascular, immune, and neural cells to create a tightly controlled microenvironment of the central nervous system. Understanding how BMECs work alone and in concert with these other cell types is essential to understand how the brain functions during health and disease. One of the key functional features of BMEC is barrier formation, and the strength and integrity of this barrier can be evaluated via measurement of the electrical resistance across the cell layer. In this study, the barrier function properties of human iPSC-derived BMEC were measured using different platform technologies, such as impedance and trans-endothelial electrical resistance (TEER). *METHODS / RESULTS* Human iPSC-derived brain microvascular endothelial cells (iCell BMEC) and all media with supplements were from FUJIFILM Cellular Dynamics. These cells were differentiated similar to previously published protocols from an apparently healthy normal (AHN) male donor 01279. Development of TEER assays to measure the barrier function of BMEC were performed using 24-well cell culture inserts (Corning) coated with Collagen-IV and Fibronectin (Sigma). Cryopreserved cells were thawed in the presence of iCell Plating Supplement for the best results. To increase the throughput of TEER measurements, however, BMEC were also plated on CytoView-Z plates and signal was recorded using the Maestro Z (Axion Biosystems). This technology monitors cell coverage (or confluence) as a resistance at high frequency (41.5 kHz) and a very sensitive TEER at a low frequency (1 kHz). These properties were monitored continuously over the course of 10 days. Real-time traces of impedance over time were used to demonstrate lot-to-lot consistency of iCell BMEC with uncorrected TEER resistance values of ~3500 Ω at 140 hours. This assay was used to profile molecules in 384w format (n=4 wells; 8-point dose-response curve) that disrupt the barrier, incl. bupropion, mannitol, prazosin, VEGF, and verapamil. *CONCLUSIONS* Characterization of and testing with the individual cellular components that make up the BBB provides added insight to the functional aspects of this complex system. Human iPSC-derived BMEC offer a robust and reliable source of cells to interrogate the multiple properties of this specialized cell type.

Disclosures: S. Hilcove: A. Employment/Salary (full or part-time):; FUJIFILM cellular dynamics. **C. Savic:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **M. Goedland:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **R. Fiene:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **J. liu:** A. Employment/Salary (full or part-time):; FUJIFILM cellular dynamics. **R. Vaidyanathan:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics. **C.B. Carlson:** A. Employment/Salary (full or part-time):; FUJIFILM Cellular Dynamics.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.01/A15

Topic: A.07. Developmental Disorders

Title: Alterations in neural plasticity and enhanced nicotine stress-induced reinstatement in a heritable rodent model of psychosis and drug abuse vulnerability

Authors: *A. M. CUOZZO, L. D. PEETERS, C. D. AHMED, L. J. WILLS, E. E. WALLEN, R. W. BROWN;
Biomed. Sci., East Tennessee State Univ., Johnson City, TN

Abstract: Schizophrenia (SZ) is a neurological disorder marked by hallucinations, delusions, anhedonia, and cognitive deficits. A significant cigarette smoking comorbidity exists within SZ that contributes to reduced antipsychotic efficacy, reduced life expectancy, lung cancer, and an overall diminished quality of life. Individuals with SZ smoke cigarettes at a rate roughly 3 to 4 times higher than the general cigarette smoking population. This substantial comorbidity is persistent and smoking cessation rates in this population are reduced. Contributions to low cessation consist of poor antipsychotic efficacy, as well as stressful life events. Stress has been identified as a primary risk factor for increased drug craving and likelihood of relapse in substance abuse. When individuals with SZ attempt to quit smoking, positive and negative symptoms are often exacerbated, reducing the likelihood of quit success. Our laboratory has established a heritable rodent model of psychosis and drug abuse vulnerability. In this model, male and female Sprague-Dawley rats are neonatally treated with saline (NS) or quinpirole (NQ), a dopamine (DA) D_2-like agonist (1 mg/kg), from postnatal days (P)1-21. NQ-treated rats display a lifelong supersensitization of the DAD_2 receptor, a hallmark of psychosis. In the present study, F1 generation offspring of two NS (MSxFS) or two NQ-treated (MQxFQ) rats were administrered nicotine conditioned place preference (CPP) stress-induced reinstatement. These rats were not neonatally treated and observed phenotypes are mediated through epigenetic mechanisms. On P41-42, F1 MSxFS and MQxFQ rats were given a saline pre-conditioning preference test, and then conditioned to nicotine (0.6 mg/kg base) from P43-50. On P51, animals were given a drug free post-conditioning preference test. Replicating past work, F1 MQxFQ rats demonstrated enhanced expression of nicotine CPP. On P52-P59, animals underwent extinction testing, with F1 generation MQxFQ rats demonstrating resistance to extinction compared to the F1 MSxFS group. On P60, animals were exposed to restraint stress 30 min prior to the reinstatement trial. The F1 MQxFQ group demonstrated robust reinstatement, whereas F1 MSxFS animals did not show reinstatement. Brain tissue was taken 1 h after restraint stress and will be analyzed for neural plasticity markers within the DA-ergic and glutamatergic systems. These analyses will elucidate mechanistic actions underlying nicotine stress-induced reinstatement. The present study is the first to investigate nicotine stress-induced reinstatement in a heritable rodent model of psychosis and drug abuse vulnerability.

Disclosures: A.M. Cuozzo: None. **L.D. Peeters:** None. **C.D. Ahmed:** None. **L.J. Wills:** None. **E.E. Wallen:** None. **R.W. Brown:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.02/A16

Topic: A.07. Developmental Disorders

Support: NIH R15DA046926

Title: Examining MGluR5 Positive Allosteric Modulation of Prepulse Inhibition Deficits in a Heritable Model of Psychosis

Authors: *C. AHMED, L. PEETERS, W. D. GILL, A. M. CUOZZO, J. T. GASS, R. W. BROWN;
Biomed. Sci., East Tennessee state Univ., Johnson City, TN

Abstract: Schizophrenia (SZ) is a debilitating disorder that is characterized by deficits in sensorimotor gating. Although the etiology of SZ remains elusive, identifying potential therapeutic targets is crucial. Our laboratory has established that rats administered neonatal quinpirole (NQ), a dopamine (DA) D_2-like receptor agonist (1 mg/kg), permanently increases DAD_2 receptor sensitivity. We have recently reported that offspring of NQ-treated rats also demonstrate increased DAD_2 receptor sensitivity, establishing this model as heritable. In the current study, we investigated whether 3-Cyano-*N*-(1, 3 diphenyl-1*H*-pyrazol-5-yl) benzamide (CDPPB), a positive allosteric modulation of the metabotropic glutamate receptor type 5 (MGluR5) would alleviate deficits in sensorimotor gating as measured by prepulse inhibition (PPI). There is interest in developing drugs with antipsychotic efficacy that circumvent debilitating side effects of DAD_2 antagonism. The DAD_2 receptor forms a triple heteromer with the adenosine A(2A) and mGlu5 receptors, such that stimulation of either the A(2A) or MGluR5 decreases DAD_2 signaling. Thus, this study tested efficacy of positive allosteric modulation of mGlu5 towards a behavioral deficit that is a hallmark of SZ. Founder male and female Sprague-dawley rats were intraperitoneal (IP) administered saline (NS) or quinpirole HCl (1 mg/kg) from postnatal days (P) 1-21, raised to P60 and mated with their NS or NQ-treated counterparts. The resulting offspring of these founder pairs, the F1 generation, was raised without drug treatment until P44. From P44-48 all groups were behaviorally tested on PPI. We tested during the adolescent period because of the neurodevelopmental nature of SZ and behavioral symptoms are commonly manifested in adolescence. Replicating past work, F1 generation offspring of at least one neonatal quinpirole (NQ) treated founder demonstrated robust PPI deficits across all 73, 76, and 82 dB prepulse intensities. Across all groups, rats were IP administered 20 min before each PPI trial with vehicle, 10 or 30 mg/kg CDPPB. Regardless of the dose CDPPB reversed PPI deficits at 10 mg/kg and 30 mg/kg. However, in the offspring of male quinpirole (MQ) - female saline (FS) pairs, 10 mg/kg CDPPB was ineffective, suggesting a unique change in neural plasticity in this group. DAD_2 receptor plasticity in the dorsal striatum and prefrontal cortex is being analyzed using ELISAs (data will be presented). These findings suggest NQ treatment induces a heritable SZ-like phenotype with PPI deficits and the MGluR5 may represent a potential therapeutic target for SZ.

Disclosures: C. Ahmed: None. **L. Peeters:** None. **W.D. Gill:** None. **A.M. Cuozzo:** None. **J.T. Gass:** None. **R.W. Brown:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

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Program #/Poster #: PSTR201.03/A17

Topic: A.07. Developmental Disorders

Support: NIH grant R15DA04926-01
NIH grant U01DA058278
NIH grant F99NS130827

Title: Altered dopamine responses in the nucleus accumbens shell mediate aberrant relapse-like behavior in a transgenerational model of drug abuse vulnerability in psychosis: Mechanisms of epigenetic inheritance

Authors: *L. D. PEETERS[1], L. J. WILLS[2], A. M. CUOZZO[3], C. AHMED[4], W. CHEN[5], Z. CHEN[6], C. WANG[7], R. W. BROWN[8];
[1]Biomed. Sci., East Tennessee State Univ. Quillen Col. of Med., Johnson City, TN; [2]Biomed. Sci., Quillen Col. of Med., Johnson City, TN; [3]East Tennessee State Univ., Johnson City, TN; [4]Biomed. Sci., East Tennessee state Univ., Johnson City, TN; [5]Loma Linda Univ., Loma Linda University, CA; [6]Loma Linda Univ., Riverside, CA; [7]Ctr. for Genomics, Loma Linda Univ., Loma Linda, CA; [8]Dept. of Biomed. Sci., Dept. of Biomed. Sci. East Tennessee State Univ., Johnson City, TN

Abstract: Schizophrenia and tobacco use disorder are highly comorbid neuropsychiatric disorders. In addition, quit success is significantly reduced and relapse rates following smoking cessation are high in patients with schizophrenia. However, the mechanisms underlying altered relapse-like behaviors in individuals diagnosed with psychosis are poorly understood. The present study explored altered dopamine signaling as a mechanism conferring aberrant relapse-like behavior in a novel heritable model of drug abuse vulnerability in psychosis, demonstrating increased dopamine D2 receptor sensitivity. Male and female offspring of two neonatal quinpirole-treated (QQ) and two neonatal saline-treated (SS) Sprague-Dawley rats were tested on an extended nicotine CPP paradigm to analyze changes in extinction and nicotine-primed reinstatement. QQ offspring demonstrated delayed extinction, more robust reinstatement, clinically consistent with reduced quit success and increased relapse vulnerability. A subset of rats received stereotaxic injections of the dopamine sensor dLight1.3b followed by fiber optic canula implantation into the nucleus accumbens (NAc) shell. Fiber optic recordings were conducted during the final day of extinction (day 8) and during the reinstatement trial. Integration of fiber optic recordings with time-locked behavioral data collected via Anymaze Behavioral Tracking Software were used to obtain information relative to the changes in dopamine during ingress and egress from the context paired with nicotine during each of these phases. Epigenetic mechanisms contributing to enhanced drug abuse and relapse vulnerability were additionally explored in a separate set of male QQ and SS offspring. Methyl-seq analysis revealed region-specific changes in several pathways, including nicotine addiction, dopamine synapses, and neuron projections. These results reveal epigenetic mechanisms of heritability and demonstrate altered relapse-like behavior consistent with a model of comorbid drug abuse and psychosis.

Disclosures: L.D. Peeters: None. **L.J. Wills:** None. **A.M. Cuozzo:** None. **C. Ahmed:** None. **W. Chen:** None. **Z. Chen:** None. **C. Wang:** None. **R.W. Brown:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

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Topic: A.07. Developmental Disorders

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Brain and Behavior Research Foundation Young Investigator Grant
NICHD T32HD075735

Title: Sex-specific perturbations of neuronal development caused by mutations in the autism risk gene DDX3X

Authors: *A. MOSSA[1], L. DIERDORFF[1], J. LUKIN[1], Y. PARK[1], C. FIORENZANI[2], Z. AKPINAR[3], M. GARCIA-FORN[1], S. DE RUBEIS[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Univ. of Bologna, Bologna, Italy; [3]Harvard Sch. of Dent. Med., Boston, MA

Abstract: Mutations in the X-linked gene *DDX3X* are a leading cause of DDX3X syndrome, a rare form of intellectual disability (ID) and autism spectrum disorder (ASD), that predominantly affects females. DDX3X expression in humans and mice is sexually dimorphic: females express the gene from both X chromosomes, while males have only one X chromosome. Yet, it is not clear why females with only one functional *DDX3X* allele manifest DDX3X syndrome, while males (who are physiologically hemizygous) do not. DDX3X is an RNA helicase with a wide range of functions in mRNA translation. However, the cellular and molecular mechanisms by which *DDX3X* mutations impair brain development and function are not fully understood. Neurons are extremely compartmentalized cells that require local mRNA translation in dendrites and synapses in order to maintain local protein homeostasis and ensure proper neuronal development and function. As ribosomes serve as mRNA translation machinery, ribosome biogenesis and assembly are crucial for local mRNA translation efficiency. Ribosome biogenesis begins in the nucleolus, a highly dynamic nuclear substructure. Changes in nucleolar dynamics and structure are a readout of ribosome biogenesis dysregulation and might represent a target mechanism underlying neurodevelopmental disorders such ID and ASD.New data generated in the De Rubeis lab are beginning to delineate a sex-specific role of DDX3X in regulating neuronal development and ribosome biogenesis. Employing *in vitro* and *in vivo* mouse models, we show that *Ddx3x* mediates sexual dimorphisms in brain development at molecular, cellular, and behavioral levels. *Ddx3x* deficiency has a sex-depended effect on cortical neuronal development and morphology as well as neuronal and synaptic proteomes. We found a general downregulation of core ribosomal proteins of the mRNA translation machinery and smaller nucleoli in *Ddx3x* haploinsufficient female neurons. Additionally, forebrain *Ddx3x* conditional

knock-out mice present sex-specific changes in developmental milestones and motor function.These results suggest that nucleolar changes and a general hypoactivity of the ribosomal machinery observed in *Ddx3x* haploinsufficient females might affect the synthesis of proteins necessary for proper dendritic arborization and spine formation, leading to abnormal neuronal development and morphology, as well as consequently compromising the correct integration of cortical neurons in neural circuitry for behavioral outcomes. Together, these findings outline *Ddx3x* as a significant player in sexual differentiation during neurodevelopment in health and disease.

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Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.05/A19

Topic: A.07. Developmental Disorders

Support: NIH Grant AG080472-01
Simons Foundation Autism Research Initiative
Eagles Autism Foundation

Title: Behavioral characterization of a mouse model for Marbach-Schaaf Neurodevelopmental Syndrome (MASNS)

Authors: *W. SAIDE;
Neurosci. and Pharmacol., Univ. of Iowa, Tiffin, IA

Abstract: Behavioral characterization of a mouse model for Marbach-Schaaf Neurodevelopmental Syndrome (MASNS)
Walter Saide (PhD)[1], Alex Glebov-McCloud (BA)[1], Ted Abel (PhD)[1], Marie Gaine (PhD) [2], Stefan Strack (PhD)[1]
[1]Department of Neuroscience and Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA
[2]Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, IA
Mutations of *PRKAR1B*, the gene encoding for the RIβ regulatory subunit of Protein Kinase A (PKA), are implicated in neurological and neurodevelopmental disorders. One such disorder known as Marbach-Schaaf Neurodevelopmental Syndrome (MASNS) is a recently discovered neurodevelopmental disorder that produces global developmental delay, hyperactivity, Autism Spectrum Disorder (ASD) or ASD-related behavior, dyspraxia, and pain insensitivity. Many of the patients identified with MASNS have a recurring *de novo* R335W mutation in PKA RIβ. However, the molecular and physiological mechanisms by which the R335W mutation results in

a neurodevelopmental disorder are unknown. To study these mechanisms, we generated a R335W *Prkar1b* transgenic mouse model for behavioral and molecular characterization. Here we validated the R335W PKA RIβ transgenic mouse models many of the behavioral deficits reported in children with MASNS. Using a variety of behavioral assays including the two-trial y-maze, contextual fear conditioning, von Frey and Hargreaves' assays, rotarod test, and three-chamber social interaction test, we determined the R335W PKA RIβ mouse model displays deficits in spatial memory, contextual fear conditioning, sensitivity to both noxious heat and noxious pressure, motor learning, and sociability, respectively. Our results suggest that the R335W mouse model we generated closely resembles MASNS in humans and can be used further to study the molecular and physiological mechanisms of this disorder. Furthermore, we observed the R335W mouse model results in obesity and decreased food and water seeking behavior compared to WT mice, suggesting a further impact of this mutation on metabolism that was not initially elucidated in MASNS. ANCOVA was performed to validate behavioral deficits were not significantly correlated to the mass of the mouse. Future studies aim at identifying changes in substrate phosphorylation, subcellular localization, and changes in cell morphology or gross anatomy that may explain how the R335W PKA RIβ causes neurodevelopmental disorders.

Disclosures: W. Saide: None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: A.07. Developmental Disorders

Support: NINDS (R01NS105502)
GMCMD-T32 (5T32NS041234-20)

Title: Behavioral dysfunction in a mouse model of a pathological human GRIK2 kainate receptor variant

Authors: *B. WEBB, E. BREACH, H. TRINH, G. T. SWANSON;
Pharmacol., Northwestern Univ., Chicago, IL

Abstract: Deleterious mutations in ionotropic glutamate receptor genes are causative for numerous non-syndromic neurodevelopmental disorders (NDDs). Individuals with NDD caused by a de novo point mutation in the glutamate receptor ionotropic, kainate subunit 2 (*GRIK2* c.1969 G>A, GluK2 p.Ala657Thr) exhibit intellectual disability, speech delay, and motor deficits. We are working to identify the mechanistic basis for this disorder using mice with an analogous G>A mutation in *Grik2*¸ which we refer to as GluK2(A657T). In this study we tested if heterozygous GluK2(A657T) mice display behavioral deficits analogous to those observed in the human disorder.
GluK2(A657T) mice were assayed in a variety of behavioral tests that assessed locomotion,

naturalistic behaviors, motor function, cognitive performance, and developmental behaviors. In open field assays, GluK2(A657T) animals displayed reduced locomotor, rearing, and grooming activity compared to wildtypes. GluK2(A657T) mice dug bedding and shredded nestlets significantly less than their wildtype littermates. They performed normally in rotarod testing but were slower to cross a balance beam, indicative of mild balance deficits. Their gait was characterized by shorter stride lengths and reduced hindpaw angle, and postural and balance assays revealed a mild to moderate ataxic-like phenotype. To test cognitive performance, we ran the mice through novel object recognition (NOR), T-Maze, and fear conditioning tests. GluK2(A657T) mice did not display long-term memory deficits in NOR but did display an impairment in working memory during delayed non-match to sample T-Maze testing. Furthermore, GluK2(A657T) mice demonstrated impaired fear conditioning association learning during training and the cue test and froze in a novel context significantly more than their wildtype littermates. Finally, we found that the mutant mice reached normal weight, gait, and righting reflex developmental milestones from P3-P10, but displayed impairments in hindlimb strength and posture at P10. GluK2(A657T) mice displayed reduced vocalization production, duration, frequency bandwidth, and energy variably across timepoints.
Our results demonstrate that GluK2(A657T) mice display reductions in activity, motor deficits, cognitive impairments, and developmental delay, supporting the pathological nature of the *GRIK2* p.Ala657Thr variant in humans and the face validity of our mouse model. We aim to elucidate the neural correlates of the deficits observed in GluK2(A657T) mice and anticipate that our findings will enhance our understanding of the role of kainate receptors in developmental disorders.

Disclosures: B. Webb: None. **E. Breach:** None. **H. Trinh:** None. **G.T. Swanson:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.07/A21

Topic: A.07. Developmental Disorders

Support: NINDS Grant NS108722
NINDS Grant NS125519

Title: Serotonin 5-HT2A receptor antagonists reduce tic-like responses in two mouse models of Tourette syndrome

Authors: *E. VAN LUIK, R. CADEDDU, M. BORTOLATO;
Univ. of Utah, Salt Lake City, UT

Abstract: Tics are repetitive movements or vocalizations that significantly impact daily functioning and quality of life. Current pharmacological treatments for Tourette syndrome and other tic disorders have limited efficacy and are associated with adverse effects, underscoring the

need for novel treatments. Converging evidence suggests that serotonin 5-HT_{2A} receptors may be involved in tic ontogeny. Accordingly, preliminary evidence indicates that 5-HT_{2A} receptor antagonists are well-tolerated and effective in reducing tic severity and improving the quality of life of TS patients. To better understand the impact of these receptors in TS, we tested the effects of two highly selective 5-HT_{2A} receptor antagonists, pimavanserin and volinanserin, in two mouse models capturing complementary aspects of TS pathophysiology: the D1CT-7 mice, in which the neuropotentiation of the somatosensory cortex results in tic-like motoric jerks; and mice subjected to early-life depletion of striatal cholinergic interneurons (CINs), which simulate the loss of these cells documented in postmortem striatal tissues of TS-affected subjects. In both models, pimavanserin and volinanserin produced a significant reduction of tic-like behavior. These effects were mimicked by local infusions into the PFC, but not striatum, suggesting that the ameliorative effects of 5-HT_{2A} antagonists may be mediated by cortical modulation of tics. These results confirm and expand previous evidence showing that 5-HT_{2A} receptors are involved in the pathophysiology of tics, and underscore that antagonists of these targets may be a valuable therapeutic tool for TS.

Disclosures: E. Van Luik: None. **R. Cadeddu:** None. **M. Bortolato:** F. Consulting Fees (e.g., advisory boards); Asarina.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.08/A22

Topic: A.07. Developmental Disorders

Title: Exploring the neurophysiologic and neuropathologic basis of visual deficits in a cystinosis mouse model

Authors: *H. CHANG[1], V. PRIFTI[1], A. SOLORZANO[4], Y. DING[5], J. FENG[6], C. CANAVESI[7], K. PADMANABHAN[1], E. G. FREEDMAN[2], K. H. WANG[3], J. J. FOXE[1]; [1]Neurosci., [2]Del Monte Inst. for Neurosci., [3]Dept. of Neurosci., Univ. of Rochester, Rochester, NY; [4]Univ. of Rochester Med. Ctr., Rochester, NY, ; [5]Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY; [6]City Univ. of Hong Kong, Hong Kong, Hong Kong; [7]LighTopTech Corp., Rochester, NY

Abstract: Cystinosis is a rare lysosomal storage disorder caused by mutations to the *Ctns* gene that result in a defective lysosomal transport protein called cystinosin. Cystinosin is responsible for the export of cystine - the oxidized form of cysteine - from the lysosome to the cytosol. Without a functioning transporter, cystine crystals accumulate in lysosomes and cause cell death. Typically, cystinosis presents early in childhood with kidney dysfunction that progresses to end-stage renal disease and early death. Certain medications can extend patient life span, but the disease will continue to progress over time, incorporating other body systems. Photophobia, or a painful sensation in response to light, is one of the most common and debilitating symptoms that

patients experience.
Historically, photophobia has been attributed to corneal cystine crystal accumulation in patients, as crystals are easily found within the eyes by just 16 months of age. Consistent usage of topical eye drops to remove these crystals, though, does not alleviate symptoms, suggesting a more complex etiology of patient photophobia. Interestingly, visually evoked potential (VEP) data collected in patients demonstrates an early cortical hyperexcitability, hinting that there may be a role of the central nervous system (CNS) in patient photophobia.
To understand whether the CNS plays a role in the etiology of photophobia in cystinosis, we have developed a Ctns knockout (*Ctns -/-*) mouse model using CRISPR/Cas9. This model introduces a frameshift mutation and premature stop codon early within the locus, producing a truncated 23 amino acid (AA) protein that is 344 AA shorter than the full-length CTNS. Early phenotyping with optical coherence tomography imaging, has confirmed that our *Ctns -/-* mice accumulate corneal crystals like that of patients.
To further examine if our mouse model experiences the same hyperexcitability as patients with cystinosis, we have designed a VEP paradigm that presents a flash, grating stimulus to mice at varying intensities and contrast levels. In conjunction, the molecular basis of this pathology will be studied using sectioning and staining of areas in the visual cortex and visual thalamus for evidence of lysosomal dysfunction and loss of inhibitory interneuron signaling. Finally, using a light-dark box behavioral paradigm we will assess for whether our mice exhibit photophobic responses in response to varying intensities of light.
In-depth neurophysiologic and neuropathologic characterization of this mouse model will facilitate future applications to examine the efficacy of potential gene therapies and medications for cystinosis.

Disclosures: H. Chang: None. **V. Prifti:** None. **A. Solorzano:** None. **Y. Ding:** None. **J. Feng:** None. **C. Canavesi:** None. **K. Padmanabhan:** None. **E.G. Freedman:** None. **K.H. Wang:** None. **J.J. Foxe:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.09/A23

Topic: A.07. Developmental Disorders

Title: Identification of Molecular Mechanisms and potential genomic compensatory mechanisms underlying TBCK/PPP1R21/C12Orf4-Associated Syndromes

Authors: *V. ZIMYANIN;
Univ. of Virginia, Charlottesville, VA

Abstract: TBCK syndrome, caused by autosomal recessive mutations in the TBCK protein, was the first described disease affiliated with defects in the FERRY-protein complex.The FERRY complex, also known as the Five-subunit Early endosome RNA and Ribosome intermediarY

complex, is a stable protein complex consisting of TBCK (Tre-2/Bub2/Cdc16 domain-containing kinase), PPP1R21, C12Orf4, Cryzl1, and Gatd1. Further research has shown that FERRY-affiliated syndromes include a spectrum of rare and severe childhood disorders broadly categorized as intellectual disability syndromes (IDS) or encephalo-neuronopathies. Our studies in Zebrafish show that downregulation of PPP1R21, TBCK and C12Orf4 translation by stable morpholino antisense oligos (MO) causes severe defects in early development and morphogenesis. Consistent with their proposed function on endosomes we observe defects in endosomal trafficking of cell adhesion and cell signaling proteins like E-cadherin and **β**;-catenin, leading to mislocalization of those proteins. In contrast to the knock-down, three independent CRISPR based mutants that we generated in the lab, introducing stop codons either N-terminal or in the middle of the PPP1R21 gene, show only minor phenotypes and are homozygous viable and fertile. In addition, TBCK alleles with a premature stop codon and double mutants carrying the mutated PPP1R21 and TBCK alleles also do not display any strong phenotypes. Similar discrepancy between knock-down (KD) and (premature stop codons) knock-out (KO) approaches have been observed for both TBCK and PPP1R21 in Drosophila. These data strongly argue for the presence of genetic compensatory mechanisms that function to alleviate deletion phenotypes, while abrupt downregulation of the protein results in strong developmental phenotypes. We propose that further understanding of such mechanisms in the context of FERRY complex associated diseases will ultimately benefit our understanding of these related syndrome and and will ultimately benefit patients.

Disclosures: V. Zimyanin: None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.10/A24

Topic: A.07. Developmental Disorders

Support: NIH/NINDS R01 NS097237
NIH/NINDS R01 NS126361
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NIH/NINDS R21 NS124198
NIH/NINDS F31 NS124245

Title: Neuronal and molecular mechanisms of neurofibromin-mediated modulation of metabolic homeostasis

Authors: *V. BOTERO, S. M. TOMCHIK;
Univ. of Iowa, Iowa City, IA

Abstract: Neurofibromatosis type 1 is a genetic disorder with a metabolic component, the mechanisms of which are unclear. Neurofibromatosis type 1 is a multisystemic disorder, arising from loss-of-function mutations a single gene, *NF1*. This gene encodes neurofibromin (Nf1), a large protein with a central GAP-related domain (GRD) that modulates a wide range of cellular and molecular processes. Individuals with neurofibromatosis type 1 present with a wide spectrum of clinical manifestations, including peripheral nerve-associated tumors, brain tumors, skeletal and vascular abnormalities, and neurocognitive and behavioral deficits. Evidence suggests that Nf1 regulates cellular and organismal metabolism in humans: individuals with neurofibromatosis type 1 have overall reduced stature, reduced body mass index, pituitary growth hormone deficiencies, muscle weakness, and increased insulin sensitivity. Despite indications of Nf1's involvement in metabolic regulation, the critical molecular mechanisms underlying metabolic effects remain largely unknown.
Leveraging the *Drosophila* neurofibromatosis type 1 model and its highly conserved signaling pathways, we investigated the Nf1-metabolism connection. *In vivo* genetic analysis revealed that the loss of Nf1 impairs metabolic homeostasis, resulting in heightened metabolic and feeding rates, disrupted lipid dynamics, and increased starvation sensitivity. These metabolic effects mapped to a distinct set of interneurons in the nervous system, which can be dynamically modulated to increase metabolic rate. Furthermore, investigation revealed that Nf1 regulates metabolic rate via neuronal mechanisms, with additional contributions from muscle cells. Neurofibromin's Ras GAP activity is facilitated by its central GRD, and our research identified Ras signaling as pivotal in mediating Nf1's effects on metabolism. Genetic approaches revealed that expression of a constitutively-active ERK partially phenocopied the metabolic phenotype observed with Nf1 loss. Additionally, our *in vivo* genetic experiments targeting multiple signaling molecules suggested that two signaling pathways downstream of Ras are critical in metabolic regulation, with Nf1 orchestrating metabolic control through the coordinated activity of both. These data reveal a novel interaction between Nf1 and metabolism, delineating the neural circuits and signaling pathways responsible for Nf1-metabolic regulation.

Disclosures: V. Botero: None. **S.M. Tomchik:** None.

Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.11/A25

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative

Title: Investigating mechanisms behind sex specific effects of neurodevelopmental mutations in a Myt1l+/- mouse model

Authors: *S. CHATURVEDI[1], S. SARAFINOVSKA[2], D. SELMANOVIC[4], K. B. MCCULLOUGH[5], S. E. MALONEY[6], J. D. DOUGHERTY[3], K. B. MCCULLOUGH[5], K. B.

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Abstract: Biological sex is known to impact the presentation and prevalence of neurological diseases across development. While some neurodevelopmental disabilities exhibit sex variations due to differences in mutations between male and female populations, in other conditions the same genetic mutation leads to sex specific effects. Sex-genotype interactions are key for understanding conditions like MYT1L Syndrome. MYT1L mutations cause a constellation of symptoms including intellectual disability, autism, and obesity. Mouse models of a MYT1L human point mutation have shown reduced social behavior only in males. To evaluate whether these male specific effects are governed by chromosomal or gonadal sex, I crossed a Myt1l het mouse model with the Four Core Genotype (FCG) model and ran offspring through open field and social operant behavioral protocols. The FCG model provides a way to separate chromosomal and gonadal sex, producing four different “sexes” with a combination of gonads and chromosomes. Our results show chromosomal sex and Myt1l genotype interact to drive higher sociability in mutant XX mice. On the other hand, gonadal sex also drives higher sociability in mice with ovaries independent of Myt1l genotype. These results suggest separate impacts of gonadal and chromosomal sex on sociability in the context of neurodevelopmental mutations, which can provide valuable insight into the mechanisms behind sex biased disabilities such as autism.

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Poster

PSTR201: Animal Models of Developmental Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR201.12/A26

Topic: A.07. Developmental Disorders

Support: STXBP1 Foundation
MDBR ODC Grant MDBR-23-008-SynGAP
ENDD Center
Hartwell Foundation

Title: Personalized strategies targeting Syngap1 haploinsufficiency in a mouse model of SYNGAP1-related intellectual disability

Authors: *L. SICHLINGER, M. REILLY, E. A. HELLER;
Ctr. for Epilepsy and Neurodevelopmental Disorders (ENDD), Penn Medicine, CHOP, Univ. of Pennsylvania, Philadelphia, PA

Abstract: SynGAP1 is a synapse protein and a critical regulator of neurodevelopment and brain function. Human loss-of-function variants give rise to SYNGAP1-related intellectual disability (SRID), a neurodevelopmental disorder characterized by epilepsy, developmental regression, and autism. SYNGAP1 variants are highly penetrant and therefore, provide a suitable target for personalized medicine. However, no such therapy has been developed. Haploinsufficient Syngap1 mice exhibit working memory deficits and aberrant synaptic plasticity leading to seizure phenotypes. This study applies two strategies for targeted rescue of a mouse model of a human-missense-variant of Syngap1. In this Syngap1 missense variant model, a single-nucleotide variant introduces a cryptic splice site leading to Syngap1 haploinsufficiency. In one approach, we apply Syngap1 CRISPRa to restore wildtype Syngap1 expression. Preliminary data show CRISPRa upregulates Syngap1 mRNA and protein expression in the mouse missense variant model. In a separate approach, we will apply steric antisense oligonucleotides (ASOs) to block the cryptic splice site, correct aberrant splicing and restore wildtype Syngap1 expression. This approach offers an avenue for reinstating canonical splicing and mitigating the effects of Syngap1 haploinsufficiency, potentially alleviating SRID phenotypes. In sum, these two strategies represent innovative approaches to address the molecular mechanisms underlying SRID and aim to pave the way for more effective and personalized therapeutic interventions for SRID and related neurodevelopmental disorders.

Disclosures: L. Sichlinger: None. **M. Reilly:** None. **E.A. Heller:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.01/A27

Topic: A.07. Developmental Disorders

Support: Hartwell Foundation's Individual Biomedical Award
Austin Faculty Fellowship
NIH R01 HD094715
NIH P50 HD105353
NIH U54 HD090256
NIH T32 NS105602
NIH T32 GM140935
NIH T32 T32 CA009206

Title: Brainstem and Cerebellar White Matter Tract Maturation in School-Aged Autistic Children

Authors: *M. I. DURAN[1], J. GUERRERO-GONZALEZ[2], D. DEAN III[3], N. ADLURU[4], S. R. KECSKEMETI[7], G. R. KIRK[1], A. L. ALEXANDER[5], B. G. TRAVERS[6];
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Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by social communication difficulties, repetitive behaviors, and sensorimotor differences. While prior research has shown differences in the developmental trajectories of cortical white matter (WM) tracts in ASD compared to controls, group differences and differences in the maturation patterns of brainstem/cerebellar substructures remain under explored. This is a critical gap, as brainstem/cerebellar regions have been found to be linked to autism core and sensory features (Surgent et al., 2022; Travers et al., 2024). Therefore, we examined group differences in microstructural measures and differences in the relationship between age and the microstructure of 23 brainstem/cerebellar WM tracts in 156 children (6 - 10.9 yo; 78 ASD, 42 females, 8 with repeated measures). T1 and diffusion weighted imaging data were collected with a 3T scanner. TiDi-Fused processing (Guerrero-Gonzalez et al., 2022) was used to enhance brainstem images, and microstructural features were quantified using the free water elimination diffusion tensor imaging model. We applied linear mixed effects models to assess microstructural changes as a function of age, diagnostic group, and their interaction, controlling for sex and motion. The analysis revealed age-dependent increases in fractional anisotropy (FA) for all tracts, indicative of ongoing brainstem maturation in both groups. Overall, we noted distinct rates of change across tracts with descending motor tracts exhibiting more rapid changes, and cerebellar tracts maturing more slowly. Contrary to our hypotheses, we did not see distinct developmental trajectories between groups. Instead, we saw sustained group differences (lower FA in ASD) in the lateral lemniscus, inferior and superior cerebellar peduncles, and spinothalamic tracts. These findings suggest that although maturation rates in both groups are similar and stable from 6 to 11 years of age, microstructural differences may stem from maturation deviations occurring prior to the age range examined here. These results urge the need for future longitudinal studies at younger ages to elucidate these developmental patterns further and their implications for ASD phenotypes.

Disclosures: M.I. Duran: None. **J. Guerrero-Gonzalez:** None. **D. Dean III:** None. **N. Adluru:** None. **S.R. Kecskemeti:** None. **G.R. Kirk:** None. **A.L. Alexander:** None. **B.G. Travers:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.02/A28

Topic: A.07. Developmental Disorders

Support: NINDS Grant R01AG086493
NIA Grant R21AG 065621

Title: Age effects in microstructure of white and grey matter in middle-aged and older adults with autism and neurotypical adults

Authors: *Y. SHIN[1], S. A. COOMBES[1,2], A.-M. D. ORLANDO[3,4], R. A. ROMERO[3,4], D. J. SHIRLEY[1], D. E. VAILLANCOURT[1,2], Z. WANG[1];
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Abstract: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by impairments in social interaction, restricted interests, and repetitive behaviors. Neuroimaging studies of ASD have reported altered cortical anatomy and white matter microstructure using single tensor models. Free-water (FW), a bi-tensor diffusion model, estimates the volume fraction of isotropic diffusion in extracellular space, and can be implemented to overcome partial volume effects that are inherent in conventional diffusion measures. In this study, we examined the effects of ASD and age on grey matter microstructure and white matter transcallosal tracts using conventional single tensor and novel bi-tensor diffusion measures. Middle aged ASD (n = 43) and an age-, sex, and IQ matched neurotypical (NT) participants (n = 43) completed the study. We report two novel findings. First, significant group differences were found in FW across white matter tracts with the ASD group having higher FW. Group effects were not found in white matter for FW-corrected FA measure (fwcFA). No effect of group was found in any measure in grey matter. Significant age effects were found in NT, but not in ASD. Further investigation found that large variability contributed to the weak age effect in ASD, highlighting the well-established heterogeneity in ASD samples. Our observations advance the field by showing that FW is the key factor driving changes in white matter microstructure in middle aged and older autistic adults. Our findings also show that the relationship between age and white and grey matter tissue microstructure is altered in ASD, providing new insight into brain aging in ASD.

Disclosures: Y. Shin: None. **S.A. Coombes:** None. **A.D. Orlando:** None. **R.A. Romero:** None. **D.J. Shirley:** None. **D.E. Vaillancourt:** None. **Z. Wang:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.03/A29

Topic: A.07. Developmental Disorders

Support: Ontario Brain Institute

Title: In vivo neuromelanin imaging in children with neurodevelopmental disorders

Authors: *S. AL-SAOUD, D. SEGUIN, E. G. DUERDEN;
Univ. of Western Ontario, London, ON, Canada

Abstract: Children with autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) can exhibit restricted and repetitive behaviours (RRBs), which are mediated by alterations in dopaminergic and noradrenergic processes. Recent advancements in neuromelanin magnetic resonance imaging (NM-MRI), has offered a unique and non-invasive way to examine dopamine and norepinephrine concentrations in the human midbrain, reflected in neuromelanin signal changes in the substantia nigra (SN) and locus coeruleus (LC).
The present study aimed to characterize RRBs in children with ASD, ADHD, and typically developing (TD) children though in vivo neuromelanin imaging using high-field (3T) MRI. Children with ASD (n=5, 2 males, mean age 12.2 years), ADHD (n=15, 8 males, mean age 11.9 years), and TD children (n=11, 6 males, mean age 8.3 years) were scanned with a modified NM-MRI protocol using a three-dimensional gradient recalled echo sequence with magnetization transfer (MT) contrast (~5 minutes) on a 3T Prisma fit MRI scanner (Siemens, Erlangen, Germany). RRBs were assessed using the Repetitive Behaviors Scale - Revised (RBS-R). Children with ASD had higher restricted interests compared to ADHD and TD children ($p<0.05$), but significant group differences were not observed across the remaining RBS-R scales. A generalized linear model identified participant age and RBS-R scores as significant predictors of SN NM-MRI signal change relative to the crus cerebri (all, $p<0.05$). A significant interaction was observed between participant diagnosis and total RBS-R scores, such that children with ASD who had increased RRBs were associated with higher SN NM-MRI signal changes relative to the crus cerebri ($p<0.05$).
A second generalized linear model identified participant age and RBS-R scores as significant predictors of LC NM-MRI signal change relative to the pontine tegmentum (all, $p<0.05$). A significant interaction was observed between participant diagnosis and RBS-R scores, such that children with ASD who had increased stereotypic behaviour, compulsive behaviour, ritualistic behaviour, restricted interests were associated with higher LC NM-MRI signal changes relative to the pontine tegmentum ($p<0.05$). Another significant interaction was observed between participant diagnosis and total RBS-R scores, such that children with ASD who had increased RRBs were associated with higher LC NM-MRI signal changes relative to the pontine tegmentum ($p<0.05$).
The present study demonstrates the utility of a short NM-MRI protocol in characterizing RRBs in children with and without neurodevelopmental disorders.

Disclosures: S. Al-Saoud: None. **D. Seguin:** None. **E.G. Duerden:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.04/A30

Topic: A.07. Developmental Disorders

Support: K01MH103594

Title: Brain Mapping of Coherent and Scrambled Biological Motion Processing in Autistic School-Age Children Using HD-DOT

Authors: *D. YANG[1], A. M. SVOBODA[2], T. GEORGE[3], P. MANSFIELD[4], M. D. WHEELOCK[2], A. SHERAFATI[4], K. TRIPATHY[4], T. M. BURNS-YOCUM[5], E. FORSEN[6], J. R. PRUETT, Jr.[7], N. MARRUS[4], J. P. CULVER[8], A. T. EGGEBRECHT[9], T. M. BURNS-YOCUM[5];
[1]Washington Univ. Sch. of Med. in St. Louis, saint louis, MO; [2]Washington Univ. In St. Louis, Saint Louis, MO; [3]Washington Univ. in St. Louis, St. Louis, MO; [4]Washington Univ. Sch. of Med., Saint Louis, MO; [5]Radiology, Washington Univ. In St. Louis Sch. of Medic, Saint Louis, MO; [6]Washington Univ. in St. Louis Sch. of Med., St. Louis, MO; [7]Dept Psych-Child, Washington Univ. Sch. Med., Saint Louis, MO; [8]Radiology, Washington Univ. in St Louis, Saint Louis, MO; [9]Radiology, Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: Autism spectrum disorder (ASD), a neurodevelopmental disorder defined by social communication deficitsplus repetitive behaviors and restricted interests, currently affects 1/36 children in the general population.Recent advances in functional brain imaging show promise to provide useful biomarkers of ASD diagnosticlikelihood, behavioral trait severity, and even response to therapeutic intervention. However, current gold-standard neuroimaging methods (e.g., functional magnetic resonance imaging) are limited in naturalisticstudies of brain function underlying ASD-associated behaviors due to the constrained imaging environment.To overcome these limitations, we aimed to establish high-density diffuse optical tomography (HD-DOT), anon-invasive and minimally constraining optical neuroimaging modality, to evaluate brain function in autisticand non-autistic school-age children as they performed a biological motion perception task previouslyshown to yield results related to both ASD diagnosis and ASD-associated behavioral traits. Herein, we usedHD-DOT to image brain function in 46 ASD school-age participants, 49 non-autistic controls (NAC), and 17pro-band siblings as they viewed dynamic point-light displays of coherent biological and scrambled motion.We assessed data quality with stringent thresholds for motion contamination. We assessed group-levelcortical brain function with statistical parametric mapping. Additionally, we tested for brain-behaviorassociations with dimensional metrics of autism traits, as measured with the Social Responsiveness Scale-2, with hierarchical regression models. We found that NAC participants presented stronger brain activitycontrast (coherent > scrambled) than ASD children in cortical regions related to visual, motor, and socialprocessing. Additionally, hierarchical regression models revealed multiple cortical regions in autisticparticipants where brain function is significantly associated with dimensional measures of autism traits (p <0.005) while controlling for age, sex, verbal and non-verbal intelligence, and motion levels in the data. Thisstudy demonstrates that HD-DOT is sensitive to brain function that both differentiates between NAC andASD groups and correlates with dimensional measures of ASD traits. These findings establish HD-DOT asan effective tool for investigating brain function in autistic and non-autistic children and opens the door tofuture studies on brain function underlying natural behaviors.

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Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.05/A31

Topic: A.07. Developmental Disorders

Support: UAB College of Arts & Sciences Faculty Startup

Title: Cortical Thickness Differs Between Central and Peripheral Vision Processing Areas in Autistic Individuals

Authors: *P. DEMIRAYAK[1], R. K. KANA[2];
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Abstract: In the human primary visual cortex (V1), central and peripheral vision are specialized for different functions. The central vision, which corresponds to the fovea, is associated with high acuity and detailed processing, making it useful for reading and object recognition. Peripheral vision encompasses a larger portion of the visual scene but with reduced spatial resolution. Altered fixation behavior and eye movements were reported in Autism Spectrum Disorder (ASD). Specifically, off-center fixations (Pelphrey et al., 2002) and higher variability in saccadic amplitude (Takarae et al., 2004) in the ASD population suggest that precision of eye movements is less reliable in ASD, which might affect the development of visuospatial maps. Due to altered processing in central and peripheral vision, a better understanding of the extent of neuroanatomical alterations in ASD will help to enlighten neurodevelopmental changes in autistic individuals. We investigated the cortical thickness differences in V1 both between autistic and neurotypical (NT) individuals as well as between autistic children and adults. The sample consisted of T1-weighted MRI data from: 27 (age range:18-40 yo) ASD adults, 27 (age range:8-17 yo) ASD children, 33 (age range:18-36 yo) NT adults and 23 (age range:8-16 yo) NT children. Multivariate ANOVA analyses on cortical thickness from Desikan Killiany Atlas areas were performed to assess group differences. This was followed by linear model mixed-effect ANOVA analyses on anatomical ROIs corresponding the 0.5-1.5, 1.5-3, 3-5, 5-8, 8-12, 12-18, 18-28, 28-50^0 eccentricities along dorsal and ventral V1 bilaterally according to Benson atlas (Benson et al., 2014). Given the lack of consistency and accuracy of the eye movement measures in ASD individuals, we hypothesized that cortical thickness would be altered in autistic children and adults in central and far peripheral eccentricities. We found group main effect in whole brain analysis mainly in bilateral parieto-temporal areas. In addition, in V1, we found thinner cortex in the central vision processing section (0.5-1.5^0) in autistic children compared to autistic adults in the left hemisphere. We also found thicker cortex in far peripheral vision processing sections (28-50^0) of V1 in autistic children compared to autistic adults in both hemispheres. Group

differences between autistic and NT individuals were not statistically significant. Overall, these findings support the idea that peculiarities in gaze and stereotyped visual behaviors have the potential to alter cortical thickness in different directions in central vs peripheral vision processing sections of V1 during neural development.

Disclosures: P. Demirayak: None. **R.K. Kana:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Topic: A.07. Developmental Disorders

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Title: Widespread alterations of functional brain networks in children with autism: An MVPA analysis

Authors: *A. L. FARMER[1], M. D. PRIGGE[2], S. NELSON[3], C. TRAISER[3], E. D. BIGLER[4], B. A. ZIELINSKI[3];
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Abstract: Autism spectrum disorder (ASD) is a relatively common neurodevelopmental disorder estimated to affect 3% of children in the United States and 1% of children worldwide. Despite decades of research, the underlying neurocircuitry of ASD remains poorly understood. We used a multivariate pattern analysis (MVPA) to identify differences in functional connectivity between a predominantly male sample of 47 ASD and 56 typically developing (TD) children aged 3-13 years old and examine functional connectivity differences associated with ASD severity as measured by Autism Diagnostic Observational Schedule (ADOS-2) total calibrated severity scores (CSS). fMRI data were preprocessed and analyzed in Conn Toolbox. All analyses were conducted using threshold free cluster enhancement and family-wise error corrected $p < 0.05$ for multiple comparisons. After correcting for sex, age, and performance IQ (PIQ), the whole-brain MVPA identified 34 significant clusters that collectively covered most of the brain. Seeds for subsequent seed to voxel analyses were derived from the five largest brain regions from each MVPA cluster. Seed to voxel analyses revealed reduced anticorrelation between default mode (DMN) and salience network seeds and brain regions in multiple resting state networks in children with ASD. Decreased connectivity in ASD was also observed between seeds in the salience network and brain regions in the sensorimotor network and dorsal attention network (DAN), as well as between frontoparietal network seeds and DAN, visual, sensorimotor, salience, and cerebellar network regions. After correcting for age, sex, and PIQ, a whole-brain MVPA analysis identified six clusters significantly correlated with ADOS-2 CSS scores. These included clusters in the anterior DMN regions; left temporal regions; a cluster in the left visual,

fusiform cortex, and hippocampal/parahippocampal regions; a cluster in the left nucleus accumbens and caudate, as well as clusters in the right amygdala and left temporal fusiform cortex. Subsequent seed to voxel analyses revealed that symptom severity was associated with hyperconnectivity between anterior DMN regions and a large portion of the brain encompassing all resting state networks. Symptom severity was also associated predominantly with hyperconnectivity between MVPA clusters and the cerebellum, hippocampus/parahippocampus, striatum, thalamic pulvinar nucleus, precuneous/posterior cingulate cortex, insular cortex, and frontal orbital cortex. Our results suggest that ASD and symptom severity in children is associated with widely distributed multifocal network dysfunction, particularly within anterior DMN regions.

Disclosures: A.L. Farmer: None. **M.D. Prigge:** None. **S. Nelson:** None. **C. Traiser:** None. **E.D. Bigler:** None. **B.A. Zielinski:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Title: Age-related decline in expressive language and related brain structures among autistic adults

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Abstract: Introduction: It is known that as one ages, cognitive function declines. However, cognitive aging research on adults with Autism Spectrum Disorder (ASD) is scant. Verbal fluency is a language-based executive function that is a challenge for many autistic individuals, and negatively affected by normal age-related processes. There is some cross-sectional evidence that brain regions supporting verbal fluency may experience accelerated cortical thinning in autistic adults compared to neurotypical (NT) controls, but they longitudinal trajectories of verbal fluency abilities and related brain aging are unknown.
Methods: Right-handed autistic (ASD; n=125) and neurotypical (NT; n=95) adults, ages 18 to 70 years old, were recruited for cross-sectional analyses. Participants over the age of 40 were

invited to participate longitudinally with evaluations every two years (ASD, n=55; NT, n=45; follow-up duration=2.31±0.43 (1.24-3.71) years). Commonly used behavioral measures of phonemic (i.e. letter) and semantic (i.e. category) word production and cortical thickness of language-related left hemisphere areas (pars opercularis, pars triangularis, superior parietal, supramarginal, transverse temporal and anterior cingulate cortex) via freesurfer were obtained. Group differences and associations with age were investigated in fluency behavior and cortical thickness with both cross-sectional and multi-level longitudinal regression models.
Results: In the cross-sectional analysis for fluency behavior, autistic adults demonstrated persistent challenges in initiating phonemic (p=0.026) and maintaining semantic (p=0.004), but there were no diagnosis group by age interactions. For the brain, there was a diagnosis group by age interaction in the left pars opercularis where autistic adults showed a steeper negative relationship with age than NT adults (p=0.002). In the longitudinal models, no significant aging (i.e. time) effects were observed for fluency behavior or cortical thickness.
Discussion: Behavioral findings from the present study suggest that autistic adults across a wide age range have persistent difficulties with verbal fluency production, but that these abilities may not change differently from NT adults during aging. Findings from cross-sectional cortical thickness analyses suggests some vulnerability to accelerated aging in the key language production region, Broca's area, but this was not corroborated in longitudinal analyses. More research is needed to determine whether cortical language areas may be vulnerable to accelerated aging in autistic adults and implications for maintaining independence as aging ensues.

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Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Topic: A.07. Developmental Disorders

Support: NIH/NIDCD K23 DC016639
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Title: White Matter Microstructural Differences in Adolescent Males with Fragile X and Autism Spectrum Disorder

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Abstract: Fragile X syndrome (FXS) and idiopathic autism spectrum disorder (ASD) share similar behavioral phenotypes. Roughly half of FXS individuals also meet the diagnostic criteria for ASD. In this study, diffusion MRI (dMRI) was used to investigate white matter (WM) microstructural differences between adolescent males with either FXS or ASD. We used diffusion tensor imaging (DTI) and Neurite Orientation Dispersion and Density Imaging (NODDI) methods to characterize WM microstructure using Tract-Based Spatial Statistics (TBSS). Twenty-three subjects (10 FXS, 13 ASD), ages 9-18 years, all male, were scanned on a 3T GE MR750. Anatomical T1w MPnRAGE images (1-mm isotropic resolution) and dMRI images at b = 0, 350, 800, 2000-s/mm^2, with 5, 8 19, 29 directions, respectively (2.0-mm isotropic resolution) were acquired. Images were corrected for motion, geometric distortions, noise, B1-bias, and Gibbs ringing. Following affine alignment and upsampling to the T1w image, DTI and NODDI parameter maps were obtained. A study-specific template was made using ANTs. Subjects were aligned to generate a mean fractional anisotropy (FA) template. A mean FA skeleton was extracted to minimize inter-subject variability. Finally, subject-specific FA images were aligned and projected onto that skeleton. This process was repeated for metrics other than FA, mapping the value of the track centers for different diffusion metrics to the FA skeleton for consistency. Statistical testing was performed voxel-wise using Permutation Analysis of Linear Models (PALM) accounting for age to identify group differences, correcting for multiple comparisons at $p<0.05$. There were statistically significant group differences for DTI mean diffusivity (MD), where FXS MD was higher compared to ASD MD for all age-corrected significant regions ($p<0.05$, FWE-corrected). After alignment to the JHU DTI White Matter Atlas, major overlapping significant regions included: body and splenium of the Corpus Callosum, left Posterior Limb of the Internal Capsule, right / left Superior and Posterior Corona Radiata, right posterior Thalamic Radiation and Optic Radiation, and the Temporal Superior Longitudinal Fasciculus. MD is inversely related to cellular density and could indicate lower WM integrity for FXS than ASD in these regions. This is the first study to investigate WM microstructural comparisons between FXS and ASD with DTI and NODDI. These dMRI measures highlight potential markers of difference between the groups and may indicate leads to better understand ASD etiology. Future work will expand the population sample sizes for greater power and incorporate gray matter structural associations.

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Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Topic: A.07. Developmental Disorders

Support: US army grant W81XWH-22-1-0536

Title: Sexual Differences in Cerebellar Activity and its Relationship to Sexual Differences in Autism Spectrum Disorder

Authors: *D. PARIENTE[1], Y. YAROM[2];
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Abstract: Sexual Differences in Cerebellar Activity and its Relationship to Sexual Differences in Autism Spectrum Disorder Daniel ParienteThe etiology of autism spectrum disorder (ASD) has puzzled medical researchers for several decades. A strong male bias in ASD prevalence has been observed with striking consistency, but no mechanism has yet to definitively account for it. Furthermore, although it has a strong genetic basis, only ~160 of statistically validated genes are currently considered high-confidence risk genes for ASD, while only few of them show sex differences in behavior. We focus on genetic model mice with heterozygous Pogz (POGO transposable element with ZNF domain) mutation, that is a high-confidence risk gene for ASD and our previous work showed sex differences in motor-related behavioral tests. Expression analysis showed that in the developing cerebellum Pogz is expressed dominantly in the nucleus of Purkinje cells, whereas in the granular and molecular layers, Pogz expression was observed at P15 and P30 (Suliman-Lavie et al. 2020). Therefore, we decided to focus on Purkinje cells electrophysiological activity while comparing between males and females, both in WT mice and in $Pogz^{+/-}$ mice using a multi-electrode array. Our preliminary data suggest that the female Purkinje cells simple spike rate has a significantly higher VMR values, indicating its activity is less regular and more of the cells are classified as bursters. But other comparisons we performed were not statistically significant due to big standard deviations. The cerebellum is organised in parasagittal bands of expression of different molecules, one of which is Aldolase C (Aldoc) (Eisenman and Hawkes, 1993). There is a known difference in the electrophysiological properties of Aldoc positive and negative Purkinje cells (Zhou, Haibo, et al. 2014). Therefore, we decided to use Aldoc-tdTomato mice to verify the recording location and compare the firing activity of Aldoc-positive neurons between males and females both WT and $Pogz^{+/-}$, at first in anesthetizes mice and later in awake mice. Our working hypothesis is that sex differences in cerebellar activity can account for sex differences in ASD.

Disclosures: D. Pariente: None. **Y. Yarom:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Topic: A.07. Developmental Disorders

Support: NINDS Grant R01AG086493
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Title: Static and dynamic postural control deviations in autistic adults ages 30-73 years

Authors: *H. M. GEMMELL[1], D. SHIRLEY[1], J. WANG[1], A.-M. ORLANDO[2], R. ROMERO[3], Z. WANG[4];
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Abstract: Autism spectrum disorder (ASD) persists throughout the lifespan, profoundly affecting individuals' health, independence, and quality of life. Recent epidemiological studies have identified strikingly high rates of comorbid neurodegenerative diseases in middle-to-old aged autistic adults, including an estimated prevalence of Parkinsonism as high as 25% compared to 0.9% of the general population. Postural control is a fundamental gross motor skill that supports goal-directed movements and predicts the onset and development of neurodegenerative conditions. Previous studies have identified postural control deficits in autistic children, implicating the basal ganglia circuits. The current study aimed to quantify postural control variability and complexity deviations in middle-to-old aged autistic adults during static and dynamic stances and further assess the relationship between compromised postural control and quality of life in autistic adults. 45 autistic adults ages 30-73 and 33 age-, sex-, and IQ-matched neurotypical controls completed static stance and postural sway trials in both the anterior-posterior (AP) and mediolateral (ML) directions on a force platform. Postural sway variability was quantified using the center of pressure (COP) trajectory length and COP standard deviation in the AP (COP_{AP}) and ML (COP_{ML}) directions. Postural sway complexity was quantified using the mutual information of COP_{AP} and COP_{ML} and detrended fluctuation analysis (DFA) of each COP time series. We anticipate autistic adults would show increased COP length and COP standard deviation in both directions relative to controls. We also expect increases in mutual information and reductions in DFA in autistic adults during both task conditions. Additionally, autistic adults with increased postural variability would show significant reductions in quality of life, and demographic and clinical severity confounds would show prominent effects on postural control deviations in ASD. Our data analysis is ongoing. We plan to present preliminary findings at the 2024 Annual Conference of the Society for Neuroscience.

Disclosures: H.M. Gemmell: None. **D. Shirley:** None. **J. Wang:** None. **A. Orlando:** None. **R. Romero:** None. **Z. Wang:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

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Topic: A.07. Developmental Disorders

Support: Fondation John Bost Individualisée Recherche

Title: Encoding strategy of vocal and musical sounds in autism

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Abstract: Autism Spectrum Disorder (ASD) is an early onset neurodevelopmental disorders defined by impaired social interaction and communication, and restricted and repetitive patterns of behavior. A broad impairment in voice perception may be at the heart of the communication impairment seen in autism with intrusive repercussions on everyday life. In typically developed adults, voices are encoded in reference to a stored gender-specific prototype. Yet, whether this encoding strategy is voice-specific, and whether a similar strategy is used by people with ASD remained unexplored yet fundamental questions. Here, we used a passive oddball paradigm with conjoint electroencephalographic (EEG) recordings to evaluate the encoding of voices. In the vocal sequence, the frequent stimulus was a natural recording of a male voice, and the deviant stimuli were a natural recording of a female voice and an atypical vocal sound, comprising the male fundamental frequency and the female spectral content. The musical oddball sequence includes the presentation of a clarinet sounds as the standard, and the sound of a saxophone and an atypical musical sound (combining the clarinet's fundamental frequency with the saxophone's timbre) as deviants. All sounds were edited to last 200ms. The mismatch negativity evoked by the deviant stimuli were measured in 21 typically developed and 17 autistic adults. A significant three-way interaction was observed on MMN latency, owing to a delayed MMN in response to atypical stimuli in autistic adults for both vocal and instrumental sounds, while MMN was delayed only for atypical musical sounds in neurotypical adults. MMN amplitude was not affected by group. While previous studies reported a modulation of MMN amplitude with voice typicality in magnetoencephalography, we found an effect on latency. We expected different modulations of ERPs parameters based on typicality to reflect a prototype-based encoding strategy. Accordingly, the results would suggest that a lack of difference between typical and atypical voice stimuli in TD adults suggest a specific encoding of vocal sounds that is not observed for musical sounds. In autistic adults, atypicality affected vocal and musical sounds in the same way suggesting that they were processed similarly and likely not in a reference to a norm.

Disclosures: M. Latinus: None. **F. bonnet-brilhault:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: DoD AR150091

Title: Pupillary Light Reflex as a Biomarker of the Response to Propranolol for Anxiety in Autism Spectrum Disorder

Authors: *J. BAILEY[1], M. J. PRENDERGAST[2], C. APPLING[1], B. FERGUSON[3], D. Q. BEVERSDORF[4];
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Abstract: Core features of autism spectrum disorder (ASD) include social-communication deficits and restrictive-repetitive behavior. In addition, anxiety disorders commonly co-occur in people with ASD. Researchsuggests that the noradrenergic system, which is altered in anxiety, may be developmentallydysregulated in ASD. Due to these factors and recent evidence suggesting social and language benefitsfrom the beta-adrenergic antagonist propranolol in ASD, we have become highly interested in how thenoradrenergic system also affects anxiety in this population. Furthermore, examining potentialbiomarkers that may be associated with the treatment response to anxiety may lead to more precisetreatments in those with ASD. One such biomarker of interest is pupillary light reflex (PLR), whichmeasures the dynamic changes in pupil size induced by optical luminance changes. PLR has been shownto be altered in ASD and is also sensitive to both noradrenergic functioning and anxiety. The presentstudy involved a randomized controlled trial of propranolol in participants with ASD. To identify patientsmost likely to report reduced anxiety to the beta-adrenergic antagonist propranolol, we examined ifbaseline PLR parameters, which have been shown to be attenuated in anxiety, predicted the anxietyresponse to propranolol. We hypothesized that PLR parameters (e.g., relative constriction amplitude,constriction latency) in response to a brief pulse of light would be significantly altered after the 12-weekpropranolol administration. We also hypothesized that baseline PLR latency would be correlated withsignificant improvements on clinician-rated Clinical Global Impact Measure (CGI) for anxiety. A total of 69participants, aged 7-24, completed the trial and the majority had valid PLR data. All participants metcriteria for ASD, were native English speakers, and were verbal. Participants were not taking anymedications known to interact with propranolol or adrenergic functioning. Analyses are currentlyongoing from a pilot sample of the participants who completed the trial and have valid PLR data. Furtherinvestigation into the prediction of treatment response to propranolol using PLR is of interest for futureindividualized treatment in ASD.

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Poster

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NIH Grant P30 DK3498
NIH Grant R21 MH104330

Title: Association of Salivary Metabolites with Features of the Gut Microbiome in Infancy

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Abstract: Background: We do not know the mechanisms linking microbiome composition to behavior. Broad-spectrum metabolomics represents a powerful way of identifying molecules that may mediate relationships between infant gut microbiome and behavior. In the current study, we examined the relationship of salivary metabolites with measurements of infant gut microbiome at 1-month and 1-year of age. **Study Design**: *Data Analysis:* Raw data was extracted, peak-identified and QC processed using Metabolon's hardware and software. Raw area counts were normalized by osmolality and volume of the saliva sample, rescaled to set the median equal to 1, and missing values were imputed with the minimum. Additionally, metabolites whose variance equal 0 and outliers were removed from the data set. Principal Component Analysis (PCA) was used to reduce the metabolomic data set into several components and linear models were used to determine associations between measurements of the gut microbiome and the top 3 salivary principal components. **Results**: *For the Neonate dataset***,** 22 metabolites positively associated with salivary principal component 1, 2 and 3 while 8 metabolites were negatively associated. Salivary principal component 1 was positively associated with Faith's phylogenetic diversity and negatively associated with weighted unifrac principal coordinate 1. *For the one-year-olds*, 16 metabolites were positively associated with principal components 1,2 and 3 while 14 metabolites were negatively associated. In the linear models, salivary principal component 1 was negatively associated with Shannon diversity, a measure of evenness, and positively associated with weighted unifrac principal coordinate 1. Salivary principal component 3 was positively associated with weighted unifrac principal coordinate 1.**Conclusion/Future Directions:** The current analysis identified associations between certain features of the gut microbiome and salivary metabolomic profiles, represented by top principal components, in early and late infancy. Results add to a growing body of research showing associations between the metabolome and the microbiome in infancy, which likely reflects several factors including the influence of nutrients on the diversity and composition of the microbiome, production of metabolites by the microbiome, and regulation of host metabolism by the microbiome. Our next step will be to test for associations between salivary principal components and fear reactivity.

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Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Program #/Poster #: PSTR202.14/A40

Topic: A.07. Developmental Disorders

Support: NIDCD R01 5R01DC016303-04

Title: The brain's pragmatic language network during a reading comprehension task in autistic children

Authors: *E. VALLES-CAPETILLO, M. KURTZ, R. K. KANA;
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Abstract: One of the earliest symptoms usually reported in children with Autism Spectrum Disorder (ASD) is a delay in language1, which can have a long-term impact on reading comprehension (RC). While most studies of reading have focused on decoding and comprehension, some suggest there is a relationship between reading and pragmatics3-5. Pragmatic language, the communicative use of language,6 is affected in ASD7. Yet, its underlying neural mechanisms and its relationship with reading are sparsely examined in ASD. The goal of this functional Magnetic Resonance Imaging (fMRI) study is to investigate differences in the brain's pragmatic network (PN) during two reading tasks in ASD and neurotypical (NT) children. Twenty-five ASD and fifteen NT children (8-13 years) participated in the study. Four task fMRI analyses, within the PN, were performed during the reading tasks (verbal absurdity and multi sentence): 1) a General Lineal Model ($z = 2.3$ $p < 0.05$); 2) % of signal change (Mann-Whitney U test) to assess the group differences in brain activation; 3) a Pearson correlation to determine the relationship between % signal change and RC, measured by the Gray Oral Reading Test (GORT-4); and 4) a Psychophysiological Interaction analysis to assess connectivity ($z = 2.3$ $p < 0.05$) of the Regions of Interest (ROIs) that overlap between the PN and RC (the bilateral Inferior Frontal Gyrus (IFG) and posterior Superior Temporal Gyrus (pSTG)). Results showed: 1) Increased activation in both groups in the PN, i.e., IFG, posterior middle temporal gyrus (pMTG) and premotor + supplementary motor area. ASD also recruited the fusiform gyrus (FFG), frontal eye fields (FEF), pSTG, angular gyrus, insula, supramarginal gyrus (SMG), anterior cingulate cortex, and the medial prefrontal cortex (mPFC). 2) The ASD group, relative to NT, demonstrated significantly greater % of signal change in the PN. 3) The % of signal change showed a negative relationship with RC in the ASD. 4) There was also increased connectivity of the ROIs (IFG, pSTG) with the mPFC. In conclusion, although both groups recruited the PN, ASD participants engaged additional PN areas that overlap with language (e.g., FFG, FEF and insula)8, integrating linguistic information into context (e.g, SMG) 9,10, and theory of mind (ToM; e.g., mPFC)11. Only the ASD showed a relationship between % of signal change and RC and increased connectivity between the IFG and pSTG, with the mPFC which is associated with ToM 11. This study may reflect the involvement of the PN during RC,

and that ASD, given some difficulties in these skills, may need to recruit additional areas of the PN.

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Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

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Topic: A.07. Developmental Disorders

Support: McKnigtht Scholar Awward, Rita Allen Scholar Award,Kingestein-Simons Fellowship Award in Neuroscience

Title: Insights into susceptibility and resilience to neurodevelopmental disorders from a mouse model of maternal immune activation

Authors: *I. SANCHEZ MARTIN[1], Q. LIN[2], D. DIMARTINO[2], C. KANG[2], A. FERRO[3], J. A. KAHNG[3], L. CHEADLE[4];
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Abstract: Viral infections in mothers during pregnancy elicit deficits in neurodevelopment leading to disorders such as autism in the offspring. While the association between maternal inflammation and neurodevelopmental dysfunction has been well-documented in the clinic and can be modeled effectively in mice via the Maternal Immune Activation (MIA) paradigm, the vast majority of studies in the model to date have focused on understanding changes observed in the postnatal brain and mouse behavior. Therefore, the explicit mechanisms linking a single, isolated prenatal inflammatory event to neurodevelopmental disorders remain fuzzy. One major gap in knowledge is why some offspring appear to be sensitive to the impact of prenatal inflammation while others are protected. To address this question, we harnessed the MIA mouse model in which the viral mimetic poly(I:C) is injected into pregnant mice at E12.5, as this model has been shown to induce robust phenotypes in adult mice reminiscent of the symptomology of autism in humans. However, instead of assessing the impact of inflammation on the offspring after birth, we analyzed the effects of prenatal inflammation on embryos 24 hours after the induction of inflammation. We observed teratogenic effects on large-scale aspects of fetal development in a subset of embryos. For example, some fetuses in MIA mice exhibited relatively normal development while others were in the process of breakdown and re-absorbtion, and still others exhibited less extreme but still obvious developmental impairments. These effects became apparent as early as 6 hours post-treatment, indicating an innate versus an adaptive response. Remarkably, we also discovered that the position within the uterus does not influence the severity of the phenotype, however sex played a powerful role: whereas all female embryos in

the MIA model were protected from deficits in fetal development, a third of the male embryos were strongly affected. Transcriptomic and proteomic data indicate that these large-scale and heterogeneous effects of prenatal inflammation on embryonic development are tightly coupled to inflammatory break-down in the placenta as well as increased cytokine expression in the amniotic fluid surrounding the fetus. Finally, initial analysis of microglial organization in the brains of affected male fetuses revealed the aberrant clustering of microglia resulting from inflammation, emphasizing the intricate relationship between immune activation and neurodevelopmental outcomes. These findings shed light on the biological processes underlying neurodevelopmental disorders, offering potential new avenues for their diagnosis and treatment.

Disclosures: I. Sanchez Martin: None. **Q. Lin:** None. **D. DiMartino:** None. **C. Kang:** None. **A. Ferro:** None. **J.A. Kahng:** None. **L. Cheadle:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.16/A42

Topic: A.07. Developmental Disorders

Support: R01 MH097949-01

Title: Elucidating the role of pten in regulating electrical activity in asd brain

Authors: *A. ABDULKAREEM;
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Abstract: Pten is tumor suppressor and regulatory antagonist of PI3K/Akt/mTOR pathway that is involved in cellular differentiation and growth. Loss of function mutation in Pten is one of the most common genetic aberrations associated with autism spectrum disorder (ASD), which is an increasingly diagnosed neurodevelopmental disorder. Pten depleted neurons have shown an increase in soma size, dendritic arborization, migration, and hyperexcitation. Knocking out Pten in granule neurons of dentate gyrus in mice has resulted in an increased burst-firing phenotype as well as a smaller fast AHP (after hyperpolarization) of the action potentials. While burst-firing accomplishes numerous functions in the brain such as emotional regulation, release of neurotransmitters and other peptides, abnormal bursting has been detected in various neuropathies. Furthermore, over 30% of ASD patients have epilepsy and seizures, and the link between seizures and burst-firing is still very ambiguous. Therefore, understanding the underlying mechanism of such electrical phenomenon can help shed light on the ionic dysregulation detected in a slew of neurodevelopmental and electrophysiological disorders. We hypothesize that Pten plays critical roles in regulating neuronal electrical activities through downstream intermediates that might directly or indirectly affect the function of voltage-gated ion channels as they are the primary mediators of neuronal excitability. Through genetic manipulation and pharmacological targeted inhibition, we evaluate changes in the ionic activities

and determine possible signaling pathways involved in burst-firing. Identifying the underlying cause of neuronal hyperexcitability in these neurons and understanding the ionic mechanism will allow a deeper insight into the electrical activity of the brain and it will also help in identifying better therapeutic targets to halt electrical abnormalities in inflicted patients.

Disclosures: A. Abdulkareem: None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.17/A43

Topic: A.07. Developmental Disorders

Title: Autism as a Disorder of Synchronization of the Central and the Autonomic Nervous Systems

Authors: *S. BEKER[1], T. VANNEAU[2], S. MOLHOLM[2];
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Abstract: Despite intensive research on Autism Spectrum Disorder (ASD), the mechanisms that underlie the cognitive and behavioral atypicalities that characterize this condition are not well understood. Accumulating evidence points to altered synchronization of brain oscillations (central nervous system; CNS), and atypical regulation of body signals by the autonomic nervous system (ANS) in ASD. Furthermore, there is evidence that the typical alignment of CNS activity with temporally predictable environmental events is reduced in this population. We hypothesize that the cognitive and behavioral rigidity and impaired social communication that characterizes ASD results from impaired synchronization between CNS and ANS physiological activity and the physical and social environments. However, to the best of our knowledge, ANS synchronization with the physical environment or within dyads have not yet been rigorously tested in ASD. Here we address these significant gaps in knowledge and show preliminary evidence for altered synchronization of ASD with the social and physical environment. In different studies, we test the alignment of electrophysiological signals from the CNS (measured by EEG) or from ANS (measured by cardiac activity and skin conductance response) with stimuli (physical environment), and with other humans (social environment), from individuals with ASD. This line of research set the stage for establishing an account of altered CNS/ANS synchronization with the environment and, as a long term goal, for establishing tractable, stable biomarkers of ASD.

Disclosures: S. Beker: None. **T. Vanneau:** None. **S. Molholm:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.18/A44

Topic: A.07. Developmental Disorders

Support: CIHR
NSERC

Title: Trajectory of auditory brainstem development in autism

Authors: *A. SEIF[1], S. SCHMID[3], R. A. STEVENSON[2];
[2]Psychology, [1]Univ. of Western Ontario, London, ON, Canada; [3]Univ. of Western Ontario Grad. Program In Neurosci., London, ON, Canada

Abstract: *Introduction*Autistic children often display sensory sensitivities, sensory seeking/avoidance behaviors, and a range of other issues related to auditory sensory processing disruptions. It has been shown that autistic individuals present abnormal cortex activation when processing acoustic stimuli and it is proposed that the brainstem is involved in that process. We have hypothesized that autistic children display delays in auditory brainstem maturation, which has been structurally observed by neuroimaging and post-mortem analysis. Functionally, this delay is measured through the auditory brainstem responses (ABR), an auditory evoked potential recorded through electrodes on the scalp.
*Objective*To investigate brainstem development of autistic children indexed via ABR
*Methods*Autistic (n = 43) and non-autistic (n = 72) children and adults completed a hearing evaluation and an ABR recording session. The hearing evaluation consisted of a visual inspection using an otoscope, tympanometry (226Hz probe), audiogram (250-8kHz), and distortion product otoacoustic emission evaluation (2kHz-8KHz). The ABR paradigm consisted of a slow click-rate (19.1clicks/second) and a fast click-rate (59.1clicks/second), and acoustic stimuli at 80dBnHL intensity across both ears. Participants listened passively while watching a silent video.
We conducted three-way, mixed-model ANOVAs with within-subject factors of stimulus click-rate, and a between-subject factor of diagnostic group, age group and sex. Absolute latencies and amplitude of peaks I, III, and V were evaluated.
*Results*The overall ABR waveform showed a trend towards prolongation in the autistic relative to the non-autistic group. Simple main effects of group were observed for peak I ($F_{(1,93)} = 4.109$, $p = 0.046$). The was an interaction of group and age for peak V latency. Autistic children ($p = 0.0032$) and adolescents ($p = 0.004$) had a shorter latency compared to their age matched counterparts. In regards to amplitude, there was a significant main effect of group on peak III amplitude ($F_{(1, 86)} = 4.898$, $p = 0.030$).
ConclusionAutistic participants showed a prolonged ABR waveform with increased latency of peak III which is indicative of reduced conduction speed of neural conduction. However, some of the differences are limited to autistic children and not adults, supporting the hypothesis of a delay in auditory brainstem development.

Disclosures: A. Seif: None. **S. Schmid:** None. **R.A. Stevenson:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.19/A45

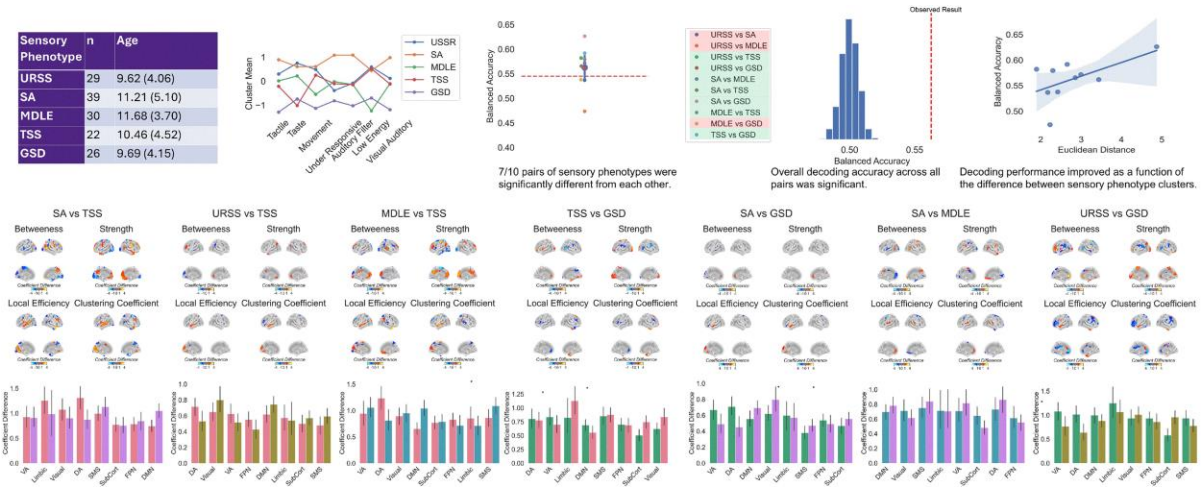
Topic: A.07. Developmental Disorders

Support: Ontario Brain Institute
CIHR Project Grant (Stevenson)
SSHRC Insight Grant (Stevenson)
NSERC Discovery Grant (Stevenson)

Title: Differences in fuctional neural networks across sensory phenotypes in Autism

Authors: *R. A. STEVENSON[1], M. KOLISNYK[2], B. STOJANOSKI[3], H. LIANG[4], E. CHOI[5], E. ANAGNOSTOU[6], R. NICOLSON[4], S. GEORGIADES[7], E. KELLEY[8], K. M. LYONS[1]; [1]Psychology, Univ. of Western Ontario, London, ON, Canada; [2]Western Univ., London, ON, Canada; [3]Ontario Technol., London, ON, Canada; [4]Univ. of Western Ontario, London, ON, Canada; [5]Dept. of Psychology / Fac. of Educ., Univ. of Western Ontario, London, ON, Canada; [6]Pediatrics, Univ. of Toronto, toronto, ON, Canada; [7]McMaster, Hamilton, ON, Canada; [8]Queen's Univ., Kingston, ON, Canada

Abstract: Autistic individuals report sensory differences across modalities. While symptoms vary across individuals, we recently identified five distinct sensory phenotypes that differed in behavioral and clinical profiles. The neural mechanisms underlying sensory phenotypes in autism are unknown. We used resting-state functional connectivity to examine neural differences between sensory phenotypes in Autism. Data were extracted from the Province of Ontario Neurodevelopmental Disorders Network. 638 Autistic participants' (Mage=9.8) parents completed the Short Sensory Profile (SSP). K-means clustering analyses grouped participants patterns of SSP subdomains. Five phenotypes were identified, 1) sensory adaptive, 2) generalized sensory differences, 3) taste/smell sensitivity, 4) under-responsive/sensory seeking, and 5) movement difficulties. We analyzed resting-state fMRI data in a subgroup of participants (N=147, Mage=11.8). We parcellated the brain based on the Schaefer Atlas and calculated functional-connectivity matrices for each participant. We calculated strength of connectivity across 7 functional networks from the Yeo parcellation. Pairwise comparisons for strength of within- and between-network connectivity were conducted across each phenotype ($p<0.05$, FRD corrected). Machine-learning algorithms were used to identify brain regions with the greatest ability to differentiate sensory phenotypes. Numerous differences in network connectivity were observed across phenotypes, including differences in limbic, default-mode, visual, and sensorimotor networks, including selective hyper- and hypo-connectivity. These results suggest that these distinct sensory phenotypes are associated with broad differences in the brain's functional architecture, not only in low-level sensory networks, but also networks associated with higher-level cognitive processes. This reflects findings over the past decade that have shown that sensory differences cascade to influence higher-level cognitive development.



Disclosures: R.A. Stevenson: None. **M. Kolisnyk:** None. **B. Stojanoski:** None. **H. Liang:** None. **E. Choi:** None. **E. anagnostou:** None. **R. Nicolson:** None. **S. Georgiades:** None. **E. Kelley:** None. **K.M. Lyons:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.20/A46

Topic: A.03. Stem Cells and Reprogramming

Title: Generation and characterization of a novel human pluripotent stem cell derived medial ganglionic eminence brain organoid model

Authors: *M. C. VARELA[1], M. WALKER[2], L. GOLDSTEIN[2], J. BOK[2], J. FU[2], M. D. UHLER[3], J. M. PARENT[4];
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Abstract: Inhibitory interneurons (INs) are important regulators of brain network activity and predominantly arise from the ganglionic eminences during development and migrate into the cortex. Dysfunction of IN's arising from the medial ganglionic eminence (MGE) have especially been implicated in neuropsychiatric and developmental disorders, including epilepsy. Precise temporal exposure to patterning factors and a delicate balance of WNT and sonic hedgehog signaling is required to produce ventral telencephalic fates, including the formation of the MGE. Developing *in vitro* brain organoid models that strongly express markers predominantly specific for the MGE, however, has been notoriously difficult in the brain organoid field. We hypothesized that systematic manipulation of concentration and timing of patterning factors will produce brain organoids that strongly resemble the developing MGE. To test this hypothesis, we

manipulated the timing and concentrations of WNT inhibition and SHH pathway activation in our single rosette organoid model over the span of several days to identify the optimal protocol that generated the most MGE-like organoids. We validated our protocol using three different human pluripotent stem cell (hPSC) lines derived from human embryonic stem cells, human blood samples, and human fibroblasts. Both males and female lines were used in this study. We characterized MGE-like IN development and specification in our model at various timepoints ranging from 18-250 days in vitro (DIV) using scRNAseq, RT-qPCR, immunostaining, and functional assays including MEA and patch clamp recordings. Our protocol generated MGE-like organoids that robustly expressed MGE markers including NKX2.1, LHX6, PV and SST. Upon fusion with cortical patterned organoids, we observed rapid and extensive migration of inhibitory neurons from the MGE organoid into the cortical organoid occurring within less than one-week post-fusion. Long term cultures of assembloids produced electrophysiological signals with robust synchronous network level activity which was altered by the application of bicuculine. Our MGE-specific organoid model will broadly benefit the brain organoid field by providing a valuable tool to study IN development and for modeling IN-related phenotypes in epilepsy, neurodevelopmental and neuropsychiatric disorders.

Disclosures: M.C. Varela: None. **M. Walker:** None. **L. Goldstein:** None. **J. Bok:** None. **J. Fu:** None. **M.D. Uhler:** None. **J.M. Parent:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.21/A47

Topic: A.07. Developmental Disorders

Support: Institute of Information & communications Technology Planning & Evaluation (IITP) grant funded by the Korea government (MSIT) (No. 2022-0-00234)

Title: Subclassifying autism spectrum disorder: mapping joint attention behaviors to neural connectivity using a graph neural network

Authors: *C. KO, D. SEO, Y. PARK;
Dept. of Biomed. Systems Informatics, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Neurodevelopmental conditions like autism spectrum disorder (ASD) exhibit diverse phenotypes and complex neurobiologies, making traditional diagnostic approaches inadequate. Our study employs data-driven methods, using computer vision and deep learning to analyze behavioral video data focused on joint attention, a key behavioral trait. We collected video data and resting-state functional MRI (rs-fMRI) from 27 participants to explore neural connectivity. Using a graph neural network (GNN) model pretrained on ABIDE data, we analyzed rs-fMRI data dynamically adjusted for BOLD signals' timing and frequency. Our analysis revealed distinct neural connectivity patterns corresponding to the behavioral clusters. In our study, we

identified distinct network patterns across three clusters differentiated by their behavioral profiles in joint attention tasks. Cluster 1, marked by the poorest initiation of joint attention, exhibited the highest segregation (Clustering Coefficient: 0.522) with notable disconnection at key hubs 'Right Anterior Intraparietal Sulcus' and 'Right Frontopolar Cortex' (Eigenvector Centrality: 0.164, 0.161), suggesting impaired neural connectivity. Cluster 2, which showed joint attention behaviors similar to those of typical development, demonstrated moderate integration and high segregation (0.502); 'Right Anterior Insula' (Betweenness Centrality: 0.0014) served as an essential but isolated bridge, indicative of specialized processing. Cluster 3, with a low response in joint attention tasks, also displayed high segregation (0.500), with 'Cingulate Cortex' and 'Right Frontopolar Cortex' acting as critical but isolated connectors, pointing to weaker, localized connections. These findings underscore that integration and segregation degrees within neural networks correspond closely with behavioral performances, revealing potential neural markers for ASD subclassification based on social cognitive functions. Our results indicate that specific neural connectivity profiles correlate with distinct behavioral phenotypes in ASD. Integrating behavioral data with neural imaging enhances our understanding of ASD's neurobiology and supports more targeted interventions based on precise behavioral-neurobiological correlations. This approach advances ASD subclassification and treatment strategies.

Disclosures: C. Ko: None. **D. Seo:** None. **Y. Park:** None.

Poster

PSTR202: Structural, Physiological and Behavior Defects in Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR202.22/Web Only

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Autism and higher levels of BDNF was associated with rs6265 polymorphism but not Val66Met polymorphism of BDNF gene

Authors: *M. SHAF'EI[1], O. IHMOUD[4], L. ALZGHOUL[2], S. HAYEK[4], S. ALQUDAH[5], S. DABABSEH[5], N. ABU TARBOUSH[3], Q. ALSHAWABKEH[5];
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Abstract: Autism spectrum disorder (ASD) is a developmental condition marked by social interaction difficulties, communication challenges, and restricted, repetitive behaviors. Despite its high prevalence, the pathophysiology of ASD remains largely enigmatic. Recent studies on ASD brain characteristics have revealed structural, functional, and connectivity abnormalities in gray and white matter, with notable regional differences compared to typically developing controls. Cognitive impairments, learning difficulties, and issues with memory binding in ASD individuals suggest atypical brain development across their lifespans.

Neurotrophins are crucial for the nervous system's development, survival, function, and plasticity. Brain Derived Neurotrophic Factor (BDNF), the most abundant neurotrophin in the mammalian central nervous system, plays a vital role in neuronal survival, differentiation, and plasticity. BDNF is thought to aid in inhibiting apoptosis and promoting neuronal reorganization. Studies have linked higher BDNF serum levels with autism. **This study first aimed to compare serum BDNF levels in ASD patients and healthy controls.**
Additionally, the BDNF gene features polymorphisms impacting its levels and function, including a common non synonymous G to A single nucleotide polymorphism (SNP) exists at position 196 of exon 2 (rs6265) of BDNF gene, which affects intracellular packaging of pro-BDNF, its axonal transport and, in turn, activity-dependent secretion of BDNF at the synapse. And the Val66Met polymorphism (Met substitution for Val at codon 66 in the prodomain), which affects regulated release of BDNF. **Hence, the second aim of this study was to investigate the potential association between these two polymorphisms and ASD, as well as if these polymorphisms correlate with BDNF levels is ASD**.
In this study, blood samples from 326 ASD patients and 342 controls were collected; serum was separated for BDNF level assessment via ELISA, and gene polymorphisms were analyzed using PCR-RFLP. Statistical analysis was conducted using SPSS.
Findings revealed significantly higher serum BDNF levels in ASD patients compared to controls, association of elevated BDNF levels with EEG abnormalities (but not with GI symptoms in ASD), and a significant correlation between rs6265 polymorphism and ASD. However, no significant link was found between Val66Met polymorphism and ASD or BDNF levels.
These results suggest that the Val66Met polymorphism may elevate BDNF levels, potentially contributing to ASD pathophysiology and associated symptoms like abnormal EEG often observed in ASD patients.

Disclosures: M. Shaf'ei: None. **O. Ihmoud:** None. **L. Alzghoul:** None. **S. Hayek:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.01/A48

Topic: A.07. Developmental Disorders

Support: R01-NS096976
U54-NS117170

Title: Neurodevelopmental deficits in a zebrafish model of ARX epileptic encephalopathy

Authors: *B. ZHU[1], S. C. BARABAN[2];
[1]Univ. of California, San Francisco, San Francisco, CA; [2]Dept Neurolog Surgery, Univ. California San Francisco, San Francisco, CA

Abstract: ARX encodes for the aristaless-related homeobox transcription factor. It plays a pivotal role in the development of telencephalic GABAergic interneurons. Mutations in *ARX* have been linked to a broad spectrum of neurodevelopmental disorders in human, including infantile spasm, epilepsy, autism, and intellectual disability. During development, *ARX* is expressed in migrating GABA progenitor cells from the medial ganglionic eminence (MGE). To date, no effective treatments have been established, in part, due to the time and cost required to model single gene mutations in rodents or human neural organoids. Zebrafish (*Danio rerio*) provide a powerful alternative vertebrate system to model ARX-related disorders. Besides the high degree of genetic similarity with human (over 80%; Howe et al. Nature 2013), zebrafish have external, rapid and visually accessible developmental advantages. Zebrafish also allow efficient genetic manipulations and high-throughput scalability. Here we describe studies to evaluate (i) functional consequences of *arxa*/*arxb* knockdown in larval zebrafish (4-6 days post-fertilization, dpf) using behavioral and electrophysiological assays and (ii) expression of developing interneurons in larval zebrafish (1-6 dpf) using multiplexed *in situ* hybridization chain reaction (HCR). To study *arx* genes in zebrafish, we use a stable F7+ generation CRISPR-generated *arxa* mutant (Griffin et al. Comm Bio. 2021) and a multi-guide CRISPR-Cas9 approach for generation of biallelic F0 knockout *arxa*/*arxb* mutants (Kroll et al. eLife 2021). First, using a minimally invasive local field potential recording technique, we confirmed epilepsy phenotypes consisting of spontaneous ictal-like (Type II) epileptiform events at 5-6 dpf. Second, using a DanioVision locomotion tracking system and a light/dark assay, we confirmed a hypoactivity movement phenotype at 4 dpf. Next, using whole-mount HCR, we observed *arxa* expression in telencephalon and diencephalon partially overlapped with *nkx2.1* (a highly specific MGE marker) at 1 dpf. Between 2 and 5 dpf, expression of *arxa* and *arxb* persisted and largely overlapped in developing zebrafish forebrain. Taken together, our work suggests that zebrafish offer an exciting new model to better understand the pathogenesis of ARX epileptic encephalopathies during early brain development.

Disclosures: B. Zhu: None. **S.C. Baraban:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.02/A49

Topic: A.07. Developmental Disorders

Support: NIH T32MH112510

Title: The DEAD-box encoding gene, EIF4A2, alters neurodevelopment in zebrafish

Authors: *A. R. DUNCAN[1,2,4], J. LEE[2], P. AGRAWAL[5], A. PODURI[3,4];
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Abstract: The DEAD-box family of RNA helicases is critical for neurodevelopment. We have previously shown that variants in the DEAD-box encoding gene, *EIF4A2*, lead to a neurodevelopmental disorder (NDD) characterized by intellectual disability, epilepsy, hypotonia and structural changes in the brain (Paul, Duncan et al AJHG, 2023). The specific mechanisms by which *EIF4A2* alters neurodevelopment, however, remain largely unknown. *EIF4A2* is an essential regulator of protein translation, interacting with multiple critical genes during development, including several genes that are essential for interneuron differentiation and migration. These interactions suggests that *EIF4A2* serves a critical role in interneuron development as well. To understand the importance of *EIF4A2* in neurodevelopment, we created *eif4a2*$^{-/-}$ zebrafish with CRISPR/Cas9 gene editing. Since individuals with variants in *EIF4A2* have intractable seizures and motor delays, we assessed hyperexcitability and motor function in wild type (WT) vs. *eif4a2*$^{-/-}$ zebrafish. We also generated acute knockouts in transgenic zebrafish with GFP-labeled GABAergic interneurons. Tail coiling assays were first performed at 17-24 hours post fertilization (hpf) to capture muscle contractions that are generated by the primary motor neurons in zebrafish. In *eif4a2*$^{-/-}$ zebrafish, there was a significant increase in the number and duration of tail coil bursts generated when compared to WT (WT n=337, eif4a2-/- n=440, $p< 0.01$), suggesting that *eif4a*$^{-/-}$ zebrafish are hyperexcitable starting as early as 17 hpf. Swimming was then assessed at 5 days post fertilization (dpf) and the *eif4a2*$^{-/-}$ zebrafish consistently swim slower than WT (WT n=360, eif4a2-/- n=360, $p< 0.0001$). Since a majority of individuals with variants in *EIF4A2* have intractable seizures, type II seizures were recorded in zebrafish through the Nodulus Danio Vision. At 6 dpf, *eif4a2*$^{-/-}$ zebrafish had increased spontaneous seizures when compared to WT (WT n=185, *eif4a2*$^{-/-}$ n=314, $p< 0.05$). To understand how loss of eif4a2 function impacts GABA interneurons, acute CRISPant knockouts of *eif4a2* were performed. Preliminary results demonstrate that GABA interneurons are significantly reduced at 6 dpf (WT n=4, *eif4a2*$^{-/-}$ n=4, * $p<0.05$) in the zebrafish optic tectum. This study shows that similar to the individuals with pathogenic variants in *EIF4A2*, *eif4a2*$^{-/-}$ zebrafish exhibit hyperexcitability and motor impairments. Preliminary studies suggest that GABA interneurons are impacted by loss of eif4a2 function, suggesting that variants in *EIF4A2* may lead to a novel interneuronopathy.

Disclosures: A.R. Duncan: None. **A. Poduri:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.03/A50

Topic: A.07. Developmental Disorders

Support: R01 AA013440

Title: Prenatal alcohol and cannabinoid exposures impose distinct, sex-specific alcohol-seeking behaviors in adult mice.

Authors: *A. BOWRING[1], S. ROUZER[2], R. C. MIRANDA[3];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Sch. of Med., Bryan, TX; [2]Texas A&M Col. of Med., Bryan, TX; [3]Neurosci. and Exptl. Therapeut., Texas A&M Univ., Bryan, TX

Abstract: Background: Individuals with prenatal exposure to alcohol or marijuana are at greater risk of engaging in drug-seeking behaviors, however little is known about the effects of simultaneous alcohol and cannabinoid (SAC) exposure. We investigated whether SAC augments alcohol-seeking behavior in offspring compared to single-drug exposure alone. Method: Pregnant C57Bl/6J mice were assigned to one of four groups: drug-free control, alcohol-exposed, cannabinoid-exposed, or SAC-exposed. From Gestational Days 12-15, dams received cannabinoid agonist CP-55940 (750μg/kg) or saline via intraperitoneal injection. Dams were then placed in vapor chambers for 30 minutes of inhalation of 95% ethanol or room air. Adult offspring (Postnatal Days 120+) were assessed for differences in alcohol-seeking activity within operant chambers. Results: Self-administration experiments indicate that all drug-exposed female offspring drank significantly more ethanol than control offspring under a fixed ration paradigm (20% ethanol while no difference was observed across drug-exposed males. However, under a progressive ratio paradigm with daily increased ethanol concentrations, male SAC and all substance-exposed female offspring consumed more ethanol in comparison to controls, particularly during the 40% ethanol trial. This may indicate greater willingness to work for alcohol and greater preference for higher alcohol concentrations. SAC males and AE females also persisted in lever-pressing for ethanol during a three-day extinction period, while all other groups reduced their alcohol-seeking behaviors. Conclusion: Prenatal SAC exposure imposes distinct, sexually dimorphic changes in alcohol-seeking behaviors compared to single drug exposure. Subsequent RNA-sequencing assessments will determine if behavior corresponds with changes in gene expression related to CB1 receptor signaling.

Disclosures: A. Bowring: None. **S. Rouzer:** None. **R.C. Miranda:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.04/A51

Topic: A.07. Developmental Disorders

Support: NIH P20GM139762-01
NIH P20GM139762-03S1

Title: Effects of prenatal alcohol exposure on the auditory system

Authors: S. HUNTWORK, ***P. RAGUNATHAN**;
Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fetal alcohol spectrum disorders (FASD) are one of the leading causes of developmental abnormalities worldwide. Individuals with FASD have impaired auditory

processing and frequently exhibit atypical auditory behaviors including low auditory filtering scores. Our studies using a chronic low-level prenatal alcohol exposure (PAE) model show that PAE mice exhibit auditory brainstem response abnormalities suggesting that chronic exposure to alcohol during fetal development can result in perturbations in auditory processing. GABA-mediated inhibition plays a key role in auditory processing, and therefore a decline in GABA-mediated inhibition may significantly contribute to auditory processing deficits. Given the important role that interneurons play in processing of auditory information, it is important to address the impact of PAE on interneurons in the auditory neuronal circuits. In this study, we use a model of maternal voluntary alcohol consumption throughout gestation in a mouse model to investigate the effects of PAE on the GABAergic interneuron populations in the primary auditory cortex. We performed immunohistochemical analysis in 4-week-old male and female mice to examine the impact of PAE on the density and laminar distribution of GABAergic interneurons in the primary auditory cortex. Our study mainly focuses on the effects of PAE on parvalbumin, somatostatin and vasoactive intestinal peptide-expressing interneurons. The results of the study indicate altered densities of parvalbumin and somatostatin interneurons in the primary auditory cortex in PAE mice. Analysis is currently ongoing to determine whether there are differences in the laminar distribution of these interneurons in the primary auditory cortex. Our hypothesis is that PAE alters interneuron populations in the primary auditory cortex that could contribute to auditory processing alterations.

Disclosures: S. Huntwork: None. **P. Ragunathan:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.05/A52

Topic: A.07. Developmental Disorders

Title: S-adenosylmethionine prevented neural tube defects induced by valproic acid in mice.

Authors: *B. ECHEFU[1], M. BECKER[1], A. ORNOY[2,1];
[1]Morphological Sci. and Teratology, Ariel Univ., Ariel, Israel; [2]Dept. of Med. Neurobio., Hebrew Univ. Hadassah Med. Sch. Jerusalem, Jerusalem, Israel

Abstract: Valproic acid (VPA) is an effective anticonvulsant and mood stabilizer, proven to cause congenital malformations in about 10% of exposed human pregnancies. S-adenosylmethionine (SAMe) is the classical methyl donor for normal cell functioning and synthesis of various molecules (Ornoy et al., 2023). SAMe was found to antagonize VPA's effects when administered on gestation day (GD) 12 (Weinstein-Fudim et al., 2020), which is post-organogenesis for most organs except the brain. The exact mechanism of their interaction is presently unknown. One-carbon metabolism (OCM) changes, oxidative stress, gene expression changes, apoptosis, and proliferation regulations are among presumed mechanisms for these interactions. In the current study, we aimed to assess the effect of SAMe on mouse embryos

exposed prenatally to VPA during neurulation, at the time of major organogenesis in most organs. Twenty- four pregnant ICR mice were divided into four groups of 6 mice each as follows: saline control, VPA, SAMe and VPA+SAMe group. On GDs 8 and 9, mice were treated intraperitoneally with saline, VPA, or SAMe while the VPA+SAMe group was co-treated with SAMe one hour before VPA injection. Dams were sacrificed on GD 15 and fetuses were examined for the presence of gross external malformation and viability. We found that VPA induced exencephaly in 27% (12/44) of fetuses compared to 1.9% (1/53) by SAMe and 0.00% (0/70) by saline. However, when VPA was co-administered with SAMe, exencephaly was completely prevented 0.00% (0/74). The VPA or SAMe only groups had significantly higher number of fetal deaths (26.56% and 27.34%) respectively when compared to the control (2.78%) and to VPA+SAMe (3.90%). Our results also show that SAMe significantly ameliorated VPA - induced growth retardation. We have also found similar phenomena on GD 12 embryos. These studies show that SAMe prevents the neural tube malformations induced by VPA, similar to the prevention of neurobehavioral abnormalities observed in our previous studies. Additional investigations are currently carried out to elucidate the possible mechanism/s underlying the interaction between VPA and SAMe at that early developmental stage.

Ornoy, A., Echefu, B., & Becker, M. (2023). Valproic Acid in Pregnancy Revisited: Neurobehavioral, Biochemical and Molecular Changes Affecting the Embryo and Fetus in Humans and in Animals. https://doi.org/10.3390/IJMS25010390

Weinstein-Fudim, L., Ergaz, Z., Szyf, M., & Ornoy, A. (2020). Prenatal s-adenosine methionine (SAMe) induces changes in gene expression in the brain of newborn mice that are prevented by co-administration of valproic acid (VPA). https://doi.org/10.3390/ijms21082834

Disclosures: B. Echefu: None. **M. Becker:** None. **A. Ornoy:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.06/A53

Topic: A.07. Developmental Disorders

Support: Goldstein Research Innovation Grant
IDDRC funding 113369-0624-01
Faneca 66 Foundation
Celebrate Hope Foundation

Title: R183q gnaq sturge-weber syndrome leptomeningeal and cerebrovascular developmental mouse model

Authors: *C. R. SOLOMON[1], M. MCCANN[2], P. SINGH[2], C. L. NEMETH[3], A. M. COMI[2]; [2]Neurol. and Developmental Med., [3]Neurogenetics, [1]Kennedy Krieger Inst., Baltimore, MD

Abstract: Objectives: Sturge-Weber syndrome (SWS), a rare neurovascular malformation disorder, is usually caused by the R183Q GNAQ somatic mosaic mutation enriched in brain endothelial cells. A developmental mouse model of SWS brain involvement is needed to investigate mutation impact upon brain vascular development and to facilitate preclinical drug studies. **Methods:** A new Tet-ON R183Q GNAQ transgenic mouse line was paired with rtTA tet transactivator mice under the *Tie2* promoter to generate mice expressing endothelial R183Q GNAQ in the presence of doxycycline. Litters were perfused at P14-17; half received a sub-seizure dose (1.5 mg/kg; i.p.) of kainate an hour before perfusion. A subset was perfused with Evans blue and assessed for staining intensity relating to blood brain barrier permeability. Fixed mouse brains were stained with X-gal, DAPI, and antibodies for Gαq, Tie2, phosphorylated-S6, claudin-5, and p-ERK. Images of tissue sections were scored for blood vessel staining intensity. Microvessels at the level of the anterior hippocampus were analyzed in ImageJ for vessel length and diameter, comparing between genotype (Control mice and Mutant mice) and drug treatment groups (non-treated mice and kainate-treated mice) as well as comparing between genotype within each drug treatment group. **Results:** X-gal staining was seen only in mutant mice; leptomeningeal endothelial X-gal staining was more frequent in kainate-treated mice ($p < 0.001$). When perfused with Evans blue, only mutant brains showed severe staining ($p = 0.028$). Median phosphorylated-S6 vessel scores were significantly higher in the leptomeninges of mutant mice ($p = 0.035$). Mutant cortical microvessels demonstrated discontinuous claudin-5 and phosphorylated-S6 staining, and abnormal co-localization of Tie2 and Gαq protein expression with X-gal staining ($p = 0.008$). Microvessel morphology analysis showed that microvessels stained with Tie2 had a significantly increased vessel length in mutant, kainate-treated mice compared to control, kainate-treated mice ($p = 0.031$). **Conclusions:** The new R183Q GNAQ Tet-ON developmental mouse brain model of SWS demonstrates endothelial expression of mutant Gαq associated with blood brain barrier breakdown, altered vascular mTOR activity, and abnormal cortical microvessel structure. The finding of increased microvessel length in mutant, kainate-treated mice compared to control, kainate-treated mice suggests further exploration on the effects of kainate on this mouse model. This new translational model can be used to develop new drug targets and treatments for SWS.

Disclosures: C.R. Solomon: None. **M. McCann:** None. **P. Singh:** None. **C.L. Nemeth:** None. **A.M. Comi:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.07/A54

Topic: A.07. Developmental Disorders

Support: CNPq
UFCSPA

Title: Cannabidiol improves maternal obesity-induced behavioral, neuroinflammatory and neurochemical dysfunctions in the juvenile offspring

Authors: *V. SILVA DIAS, F. RODRIGUES, J. JANTSCH, G. FRAGA, C. PEREIRA MEDEIROS, F. WICKERT, Y. BITENCOURT, S. P. DE MATOS, M. GIOVENARDI, R. P. GUEDES;
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Abstract: Introduction: Maternal obesity, marked by prolonged inflammation, may result in disturbances in the central nervous system development, potentially leading to neuropsychiatric disorders like anxiety, depression, and autism spectrum disorder. Despite extensive research on preventive measures, studies that target both male and female offspring throughout life are still lacking. Thus, we aimed to assess the effects of cannabidiol treatment on maternal obesity-related behavioral, neuroinflammatory, and neurochemical alterations in male and female offspring. **Methods:** Offspring of female Wistar rats fed a Cafeteria diet (CAF) were treated with CBD (50mg/kg) via oral gavage for three weeks. Behavioral tests included Elevated Plus Maze (EPM), Open Field (OF), Social Preference, and Social Recognition. Offspring were euthanized at PND42 for tissue collection. Offspring's gene expression, neurotransmitter and endocannabinoid content were assessed in the prefrontal cortex (PFC) and hippocampus. Data were analyzed by Two-way ANOVA with Bonferroni post hoc using Graphpad Prism 9. **Results**: Both male and female offspring of obese dams presented increased freezing behavior in the OF. Furthermore, males expelled a higher number of fecal boli, while females had a higher latency to enter the center zone. In the EPM, male CAF-Veh had decreased entries and time in the open arms, while females had increased latency to enter the open arms. Lastly, female CAF-Veh showed impaired social preference, while males showed decreased social memory. CBD treatment was able to rescue all the behavioral alterations. Maternal obesity impacted gene expression of IBA-1, GFAP, CB1R, DRD2R and OXTR in the PFC and hippocampus of the offspring. CBD treatment was able to attenuate the disturbances in IBA-1, GFAP, CB1R and OXTR in a sex-dependent manner. Furthermore, both male and female offspring of obese dams presented increased glutamate and reduced endocannabinoid concentrations in the PFC, with a positive effect of CBD. **Conclusion**: Our findings highlight CBD's therapeutic potential in mitigating behavioral, neuroinflammatory, and neurochemical changes associated with maternal obesity, featuring sexual dimorphism and the involvement of the endocannabinoid system.

Disclosures: V. Silva Dias: None. **F. Rodrigues:** None. **J. Jantsch:** None. **G. Fraga:** None. **C. Pereira Medeiros:** None. **F. Wickert:** None. **Y. Bitencourt:** None. **S.P. de Matos:** None. **M. Giovenardi:** None. **R.P. Guedes:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.08/A55

Topic: A.07. Developmental Disorders

Title: Nkapl deletion cause schizophrenia-like cognitive deficits in mice

Authors: *Y. YANG;
Peking Univ. Sixth Hosp., Beijing, China

Abstract: Nkapl deletion cause schizophrenia-like cognitive deficits via inhibitory synaptic transmission inmPFC in miceAuthors* Yang Yang; Peking University Sixth Hospital, Peking University Institute of Mental Health,NHC Key Laboratory of Mental Health (Peking University), Beijing, China**DisclosuresYang Yang:** None.**Abstract**Schizophrenia (SCZ) is a severe brain disorder. Cognitive deficits as core feature of SCZ appearsin a prodromal period during late adolescence and early adulthood. Mechanism of cognitivedeficits are poorly understood. The SNP rs1635, located on the exon of *NKAPL* gene, has beenidentified with SCZ risk and cognition in early-onset SCZ. In this study, we have developed*Nkapl* knock out (KO) mouse model to investigate the mechanism of *Nkapl* under the cognitionassociated with SCZ. We found that KO mice exhibited deficits in short-term memory andworking memory. To further determine the key brain region contributes to cognitive deficitscaused by *Nkapl* deletion, we knocked down the *Nkapl* expression in mPFC, dorsal and ventralhippocampus, respectively, and performed behavior test. Mice with *Nkapl* deletion in mPFCshowed cognitive deficits consistent with KO mice. The bulk RNA-Seq in KO mice showedsignificantly elevated Aldh5a1 encoding GABA metabolic enzyme SSDH and this was verifiedby RT-qPCR and western blot. The KO mice showed higher GABA metabolic rate and decreasedfrequency of mIPSCs in pyramidal cells in mPFC, suggesting impaired inhibitory synaptictransmission. To determine the cell types with functional abnormalities, we deleted *Nkapl* ininterneurons or astrocytes in mice and carried out behavior tests and whole-cell recording.Deletion *Nkapl* in interneurons could result in cognitive deficits and decreased frequency ofmIPSCs consistent with KO mice. Overexpression SSDH in interneurons in mPFC in wild typemice also resulted in decreased frequency of mIPSCs. Knock down SSDH or re-expression *Nkapl*in interneurons can rescue the decreased frequency of mIPSCs and cognitive deficits in KO mice.We also conducted Dual-luciferase Reporter and chromatin immunoprecipitation assay todetermine that Nkapl is a transcriptional repressor of SSDH and the T153N variant is crucial forthe process. Together, we demonstrated that *Nkapl* KO mice exhibited cognitive deficitsassociated with SCZ and could be a mouse model for this. Nkapl as a transcriptional repressor ofSSDH regulates the rate of GABA metabolism in interneurons, that plays a role in inhibitorysynaptic transmission in mPFC and contributes to cognitive behaviors, suggesting GABAmetabolism in interneurons could be a target for therapeutic intervention of cognitive deficits inSCZ.

Disclosures: Y. Yang: None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.09/A56

Topic: A.07. Developmental Disorders

Support: 1R01NS119251-01A1
R01 NS107383
R01 GM112715

Title: Abnormal local cortical functional connectivity due to interneuron dysmaturation after neonatal intermittent hypoxia

Authors: I. GOUSSAKOV, S. SYNOWIEC, R. B. FABRES, D. P. AKSENOV, *A. DROBYSHEVSKY;
NorthShore Univ. HealthSystems, Evanston, IL

Abstract: Background: Premature infants are often experience frequent hypoxic episodes due to immaturity of respiratory control that may result in disturbances of gray and white matter development and long term cognitive and behavioral abnormalities. We hypothesize that neonatal intermittent hypoxia can modulate abnormal cortical maturation of excitatory and inhibitory circuit that can be detected early with functional MRI.**Methods.** C57BL/6 mouse pups were exposed to intermittent hypoxia (IH) regiment consisting 12 to 20 hypoxic episode daily of 5% oxygen exposure for 2 min at 37C from P3 to P7, followed by MRI at P12 and patch clamp recording in cortical slices performed at several time points between P7 and P13. Behavioral tests were conducted at P41-P50 to assess animal activity and motor learning.**Results.** Adult mice after neonatal IH exhibited hyperactivity on open field and impaired motor learning in complex wheel task. Electrophysiology revealed increased glutamatergic transmission both on patch clamp and field potential recording with Picrotoxin. Decreased inhibitory drive was evidenced by EPSC frequency on pyramidal cells, on MUI recording in vivo in motor cortex with Picrotoxin injection, as well as by the decreased interneuron density at P13. Amplitude of low frequency fluctuation on resting state fMRI were larger in IH group and significantly increased after Picrotoxin injection. **Conclusion.** Increased excitatory glutamatergic transmission and decreased numbers and activity of inhibitory interneurons after neonatal IH may result in long-term changes in cortical network maturation and results in hyperactivity and impaired motor learning. Increased intrinsic connectivity in sensorimotor cortex on functional MRI is indicative of neuronal dysfunction in cortical maturation after neonatal IH brain injury.

Disclosures: I. Goussakov: None. **S. Synowiec:** None. **R.B. Fabres:** None. **D.P. Aksenov:** None. **A. Drobyshevsky:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.10/A57

Topic: A.07. Developmental Disorders

Support: Roy J. Carver Charitable Trust Grant# 23-5683

Title: Leakage of the blood-borne neuroinflammatory molecule fibrinogen correlates with microglial immunofluorescence in the fetal brain during maternal immune activation

Authors: *R. GONZALEZ-RICON[1], A. M. OTERO[2], I. CHALEN[3], A. M. ANTONSON[4]; [1]Neurosci., Univ. of Illinois Urbana-Champaign Champaign, Champaign, IL; [2]Neurosci., Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Dept. of Animal Sci., Univ. of Illinois Urbana-Champaign, Champaign, IL; [4]Animal Sci., Univ. of Illinois, Urbana-Champaign, Urbana, IL

Abstract: Background: Influenza A virus (IAV) infection during gestation is associated with an increased risk of neurodevelopmental disorders (NDDs) in offspring, like schizophrenia and autism spectrum disorders. The pathophysiological mechanisms underpinning this association remain a subject of investigation. We posit that maternal immune activation (MIA) precipitated by IAV infection may disrupt selective permeability at the maternal-fetal interface, consequently compromising the integrity of the fetal blood-brain barrier (BBB). This compromised barrier function could lead to aberrant trafficking of neuroinflammatory blood-borne molecules into the fetal compartment, predisposing offspring to neuroinflammatory insults implicated in NDD etiology. Our study aims to: (1) investigate whether fibrinogen, a neuroinflammatory mediator, breaches the placental and fetal brain barriers (vascular or ventricular) during MIA, and (2) determine if this correlates with microglial immunofluorescence.
Methods: C57Bl/6NTac pregnant mice were inoculated with mouse-adapted IAV (10^3 or 10^4 $TCID_{50}$) or saline at gestational day 9.5. Expression of tight junction proteins (TJPs) and $CD31^+$ endothelial cells in placentas and fetal brains was assessed via qPCR and immunohistochemistry. Co-staining of fibrinogen and $Iba1^+$ cells (microglia/macrophages) was assessed in the subventricular zone (SVZ) of the lateral ventricle in fetal brains.
Results: No statistical differences were seen regarding TJPs and $CD31^+$ quantification in placentas or fetal brains. A significant increase in fibrinogen levels was noted in fetal brains exposed to 10^4 $TCID_{50}$ IAV versus other groups. Moreover, a positive correlation between fibrinogen and $Iba1^+$ staining at the SVZ was noted.
Conclusion: Large neuroinflammatory glycoprotein fibrinogen can reach the fetal brain during IAV infection. We detected increased correlation in fibrinogen leakage and $Iba1^+$ cell immunofluorescence mainly at the SVZ. Ongoing experiments will determine potential origin and trafficking of other blood-borne molecules that might be implicated in fetal neuroinflammation upon MIA.

Disclosures: R. Gonzalez-Ricon: None. **A.M. Otero:** None. **I. Chalen:** None. **A.M. Antonson:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.11/A58

Topic: A.07. Developmental Disorders

Support: CNCDP-K12 NS098482
R01MH129970
R01DK135871

Title: Microglial depletion worsens lesion in female but not male C57BL/6J mice after hypoxia ischemia

Authors: D. H. GUEZ-BARBER[1], ***S. NICOLAYEVSKY**[2], K. J. D. JOHNSON[3], S. YUN[4], A. J. EISCH[5];
[1]Neurol., CHOP, Philadelphia, PA; [2]Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Univ. of Pennsylvania, Philadelphia, PA; [4]Anesthesiol. and Critical Care, CHOP, Philadelphia, PA; [5]Anesth & Crit Care and Neurosci, UPenn & CHOP, Philadelphia, PA

Abstract: Rodent models for perinatal hypoxic ischemic (HI) encephalopathy have reported sex differences such as the same injury causing larger lesions in the brains of males compared to females. Microglia, the resident immune cells of the brain that have sex-dependent developmental trajectories and gene expression patterns, likely play a different role in females and males following HI. However, there is conflicting literature on whether depletion of microglia worsens or improves HI-induced lesions and whether this differs by sex. Here we tested the effect of pharmacologic microglia depletion on HI lesion size in male and female mice. An initial cohort of C57BL/6J mouse pups underwent HI at postnatal day 10 (P10) using a modified Vannucci procedure or a Sham insult followed by brain collection at P13. Another cohort of mice received daily intraperitoneal injections from P7 to P12 of either 25mg/kg PLX3397 (PLX, a CSF1R inhibitor) or vehicle (Veh). These mice also underwent HI or Sham at P10, resulting in four groups (Veh-Sham, Veh-HI, PLX-Sham, PLX-HI). All groups included female and male mice. Behavioral testing was performed both pre-HI (forelimb grasping [P8, P9]) and post-HI (open field traversal [P12], behavior and appearance observations [P13]). P13 brain sections underwent immunohistochemistry for Iba1 or cresyl violet staining for lesion scoring. P13 HI hippocampal sections had more Iba1 signal than Sham, with more variance in Male-HI vs Female-HI mice. PLX led to >95% depletion of Iba1+ cells at P10 or P13, and effective elimination of microglia did not differ by sex. In the hippocampus, Female-PLX-HI mice had worse lesion scores than Female-Veh-HI mice; this was not true in male mice, where there was a trend in the opposite direction. Female-PLX-HI mice also had worse lesion scores than Male-PLX-HI mice. In contrast to this sex-dependent effect of PLX on lesion score, there was no difference among groups in developmental milestones. PLX3397 injection P7-P9 or P7-P12 effectively depletes microglia by P10 or P13, respectively. Microglial depletion via PLX worsens HI-induced injury in female mice but not in male mice.

Disclosures: D.H. Guez-Barber: None. **S. Nicolayevsky:** None. **K.J.D. Johnson:** None. **S. Yun:** None. **A.J. Eisch:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.12/Web Only

Topic: A.07. Developmental Disorders

Support: NIDA 1U01DA058278
NIH S10OD019960

Title: Region specific DNA methylome alterations in neonatal rat brain following maternal e-cigarette exposure

Authors: W. XIONG[1], ***W. CHEN**[1], Z. CHEN[1], C. WANG[2];
[2]Ctr. for Genomics, [1]Loma Linda Univ., Loma Linda, CA

Abstract: DNA methylation (DNAm) is a pivotal epigenetic mechanism regulating various processes involved in the brain development and its susceptibility to different brain diseases. Profiling DNAm patterns in different brain regions may help understand their unique functional specialization, and potentially offering insights into differential pathological mechanisms across brain regions. Growing evidence suggests that prenatal exposure to electronic cigarette (e-cig) may impact brain development. However, it remains unclear whether this abnormal development displays a region-specific susceptibility regulated by differential DNA methylations across different brain regions. Here, using a unique well-established intrauterine e-cig exposure rat model, we examined DNAm by reduced representation bisulfite sequencing (RRBS) in postnatal day 7 developing rat brain from three brain regions, i.e., olfactory bulk, prefrontal and occipital cortex, following prenatal e-cigarette exposure. We found significant differences in both CpG and non-CG methylation (mCH) across brain regions. The prefrontal cortex exhibited the most significant methylation alteration following prenatal e-cigarette exposure, while there is a minimal DNAm change detected in the olfactory bulk region. Moreover, a gender-specific response to e-cigarette exposure was found in the prefrontal cortex region, while no such distinction was observed in the other two regions. Gene ontology analysis revealed that prenatal e-cigarette exposure not only affected GABAergic synapse and cholinergic synapse, but also altered the pathways related to calcium signaling and morphine addiction. In conclusion, this study provides valuable insights into the differential region-specific epigenetic regulation in the developing brain induced by prenatal e-cigarette exposure.

Disclosures: W. Xiong: None. **W. Chen:** None. **Z. Chen:** None. **C. Wang:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.13/A59

Topic: A.07. Developmental Disorders

Support: Jordan's Guardian Angels

Title: Behavioral effects of phosphodiesterase 4D inhibition in a mouse model of Houge-Janssens Syndrome 1, an autosomal dominant neurodevelopmental disorder, caused by de novo mutations in a protein phosphatase 2A regulatory subunit

Authors: *C. JONG[1], C. CHEN[2], S. STRACK[3];
[1]Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA; [2]Pharmacol. and Neurosci., Univ. of Iowa, IOWA CITY, IA; [3]Neurosci. and Pharmacol., Univ. Iowa, Iowa City, IA

Abstract: Houge-Janssens Syndrome 1 (HJS1) or autosomal-dominant mental retardation 35 (MRD35) is caused by *de novo* germline mutations in the protein Ser/Thr phosphatase 2A (PP2A) regulatory subunit B'δ (PPP2R5D). Relatively common among monogenic causes of neurodevelopmental disorders, HJS1 typically presents with intellectual disability, human overgrowth, and autism. There is an urgent need for a drug in the treatment of HJS1. Because our biochemical data show enhanced PP2A activity in HJS1, we therefore explored if zatolmilast or BPN14770, a PDE4D inhibitor, offers therapeutic benefits in our mouse model of HJS1, potentially by boosting the cAMP-mediated PKA activity. In our mouse model of HJS1 harboring the constitutive E198K knock-in mutation, which is the most common and the most severe mutation, the mice recapitulate most of the common features of HJS1 which includes growth retardation, craniofacial anomalies, seizure occurrences, hyperactivity and cognitive deficits. When E198K mice were given subcutaneous injection of 5mg/kg zatolmilast one hour prior to behavioral tasks, cognition was restored in the 2T-ymaze task as evidenced by an increase in the ratio of the time spent in the novel to the familiar arms. The open field test, however, did not show a reduction in the hyperactivity in E198K treated with zatolmilast, as evidenced by an increase in the distance traveled, the velocity and the number of highly mobile and mobile events. On the other hand, daily treatment of 5mg/kg zatolmilast for 14 days restored cognition and reduced hyperactivity in E198K mice. When zatolmilast was withdrawn for 14 days, the improvement in the cognition waned while the reduced hyperactivity persisted. Our findings suggest acute and chronic phosphodiesterase 4D inhibition exerts different behavioral effects in our HJS1 mice. Further examination of the neuroanatomical changes in response to acute or chronic phosphodiesterase 4D inhibition may help to understand the different behavioral effects observed in our HJS1 mice.

Disclosures: C. Jong: None. **C. Chen:** None. **S. Strack:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.14/A60

Topic: A.07. Developmental Disorders

Support: Jordan's Guardian Angels

Title: Aberrant phosphorylation and neurogenesis in mouse models of PPP25D-related neurodevelopmental disorder

Authors: *C. CHEN[1,2], C. JONG[1], R. A. MERRILL[1], S. STRACK[3];
[1]Neurosci. and Pharmacol., Univ. of Iowa, IOWA CITY, IA; [2]Human toxicology, University of Iowa, Iowa, IA; [3]Neurosci. and Pharmacol., Univ. Iowa, Iowa City, IA

Abstract: Protein phosphatase 2A (PP2A) is one of the major Ser and Thr phosphatases and constitutes up to 1% of total protein in mammalian cells. PP2A is a trimeric holoenzyme consisting of a scaffolding A subunit, a regulatory B subunit and a catalytic C subunit. A de novo single nucleotide mutation in PPP2R5D, which encodes a regulatory subunit of a PP2A, generates a heterozygous dominant missense variant resulting in a neurodevelopmental disorder characterized by intellectual disability, autism spectrum disorder, recurrent seizures, hypotonia, macrocephaly and other features. We used a CRISPR/Cas9-mediated gene editing to introduce the E198K/E200K/E420K mutation, in the murine Ppp2r5d locus to generate three knock-in mice model of this disease, which are three most common mutations in human. To evaluate if these mice model can recapitulate most of the common symptoms of patients, we used standard behavior tests to assess developmental milestones and adult behaviors, as well as CT scan to capture early postnatal changes in brain development. Our data revealed that mice harboring the E198K, E420K and E200K recapitulate cardinal symptoms and severity of the disease and may be a good model for studying the effects of the disease cause alleles. To evaluate the biochemical phenotype in mice, we screened for differentially phosphorylated proteins by immunoblotting sub-dissected brain regions with phospho-specific antibodies. Our results revealed that mice harboring mice harboring the E198K, E420K mutation has significant decrease phosphorylation of PKA substrates. To investigate the pathogenesis of the disease, we used immunohistochemical method to stain the brain tissue from E198K mouse. Our results revealed that dendrite complexity of doublecortin (DCX)-labeled cells was significant increase while the number of DCX+ cells remain unchanged in the dentate gyrus of E198K mouse. However, the immunofluorescence against SOX2 revealed that SOX2+ nuclei was depleted from the inner blade of granule layer, accompanied by a significant decrease in the number of SOX2+ cells. Together, these data suggested aberrant phosphorylation and neurogenesis in mouse models of PPP25D-related neurodevelopmental disorder.

Disclosures: C. Chen: None. **C. Jong:** None. **R.A. Merrill:** None. **S. Strack:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.15/A61

Topic: A.07. Developmental Disorders

Title: Prenatal maternal infection induced with viral mimetic Poly I:C alters neonatal neurodevelopment and communicative behavior in a dose-dependent manner

Authors: *A. PHAN[1], L. CUPO[1], D. R. GALLINO[2], G. A. DEVENYI[3], M. CHAKRAVARTY[2];
[1]McGill Univ., Montreal, QC, Canada; [2]Cerebral Imaging Ctr., Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [3]Cerebral Imaging Ctr., Douglas Univ. Mental Hlth. Inst., McGill, Montreal, QC, Canada

Abstract: Epidemiological studies have consistently described an association between prenatal maternal immune activation (MIA) and an increased prevalence of neurodevelopmental disorders. Previous animal studies using polyinosinic: polycytidylic acid (Poly I:C), a viral mimetic, found that MIA-exposure promotes subtle alterations in the neonatal brain and impairment in communicative ability. In the Poly I:C model, factors including differences in doses, batches, and timing influence developmental outcomes; however, the dosing effect remains ambiguous.On gestational day 9, pregnant dams were injected with vehicle (n=5) or Poly I:C (2.5, 5, or 7.5 mg/kg, n= 4,4, and 5). On postnatal days (PND) 3, 5, 7, and 9, structural manganese-enhanced MRI scans were acquired using 2 of each sex from each litter. On PND 12, ultrasonic vocalizations (USVs) were measured after separation. Longitudinal deformation-based morphometry was used to assess trajectories of neuroanatomical development. Group differences were assessed with linear mixed effects models examining fixed effects of sex and the interaction between treatment and a quadratic trajectory of age and random effects for subject and litter. USVs were assessed with a shift-function to compare the distributions of call length based on decile differences.We identified altered developmental trajectories due to MIA exposure relative to control. In many affected brain regions, the 5 and 7.5 group showed trends of accelerated growth rate in a dose-dependent manner, such as in the cingulate cortex, CA3 of the hippocampus and nucleus accumbens. In other affected regions, only the 7.5 group showed significantly different trajectories such as in the secondary motor area and caudoputamen. MIA-exposed (5 and 7.5) pups had a significantly different distribution of USV call lengths compared to control (Fig 1D). MIA exposure induces changes in brain and behavior during neonatal development in a dose-dependent manner. A higher dose of Poly I:C leads to more global differences and different trajectories of growth in multiple brain regions.

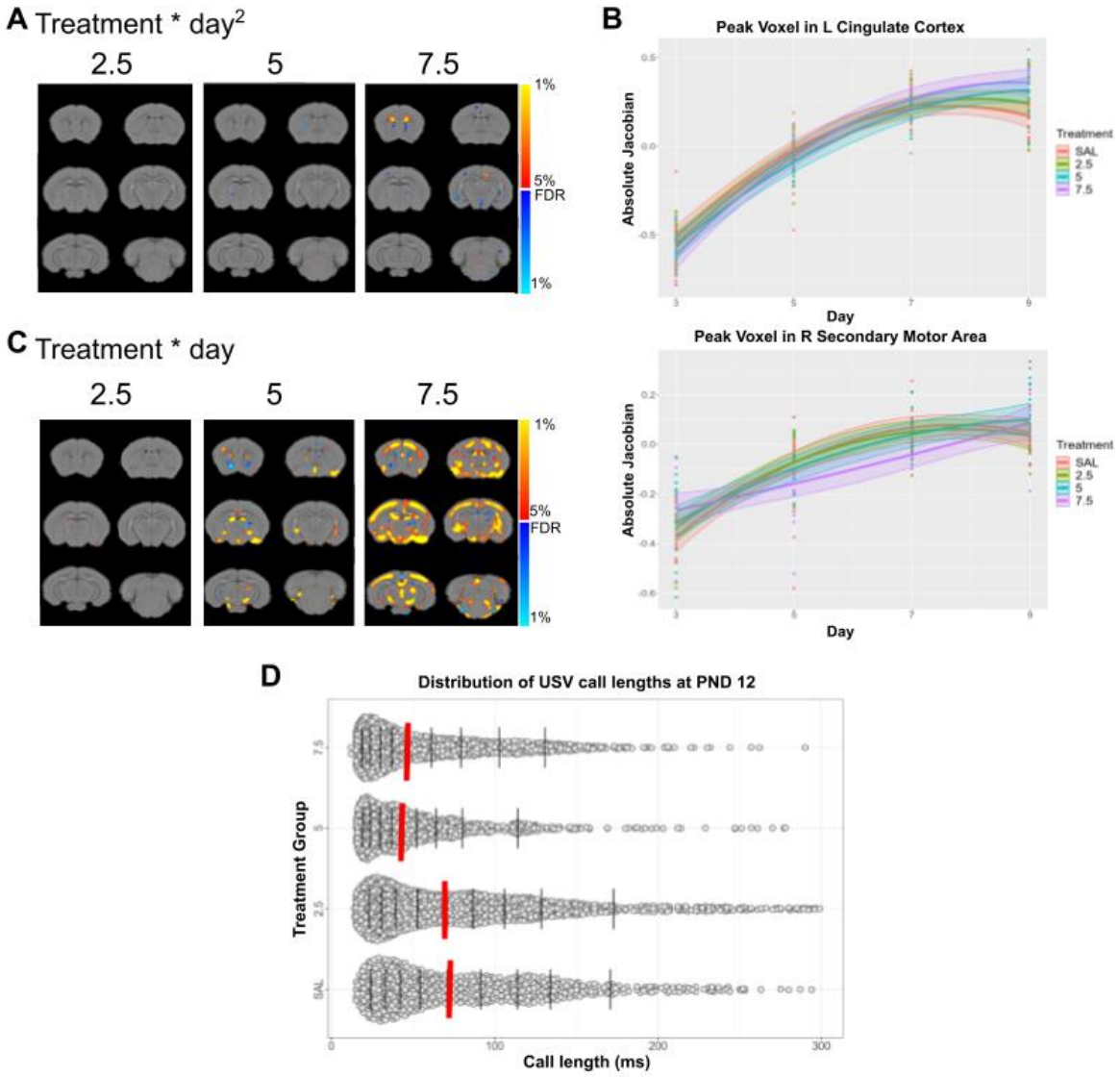

Figure 1 A) Heatmap of the interaction between Treatment and Age (quadratic term) in 2.5, 5, and 7.5 mg/kg groups compared with SAL group over time. Multiple comparisons were corrected with the false discovery rate (FDR)
B) Plots of peak voxels indicate group differences in quadratic trajectories of growth in the cingulate cortex and secondary motor area.
C) Heatmap of the interaction between Treatment and Age (linear term) in 2.5, 5, and 7.5 mg/kg groups compared with SAL group over time.
D) Distribution of USV call lengths by group. Each pup was recorded for 5 minutes upon separation from dam and littermates. Red line indicates median for each group, and thin lines indicate deciles. The shift function describes how each decile should be shifted to match one distribution to another. The leftward shift of distribution in call lengths relative to SAL suggests MIA-exposed (5 and 7.5) pups make more short calls under 50 ms.

Disclosures: A. Phan: None. **L. Cupo:** None. **D.R. Gallino:** None. **G.A. Devenyi:** None. **M. Chakravarty:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.16/A62

Topic: A.07. Developmental Disorders

Support: R01NS113141

Title: Single cell molecular profiling in the Christianson Syndrome rat brain identifies a critical role for neuroinflammation in disease progression.

Authors: *H. AAMIR RIAZ, E. M. MORROW;
Brown Univ., Providence, RI

Abstract: Christianson syndrome (CS) is an X-linked mixed neurodevelopmental and neurodegenerative disorder caused by loss-of-function mutations in the SLC9A6 gene which encodes the endosomal Na+/H+ Exchanger 6 (NHE6). NHE6 deficiency has been reported to significantly impact various cell types within the brain, resulting in impaired endolysosomal function, neuronal arborization and circuit function in neuronal cell types, as well as elevated glial responses from microglia and astrocyte cell types; however, the specific regulatory programs unique to each cell type have yet to be identified. To study cell type specific responses in CS, we performed single-nuclei RNA-seq (snRNAseq) on the hippocampus of 1, 8.5 and 17-month-old control and Nhe6-/y male rats, and identified neuronal and non-neuronal cell types with significantly different transcriptional signatures. Differentially expressed genes (DEGs) between the Nhe6-/y and control rats were enriched for the activation of innate immune pathways and synaptic dysfunction particularly in the 8.5- and 17-months old rats. The neuroinflammatory mechanisms revealed by astrocytes and microglia implicate disease-associated pathways in Nhe6-/y rats with parallels to glial responses seen in Alzheimer's Disease. We have also observed significant demyelination in Nhe6-/y rats at 8.5 and 17 months of age. These findings unveil a new mechanism underlying the progression of CS and endolysosomal disease, where the absence of the NHE6 protein triggers innate immune pathways, leading to demyelination and other neurodegenerative associated symptoms. In addition, we are currently expanding this molecular profiling to include studies of the hippocampus in female rats who are carriers of NHE6 mutations, in order to define pathogenesis in the understudied Female Carrier Syndrome.

Disclosures: H. Aamir Riaz: None. **E.M. Morrow:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.17/A63

Topic: A.07. Developmental Disorders

Support: 1K08 NS131572

Title: Assessing the Cause of Epileptogenesis in Focal Cortical Dysplasia and Similar Malformations of Cortical Development

Authors: *P. HOFFMAN;
Univ. of Colorado Anschutz Med. Campus, Denver, CO

Abstract: Malformations of cortical development (MCDs) produce drug-resistant epilepsy (DRE) in children that is commonly treated by surgery. However, over 30% of children regain seizures and may need to return for further resections. Mutations in mechanistic target of rapamycin (mTOR) pathway genes are the predominant cause of MCDs, one of which being focal cortical dysplasia (FCD). MTOR promotes cellular growth and regulates intracellular autophagy. The link between mTOR pathway upregulation and epilepsy is well-established but there is debate regarding the mechanism(s) by which mTOR activation leads to epileptogenesis. ***I am focused on determining the timeline of neurodevelopmental dysregulation in MCD to pinpoint the order in which cortical dyslamination, axonal proliferation, network activity/ synchronization, and neuron loss take place to translate this timeline to human DRE***. I generated a mouse model of FCD via in utero electroporation of a plasmid that causes a gain-of-function mutation in the mTOR pathway. This mutation produces a constitutively active form of Rheb ($Rheb^{ca}$), a downstream factor of MTORC1, which creates mice exhibiting stage 5 seizures on the Racine scale. Quantification of electroporated cortical neurons from embryonic day 18 (E18) to post-natal day 21 (P21) showed that FCD and similar MCDs display dyslamination and increased axonal proliferation as early as P2. Staining of the cortex with layer markers and quantifying the distribution of cells adjacent to electroporated neurons showed (1) there is no dyslamination of neurons without $Rheb^{ca}$ within the FCD region, and (2) electroporated neurons continue to express the layer markers for the layer they were originally destined for rather than changing expression to match the layer they ended up in. This suggests that cells adjacent to dysplastic neurons maintain a proper migratory pattern throughout development and neurons expressing $Rheb^{ca}$ do not change their expression of layer markers to match that of the dyslamination pattern. Staining for interneuron density and distribution within the malformed region revealed a decrease in inhibitory interneurons, but not their perineuronal nets, a type of extracellular matrix that maintains both the start and stop of developmental plasticity, at P21. This suggests that epileptogenesis occurs before the loss of inhibitory interneurons. ***Therefore, $Rheb^{ca}$ causes upregulation in MTORC1 by P0 that leads to irregular neuronal growth, cortical dyslamination, and axonal proliferation by P2. Irregular network activity, network synchronization, and epileptogenesis start by P7, and cortical interneuron loss is a result of DRE from MCDs.***

Disclosures: P. Hoffman: None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.18/A64

Topic: A.07. Developmental Disorders

Title: Exploring Translational Opportunities Through Phenotypic Characterization in a Mouse Model of CDKL5 Deficiency Disorder

Authors: *M. TRUJILLO[1], P. B. MARTIN[2];
[1]The Jackson Lab., Sacramento, CA; [2]Rare Dis. Translational Ctr., The Jackson Lab., Bar Harbor, ME

Abstract: CDKL5 Deficiency Disorder (CDD) is a rare X-linked neurodevelopmental disorder caused by mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene. Occurring one in 40,000 to 60,000 live births, CDD presents with cognitive and motor impairments, epileptic symptoms, and developmental delays. Therapeutic options for CDD remain limited. In the present study, a mouse model of CDD (JAX Stock#021967) was assessed as a translational model for preclinical drug discovery. Preclinical studies utilizing animal models are fundamental for understanding disease progression and evaluating potential treatments. A thorough battery of behavioral assays were used to characterize disease progression in a mouse model of CDD. Capturing the phenotypic spectrum can be used to inform relevant timing of endpoints that can be used for evaluating potential prophylactics or therapeutics that may have the potential to treat symptoms of CDD. Translationally relevant assays were chosen to recapitulate symptoms associated with humans with CDD as measured via light-dark exposure, open field, contextual fear conditioning, nesting behavior, marble burying, and hind-limb clasping. CDD model mice demonstrated enhanced anxiety-like behavior (light-dark exposure) and anxiogenic behavior (open field) as early as 5 weeks of age. Impaired learning and memory (contextual fear conditioning) and nesting behavior were significantly different from wild-type littermate controls as early as 6 weeks. The age of onset of phenotypes are important for informing the therapeutic window for evaluating potential treatments in preclinical therapeutic studies. The present study identified robust and reproducible behavioral phenotypes that are analogous to key aspects of human CDD symptoms. Leveraging reproducible phenotypes in mouse models of CDD offers a promising avenue for accelerating the development of treatment options such as small molecules and gene and cell therapies aimed at improving outcomes for individuals with CDD and potentially a cure.

Disclosures: M. Trujillo: A. Employment/Salary (full or part-time):; The Jackson Laboratory.
P.B. Martin: A. Employment/Salary (full or part-time):; The Jackson Laboratory.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.19/A65

Topic: A.07. Developmental Disorders

Title: Neonatal Opioid Withdrawal Syndrome (NOWS): Prenatal Morphine Exposure Dose-Dependently Changes Complex Ultrasonic Vocalization (USV) Characteristics in Novel Preclinical Model of NOWS

Authors: *S. STEVENS, S. MOHAN;
Biomed. Sci., Baptist Univ. Col. of Osteo. Med., Memphis, TN

Abstract: As the opioid epidemic continues to abound, opioid use in pregnant women is also on the rise. In turn, an increase in infants born with neonatal opioid withdrawal syndrome (NOWS) has also been observed. Though the incidence of NOWS continues to rise, our understanding of the short and long-term consequences of in-utero opioid exposure remains limited. Our lab developed a novel preclinical model of NOWS to understand the short-term withdrawal behaviors in a unique rodent species, *Acomys cahirinus,* more commonly known as spiny mice. Spiny mice offspring were assessed for a series of withdrawal behaviors, including ultrasonic vocalizations (USVs). Here we aim to gain a greater understanding of the effects of prenatal opioid exposure on USVs by examining differences in wave morphology in spiny mice experiencing spontaneous opioid withdrawal. To model maternal opioid use, dams were treated daily with saline or morphine 10 and 30 mg/kg S.C. beginning on G19 until day of birth. USVs were recorded daily for the first eight days of life (P0-P7), using an Echo Meter®. Recorded USVs were processed using the open-source software DeepSqueak® to quantify and characterize calls based on wave morphology, duration, and frequency. Preliminary data showed that morphine exposed offspring emitted a greater number of USVs compared to saline exposed mice. Additionally, differences in the number of calls were observed based on gender, dosage, and postnatal day in morphine exposed offspring. The trained neuronal network detected differences in wave morphology in male and female offspring exposed to morphine compared to saline; these mice were also shown to emit USVs at higher frequencies and for shorter durations. These data provide support that measuring complex wave morphology of USV's can be used to understand opioid withdrawal in a novel rodent model of NOWS.

Disclosures: S. Stevens: None. **S. Mohan:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.20/A66

Topic: A.07. Developmental Disorders

Support: NIH Grant EB028319

Title: Vascular patterning in sensory cortex following developmental seizures

Authors: *A. REID[1], Y. SHEN[2], J. A. FISHER[2];
[1]New York Med. Col., Valhalla, NY; [2]Physiol., New York Med. Col., Valhalla, NY

Abstract: Developmental mechanisms adjust cerebral microvasculature patterning to match regional metabolic demands during limited time windows that align with cortical circuit maturation. Postnatal maturation of cortical microvascular networks is influenced by transient perturbations of neuronal excitability. For example, pharmacologically induced seizures at precise times during cortical development have been show to have a lasting effect on microvascular patterning in the adult brain. Here, we aim to characterize changes in cortical microvasculature in a transgenic mouse model of non-convulsive seizures that emerge concurrent with different maturational stages of specific brain areas. We use in vivo optical coherence tomography angiography (OCT-A) imaging and 3D vessel reconstruction to relate developmental cortical hyperexcitability, documented with electroencephalography (EEG) recordings, to sustained changes in cortical vascular architecture. Our results contribute to a growing literature linking developmental experience to the long-term patterning of neuronal and vascular architecture.

Disclosures: A. Reid: None. **Y. Shen:** None. **J.A. Fisher:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.21/A67

Topic: A.07. Developmental Disorders

Support: The intellectual and Developmental Disabilities Research Center at Boston Children's Hospital
Luke Heller Foundation
Jordan Avi Ogman Foundation

Title: Newly Developed TECPR2 Knock in Mouse Model for Understanding the Mechanisms of Autophagy-Related Neurodegenerative Disorders

Authors: *N. HODGSON[1], B. TURNES[2], L. MEJIA[2], E. G. CROPPER[5], P. CASEY-CAPLAN[3], T. BERRY[2], S. DE LIMA[6], Z. ZHANG[7], B. ZHANG[2], N. ANDREWS[8], C. J. WOOLF[9], M. FAGIOLINI[4];
[1]Boston Children's Hosp., Boston, MA; [2]Boston Children's Hosp., Boston, MA; [3]Neurobio., Boston Children's Hosp., Westborough, MA; [4]Neurol., Boston Children's Hosp., Boston, MA; [5]Neurobio., [6]Neurosurg., Boston Childrens Hosp., Boston, MA; [7]Neurobio., Harvard Med. Sch. Grad. Program In Neurosci., BOSTON, MA; [8]Salk Inst., San Diego, CA; [9]Neurobio., Children's Hosp. Boston, Boston, MA

Abstract: Autophagy is a crucial cellular process for maintaining neuronal health by clearing out damaged proteins and organelles. Recent studies have identified a potential connection between

Tectonin Beta-Propeller Repeat Containing 2 (TECPR2) mutations and autophagy dysfunction in neurodegenerative diseases. TECPR2 is a key regulator of autophagy, and mutations in this gene have been associated with impaired autophagic flux and neurodegeneration. Dysregulation of autophagy has been linked to various neurodegenerative diseases, including Alzheimer's, Parkinson's, and hereditary spastic paraplegia (HSP). HSP is estimated to affect 9.6 out of 100,000 individuals and some features of the syndrome include developmental delay and later intellectual disability, decreased sensitivity to pain and autonomic dysfunction, with progressive central apneas being the most common cause of death. There are currently no approved therapies for *TECPR2*-related disorder and treatments are thus purely symptomatic. To identify behavioral abnormalities, *Tecpr2* KI mice and WT littermates underwent behavioral analyses, measuring motor (grip strength and gait), sensory (tactile sensory threshold) and auditory (acoustic startle responses and auditory brainstem reflexes) function. To longitudinally assess the histopathological phenotype of the animals *Tecpr2* KI and WT littermate mice were perfused and brain and spinal cord collected and fixed for histochemical (hematoxylin and eosin), immunohistochemical (autophagy, SQSMT/p62; neurofilament aggregation, NF-200) and ultrastructural analysis (transmission electron microcopy) at multiple time points (P30, P60, P90, >P120). *Tecpr2* KI mice display higher tactile sensory threshold, tested using von Frey filaments; multiple gait abnormalities measured using a treadmill-based system, Digigait®, starting as early as P45 and worsening with age; and acoustic startle and auditory brainstem reflex (ABR) responses were significantly depressed in the mutant mice at P>120 compared with WT littermate controls. *Tecpr2* KI mice also display age-related progressive appearance of axonal swellings (spheroids) in the gracile and cuneate nuclei of the medulla oblongata, as well aberrant neurofilament and SQSMT/p62 aggregation. *Tecpr2* KI mice exhibit phenotypes which parallel patient abnormalities. Taken together these data indicate the *Tecpr2* KI mouse model show common features of *TECPR2*-related disorder, providing an opportunity for rigorous testing of novel therapeutic approaches including AAV9-mediated gene replacement.

Disclosures: N. Hodgson: None. **B. Turnes:** None. **L. mejia:** None. **E.G. Cropper:** None. **P. Casey-Caplan:** None. **T. Berry:** None. **S. de Lima:** None. **Z. Zhang:** None. **B. Zhang:** None. **N. Andrews:** None. **C.J. Woolf:** None. **M. Fagiolini:** None.

Poster

PSTR203: Animal Models of Developmental Disorders Other Than Autism

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR203.22/A68

Topic: A.07. Developmental Disorders

Support: AFM-Telethon 24997

Title: The impact of polyamine imbalance on neurological manifestations and prospect of gene therapy for Snyder-Robinson syndrome

Authors: O. AKINYELE[1], M. JOHNSON[1], A. NWAFOR[2], T. MURRAY STEWART[2], ***D. KEMALADEWI**[1,3];
[1]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Johns Hopkins Univ., Baltimore, MD; [3]UPMC Children's Hospital of Pittsburgh, Pittsburgh, PA

Abstract: Snyder-Robinson syndrome (SRS) is a rare X-linked recessive disorder caused by a mutation in the *SMS* gene, which encodes spermine synthase (SMS), a protein crucial in polyamine metabolism. SRS-affected individuals have intellectual disability, thin habitus, seizure, low muscle tone/hypotonia, and osteoporosis. The SMS protein catalyzes the conversion of the polyamine spermidine to spermine; thus, the lack of SMS in SRS leads to the accumulation of spermidine with little or no spermine. How polyamine imbalances underlie many of the clinical features of SRS remains unclear, which hampers the development of therapeutic approach.
In this study, we investigate the impact of polyamine imbalance on clinical manifestations of SRS using a mouse model, as well as develop and evaluate *SMS* gene therapy as a potential treatment approach.
First, we assess the molecular and neurological presentations in the G56S mouse model, which carries a missense mutation in the *Sms* gene and mimics a variant found in patients. We found that the lack of SMS protein in the G56S mice resulted in increased spermidine/spermine ratio, failure to thrive, short stature, and reduced bone density. They showed impaired learning capacity, increased anxiety, reduced mobility, and heightened fear responses, accompanied by reduced total and regional brain volumes. Furthermore, transcriptomic analysis revealed impaired mitochondrial oxidative phosphorylation in the G56S cerebral cortex, which was recapitulated in G56S fibroblasts and SMS-null hippocampal cells.
Subsequently, we developed a gene therapy approach using adeno-associated viral vector serotype 9 (AAV9) and evaluated its efficacy in the SRS models. Upon treatment, we were able to restore the expression of SMS and polyamine levels, rescuing the mitochondrial functions. Ongoing experiments involve molecular and functional evaluations of *SMS* gene therapy in the G56S mice.
Collectively, our study establishes the suitability of the G56S mice as a preclinical model for SRS and provides a set of molecular and functional outcome measures that can be used to evaluate therapeutic interventions for SRS, including but not limited to *SMS* gene therapy described here.

Disclosures: O. Akinyele: None. **M. Johnson:** None. **A. Nwafor:** None. **T. Murray Stewart:** None. **D. Kemaladewi:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.01/A69

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: The Effects of Cortisol on Glutamatergic Transmission in Caenorhabditis elegans

Authors: *D. COATES[1], T. YAHOUEDEOU[1], A. J. KALLARACKAL[2];
[1]Mount St. Mary's Univ., Emmitsburg, MD; [2]Psychology, Mount St. Mary s Univ., Emmitsburg, MD

Abstract: Cortisol is a natural stress hormone in the human body. It is a known biomarker for the development of neurological and psychiatric disorders. Cortisol impacts the nervous system in a variety of ways, including modulating glutamatergic transmission as well as learning and memory. Corticosterone (CORT) has the capacity to mediate glutamatergic functioning underlying stress-induced behavioral phenotypes. *C.elegans* can be used as a model due to their transparency and complete mapped-out neuron count. We used a combination of calcium imaging, optogenetics, and behavioral analyses to determine the role of CORT on glutamatergic signaling. Additionally, we sought to identify signaling molecules involved with CORT-mediated effects on glutamatergic transmission using a candidate genetic screen. Results showed an indication of a moderate effect and positive trend of cortisol treatment of glutamatergic activity of neurons in the worms. CORT significantly increases reversal behavior. However, CORT is not acting through *sgk-1* to mediate glutamatergic expression.

Disclosures: D. Coates: None. **T. Yahouedeou:** None. **A.J. Kallarackal:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.02/A70

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant Z01 HD008914

Title: Neto proteins differentially modulate the gating properties of Drosophila NMJ glutamate receptors

Authors: *T. HAN[1], R. VICIDOMINI[2], M. L. MAYER[3], M. SERPE[4];
[1]NICHD/Eunice Kennedy Shriver Nat'l Inst. of Child H, NIH, Bethesda, MD; [2]Section on metabolic regulation, NIH/NCHID, Bethesda, MD; [3]LCMN NICHD, LCMN NICHD NIH, Bethesda, MD; [4]Natl. Inst. of Child Hlth. and Human Develop., NIH, Bethesda, MD

Abstract: The formation of functional synapses relies on ion channels and their accessory proteins which control where, when, and how receptors and channels function. The auxiliary protein Neto (Neuropillin and Tolloid-like) modulates the function of kainate-type glutamate receptors in vertebrates as well as at the *Drosophila* neuromuscular junction (NMJ), a glutamatergic synapse widely used for genetic studies on synapse development. The fly NMJ relies entirely on kainate-type glutamate receptors and uses at least 5 different subunits to form two types of postsynaptic receptors, type A and -B, which contain either GluRIIA or GluRIIB

subunits, plus GluRIIC, GluRIID and GluRIIE. We previously reported the functional reconstitution of fly NMJ glutamate receptors in *Xenopus* oocytes (Han et al, PNAS, 2015) and showed that Neto is an essential component required for receptor function. Here, using outside-out patch-clamp recordings and fast ligand application, we examine the biophysical properties of recombinant *Drosophila* NMJ receptors expressed in HEK293T cells, and compare them with native receptor complexes of defined composition. The two Neto isoforms, Neto-α and Neto-β, differentially modulate the gating properties of NMJ receptors and their behavior in the presence of toxins and endogenous polyamines. Surprisingly, we found that the decay of synaptic currents is much slower than the rate of iGluR deactivation. Our studies demonstrate that Neto is not only required for function of NMJ iGluRs but also increases the functional repertoire of these ion channels.

Disclosures: T. Han: None. **R. Vicidomini:** None. **M.L. Mayer:** None. **M. Serpe:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.03/A71

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: the World Premier International Research Center Initiative (WPI), MEXT, Japan
JSPS KAKENHI JP20K21122
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the Mochida Memorial Foundation for Medical and Pharmaceutical Research
the Uehara Memorial Foundation
JSPS Bilateral Program JPJSBP120247404

Title: Single-molecule nanodynamics of AMPA-type glutamate receptors by high-speed atomic force microscopy

Authors: *M. SHIBATA[1], A. SUMINO[2], T. SUMIKAMA[1], M. HATTORI[3];
[1]Nano Life Sci. Inst., Kanazawa Univ., Kanazawa, Japan; [2]Kanazawa Univ., Kanazawa, Japan; [3]Fudan Univ., Shanghai, China

Abstract: Structural biology elucidates the three-dimensional atomic structures of proteins, shedding light on their molecular mechanisms. Furthermore, the visualization of protein flexibility can enhance our understanding of how proteins function. High-speed atomic force microscopy (HS-AFM) is a capable technique to directly visualize the flexible regions of single molecules under near physiological conditions, with a temporal resolution of ~ 100 millisecond at a nanometer resolution (*1-3*).α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptors (AMPARs) are crucial for rapid excitatory synaptic transmission by

localizing to the postsynaptic density of glutamatergic spines. AMPARs possess large extracellular N-terminal domains (NTDs), which participate in AMPAR clustering at synapses. However, the dynamics of these NTDs and the molecular mechanism governing their synaptic clustering remain elusive. In this study, we employed HS-AFM to directly visualize the conformational dynamics of NTDs in the GluA2 subunit of AMPARs with TARP γ2 in lipid environments. Our HS-AFM videos of GluA2-TARP γ2 in both resting and open states showed fluctuations in NTD dimers. In contrast, in the desensitized state, the two NTD dimers adopted a separated conformation with reduced fluctuations. Notably, we observed individual NTD dimers transitioning into monomers. This NTD-dimer splitting resulted in intersubunit exchange between NTD dimers and increased the number of binding sites for the synaptic protein neuronal pentraxin 1. Consequently, our findings illuminate the significance of NTD dynamics in the synaptic clustering of AMPARs (4). References: [1] T. Ando *et al*., Filming biomolecular processes by high-speed atomic force microscopy. *Chem. Rev. 114*, 3120-3188 (2014). [2] M. Shibata *et al.*, Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy. *Nat. Commun. 8*, 1430 (2017). [3] S. Tsujioka *et al.*, Imaging single CaMKII holoenzymes at work by high-speed atomic force microscopy. *Sci. Adv. 9*, eadh1069 (2023). [4] A. Sumino *et al*., "High-speed AFM reveals fluctuations and dimer splitting of the N-terminal domain of GluA2-γ2" *bioRxiv (2023).*

Disclosures: M. Shibata: None. **A. Sumino:** None. **T. Sumikama:** None. **M. Hattori:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.04/A72

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Intramural Award to CJM

Title: Regulation of interneurons by GluN1/GluN3a excitatory glycine receptors influences hippocampal network rhythms throughout development

Authors: *J. KIM[1], A. VLACHOS[2], V. MAHADEVAN[2], R. CHITTAJALLU[2], S. HUNT[2], T. G. BANKE[3], D. LIU[3], H. YUAN[3], S. F. TRAYNELIS[3], D. LIOTTA[4], B. B. AVERBECK[5], K. A. PELKEY[2], C. J. MCBAIN[2];
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Abstract: Nonconventional GluN1/GluN3a excitatory glycine receptor (eGlyR)-mediated currents were recently reported to tonically influence excitability of neurons in the cortex, medial habenula, and amygdala. These N-methyl-D-aspartate receptors (NMDARs) are distinct from the GluN2-containing conventional NMDARs, in being voltage-independent and gated solely by

glycine. Within the hippocampus, amongst various neuronal types, somatostatin-expressing interneurons (SST-INs) express remarkably high levels of *Grin3a*, the gene encoding GluN3a, throughout development, as do *Lamp5* (putative neuropeptide Y (NPY)-expressing) neurogliaform cells (NPY-NGFCs). We found that the excitability of SST- and NPY-NGFCs is strongly influenced by eGlyRs from early postnatal to adult ages. Critically, eGlyR-mediated excitation of hippocampal interneurons dramatically increased GABAergic tone onto pyramidal neurons in the developing hippocampus, with consequences for the generation of giant depolarizing potentials (GDPs). GDPs are intrinsically generated synchronous occurring rhythmic waves necessary for synaptic maturation through activity dependent plasticity in nascent hippocampal and cortical networks between birth to about 10 postnatal days. In the mature hippocampus, eGlyR-mediated excitation of SST-INs was found to regulate sharp wave ripples (SWRs), network oscillations associated with memory consolidation. Collectively, the data suggest that eGlyRs influence SST-/NPY-IN excitability to regulate synchronized network rhythms associated with circuit and memory formation, yielding novel insight into physiological roles of the notoriously enigmatic GluN3A subunit. In addition, we confirmed eGlyR expression and excitation of SST-INs and the influence on SWRs in non-human primate hippocampus, illustrating evolutionary conservation of eGlyR function.

Disclosures: J. Kim: None. **A. Vlachos:** None. **V. Mahadevan:** None. **R. Chittajallu:** None. **S. Hunt:** None. **T.G. Banke:** None. **D. Liu:** None. **H. Yuan:** None. **S.F. Traynelis:** None. **D. Liotta:** None. **B.B. Averbeck:** None. **K.A. Pelkey:** None. **C.J. McBain:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.05/A73

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: The Intramural Research Programs of the National Eye Institute, NIH

Title: Extracellular components of the AMPA receptor complex: mutual interactions and retinal effects

Authors: *S. TOMAREV[1], N. NAKAYA[2], P. JOHNSON[2], D. KUMAR[2];
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Abstract: Olfactomedin 1-3 (Olfm1-3), brorin (Vwc2), and neuritin are extracellular components of the AMPA receptor (AMPAR) complex in the brain and retina. Previous data demonstrated that Olfm1-3 interact with AMPARs on the extracellular side as homo- and hetero-tetramers and recruit the GPI-anchored neuritin and the secreted brorin to the AMPAR complexes. AMPARs of triple *Olfm1-3* knockout mice were entirely deprived of neuritin and brorin (*Neuron* 111, 2544). The objective of this study was to investigate presumed direct

interactions of Olfm1 and neuritin with the core subunits of the AMPARs and with each other and determine effects of *Olfm1-3* knockout on the activity of retinal ganglion cells. Known 3D structures of GluA2 and Olfm1 have been used to build a model of their interaction employing molecular docking studies. According to this model, a dimer of Olfm1 interacts with the extracellular N-terminal domain of GluA2 through the olfactomedin domains. Direct interaction of Olfm1 and GluA2 was confirmed in co-transfection experiments *in vitro* using COS-7 cells. The olfactomedin domain of Olfm1 is also essential for interaction with neuritin in transfected cells and dimerization of Olfm1 enhanced their interaction. In the retina, Olfm1, neuritin, and GluA2 showed overlapping expression pattern both at RNA and protein levels. Retinas of adult *Olfm1*, *Olfm2* and *Olfm3* knockouts demonstrated partially overlapping but not identical spectra of mRNA changes as compared with wild-type retinas. *Olfm1* knockout samples demonstrated more changes compared with wild-type retinas (71 upregulated and 92 downregulated mRNAs; fold-change ≥ 1.5, FDRP ≤ 0.05, MGM ≥ 5) than *Olfm2* and *Olfm3* knockout retinas. The most affected functional changes were connected to extracellular proteins, cell adhesion, cytoskeleton proteins and glutamatergic synapses. *Olfm1* knockout and especially *Olfm1-3* triple knockout in mice lead to changes in retinal ganglion cell physiological activity and synaptic remodeling with more pronounced effects observed in several retinal ganglion cell subtypes. These data indicate that the extracellular components of the AMPAR complex play an important role in retinal physiology.

Disclosures: S. Tomarev: None. **N. Nakaya:** None. **P. Johnson:** None. **D. Kumar:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.06/A74

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH grant K99 MH132811
NIH grant R37 NS036715
NIH grant R01 GM094495
NIH grant R35 GM122528
NIH grant F99NS130928

Title: Novel Structural Features of AMPAR Auxiliary Subunit TARPγ2

Authors: *D. HALE[1], R. L. HUGANIR[2], E. TWOMEY[1], V. JAYARAMAN[3];
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Abstract: Excitatory neurotransmission is principally mediated by AMPA-subtype ionotropic glutamate receptors (AMPARs). Negative allosteric modulators are therapeutic candidates in that

inhibit AMPAR activation and can compete with positive modulators to control AMPAR function through unresolved mechanisms. Here, we show that allosteric inhibition pushes AMPARs into a distinct state that prevents both activation and positive allosteric modulation. We used cryo-electron microscopy to capture AMPARs bound to glutamate while a negative allosteric modulator, GYKI-52466, and positive allosteric modulator, cyclothiazide, compete for control of AMPARs. GYKI-52466 binds in the ion channel collar and inhibits AMPARs by decoupling the ligand-binding domains from the ion channel. The rearrangement of the ligand-binding domains ruptures the cyclothiazide site, preventing positive modulation. Our data provide a framework for understanding allostery of AMPARs and for rational design of therapeutics targeting AMPARs in neurological diseases.

Disclosures: D. Hale: None. **R.L. Huganir:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurmora Therapeutics. F. Consulting Fees (e.g., advisory boards); Neumora Therapeutics, MAZE Therapeutics. **E. Twomey:** None. **V. Jayaraman:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.07/A75

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NS113530
HD082373
AG075444
AG079956
AG081401
NS111619

Title: The GluN2C/GluN2D NMDA Receptor Positive Allosteric Modulator (+)-EU1180-453 Enhances Interneuron Output

Authors: *S. F. TRAYNELIS;
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Abstract: N-methyl-D-aspartate receptors (NMDARs) comprise a family of ligand-gated ionotropic glutamate receptors that mediate a slow, calcium-permeable component to excitatory neurotransmission. Most NMDARs are heterotetrameric assemblies of two obligate GluN1 subunits and two GluN2 subunits, which can be any combination of GluN2A-D. The GluN2D subunit shows limited expression in the adult brain and is enriched in GABAergic interneurons. Interneuron dysfunction and diminished levels of inhibition contribute to multiple neurological diseases, suggesting that GABAergic interneuron enhancing treatments have therapeutic utility, making GluN2D modulation an attractive drug target. Here, we describe the actions of an

improved GluN2C/GluN2D-selective positive allosteric modulator (PAM), (+)-EU1180-453, which has enhanced drug-like properties such as increased aqueous solubility compared to our first-in-class GluN2C/GluN2D-selective prototypical PAM (+)-CIQ. (+)-EU1180-453 doubles the NMDAR response at lower concentrations (< 10 μM) than (+)-CIQ, and produces a greater degree of maximal potentiation at 30 μM. Using *in vitro* electrophysiological recordings, we show that (+)-EU1180-453 potentiates triheteromeric NMDARs containing at least one GluN2C or GluN2D subunit, and is active at both exon5-lacking and exon5-containing GluN1 splice variants. (+)-EU1180-453 increases glutamate potency and efficacy for GluN2C/GluN2D-containing NMDARs, and prolongs the deactivation time course. We show that (+)-EU1180-453 selectively doubles synaptic NMDAR-mediated charge transfer onto P11-15 CA1 *stratum radiatum* hippocampal interneurons, but is without effect on CA1 pyramidal cells. This increased charge transfer produces enhances inhibitory output from GABAergic interneurons onto CA1 pyramidal cells by increasing sIPSC frequency by 1.4-fold and increasing the IPSP-to-EPSP ratio by 2.8-fold. Thus, (+)-EU1180-453 can enhance overall circuit inhibition, which could prove therapeutically useful for treatment of epilepsy, autism, depression, schizophrenia, and other neurological disorders.

Disclosures: S.F. Traynelis: 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.; SRA from Biogen, Janssen, and GRIN Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-Owner of Emory-owned PI. Co-Founder of NeurOp and Agrithera. F. Consulting Fees (e.g., advisory boards); SAB member for Sage Therapeutics, Eumentis Therapeutics, Neurocrine, GRIN2B Foundation, CureGRIN Foundation, and CominedBrain. Consultant for GRIN Therapeutics..

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.08/A76

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Functional characterization of GRIN2B variants using automated patch clamp technology

Authors: C. KADI[1], E. PARKER[1], C. BROWN[1], L. PISAREK[1], S. RICE[1], R. LONG[1], R. FINOCCHIARO[1], D. DALRYMPLE[1], R. MACNAIR[3], I. MCPHEE[1], S. F. TRAYNELIS[4], D. PAU[2], *L. HUTCHISON[1];
[1]SB Drug Discovery, Glasgow, United Kingdom; [2]SB Drug Discovery, Glasgow, ; [3]GRIN Therapeut., New York, NY; [4]Dept Pharmacol, Sch. of Med., Atlanta, GA

Abstract: N-methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptors composed of two Glycine-binding NR1 subunits (encoded by GRIN1 gene) in combination with

two Glutamate-binding NR2 subunits (encoded by GRIN2A, GRIN2B, GRIN2C or GRIN2D genes). Rare de novo variants of the GRIN genes have been associated with neurodevelopmental disorders (NDD) and epileptic encephalopathy resulting in seizures, behavioural symptoms and movement disorders. Current investigations are focusing on functional and pharmacological analyses to understand the properties of these variants and potentially lead to a more rapid classification of GRIN variants as gain-of-function (GoF) or loss-of-function (LoF). The classification of these variants from patients may provide diagnostic advantages and together with precision medicine could enable the development of a personalised therapy.
The functional analysis of these variants includes at least six different assays which aim to investigate the receptor's sensitivity to the agonists Glutamate and Glycine, extracellular Mg^{2+} inhibition, alterations in response time-course (e.g. kinetics), channel open probability and trafficking to the plasma membrane. The results of these assays are required to determine the variant classification (Myers *et al*., 2023).
Until now, the functional analysis of the GRIN variants has been restricted to the conventional manual patch-clamp and two electrode voltage techniques. Although this approach has successfully been used to classify variants, the low throughput slows the rapid screening that may be required for target-directed pharmacological treatments. Here, using automated patch-clamp technology, we describe the functional and pharmacological characterization of human embryonic kidney (HEK) cells transfected with GRIN1A (wild-type) or GRIN1-A652C in combination with the GoF variant GRIN2B-S810R or LoF variant GRIN2B-E413G.
The aim of this work is to demonstrate the capability of the automated patch-clamp system to characterise variants as LoF or GoF. In the future, the establishment of a higher throughput assay will enable a faster evaluation of the GRIN variants, where multiple variants can be assessed simultaneously, while obtaining robust data. Furthermore, it is expected that this time efficient approach can be applied more generally in a wide range of ion channels for the functional classification of other missense variants related to neurological disorders.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.09/A77

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant AG065813

Title: Familial Alzheimer's disease mutations in amyloid precursor protein impair calcineurin signaling to NMDA receptors.

Authors: *S. J. TAVALIN;
Pharmacology, Addiction Sci., & Toxicology, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Familial Alzheimer's disease (FAD) is frequently associated with mutations in the amyloid precursor protein (APP), which are thought to lead to cognitive deficits by impairing NMDA receptor (NMDAR)-dependent forms of synaptic plasticity. Given the reliance of synaptic plasticity on NMDAR-mediated Ca^{2+} entry, shaping of NMDAR activity by APP and/or its disease-causing variants could provide a basis for understanding synaptic plasticity impairments associated with FAD. A region of APP (residues 638-644 within APP695) processed by the γ-secretase complex, which generates amyloid β (Aβ) peptides, is a hotspot for FAD mutations. This region bears similarity to a binding motif for calcineurin (CaN), a Ca^{2+}/calmodulin-dependent phosphatase. Interaction assays confirm that APP associates with CaN in native tissue as well as in a heterologous expression system. This capacity to bind CaN extends to APP family members amyloid precursor-like protein 1 and amyloid precursor-like protein 2 (APLP1 and APLP2, respectively). Electrophysiological analysis demonstrates that APP and its family members limit NMDAR activity, in a manner consistent with CaN-dependent regulation of NMDAR desensitization. FAD mutations, in this region of APP, impair this regulation and consequently enhance NMDAR activity. Thus, by altering the landscape for CaN regulation of NMDA receptors, FAD mutations in APP may contribute to faulty information processing by modifying the dynamic range and temporal window of a critical signal for synaptic plasticity.

Disclosures: S.J. Tavalin: None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.10/A78

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH grant R01GM128195
NIH grant T32NS007433
Andrew W. Mellon Predoctoral Fellowship

Title: Membrane to channel path of NMDA receptor inhibition by intracellularly applied MK-801

Authors: *E. G. NEUREITER, A. NIGAM, P. FENZA, J. W. JOHNSON;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: N-methyl-D-aspartate receptors (NMDARs) are a type of excitatory ligand-gated ion channel that are vital in synaptic communication, learning, and memory. NMDAR hyperactivity has been implicated in a range of diseases, notably Alzheimer's disease and cell death following stroke. NMDAR hyperactivity can be ameliorated using channel blocking drugs, which physically block the flow of ions by binding near the Q/R/N site within the NMDAR channel. Traditionally, this class of drugs (which includes memantine, ketamine, and MK-801) has been understood to act by entering the NMDAR channel directly from the extracellular space. However, many channel blocking drugs are also able to access the NMDAR channel via a second path, a process known as membrane to channel inhibition (MCI). In MCI, uncharged drug molecules first access the plasma membrane before moving into the activated NMDAR channel through a path in the side of the receptor. MCI has only been studied using extracellular drug application; it is unknown whether MCI can occur with intracellular drug application. However, intracellular application of MK-801 (iMK-801) is a common experimental method to inhibit NMDARs, even though it is unknown whether iMK-801 inhibits NMDARs via traditional block, MCI, or another mechanism. We used whole-cell patch-clamp electrophysiology in tsA201 cells transfected to express NMDARs to investigate our hypothesis that NMDAR inhibition by iMK-801 is mediated by MCI. We developed a protocol to quantify NMDAR inhibition by iMK-801, with which we measured peak NMDAR current and steady state current after iMK-801 induced inhibition. MK-801 does not inhibit NMDARs prior to channel opening and binds to the channel relatively slowly; therefore, we could measure an uninhibited peak current before inhibition by iMK-801. We studied whether iMK-801 binds to the blocking site that mediates MCI using site-directed mutagenesis of the GluN1 Q/R/N site. We also found that iMK-801 IC_{50} depended on NMDAR subtype: GluN1/2D receptors had an approximately 8-fold higher IC_{50} as compared to GluN1/2A receptors, while GluN1/2B and GluN1/2C receptors had approximately 2-fold higher IC_{50}s as compared to GluN1/2A receptors. NMDAR subtype dependence of MCI by extracellular MK-801 appears to be similarly strong, in contrast to the weaker subtype dependence of traditional block by MK-801. Finally, we developed a kinetic model of GluN1/2A receptor inhibition to determine whether properties of inhibition by iMK-801 are consistent with MCI. The data from these investigations support our hypothesis that inhibition by iMK-801 acts through MCI and will deepen our understanding of an important class of drug.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.11/A79

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR
NSERC

Title: Developmental defects in nanoscale organization of AMPARs and quantal transmission in a mouse model of fragile X syndrome

Authors: *M. GURMA[1,2], A. BODALIA[3], A. FEKETE[3], L.-Y. WANG[1,2];
[1]Dept. of Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Neurosciences & Mental Health, The Hospital for Sick Children, Toronto, ON, Canada; [3]Neurosciences & Mental Hlth., The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Excitatory synapses undergo rapid remodeling during early sensory development by changing the abundance, composition, and nano-organization of postsynaptic glutamate receptors to enable fast neurotransmission. Dysregulation of synaptic remodeling can lead to neurodevelopmental disorders such as fragile X syndrome (FXS), caused by a mutation in the *Fmr1* gene encoding the fragile X messenger ribonucleoprotein 1 (FMRP). It is entirely unknown how FMRP deletion impacts the nano-organization of AMPARs and quantal transmission during early synaptic development. To answer this question, we used the calyx of Held synapse in the auditory brainstem, where AMPARs undergo a developmental subunit switch from slow-gating GluA1-dominant to fast-gating GluA4-dominant. We applied expansion microscopy (ExM) to map nanoscale differences in subsynaptic localization of homomeric GluA1- and GluA4-AMPARs between wild-type (WT) and *Fmr1*$^{-/-}$ mice at pre- (P8-10) and post-hearing (P16-19) stages. Patch-clamp recordings from the principal MNTB cell in the calyx of Held synapse were performed for each age group and genotype to measure miniature excitatory postsynaptic currents (mEPSCs). We found that nanoclusters of GluA1- and GluA4-AMPARs in peri-synaptic and synaptic domains are localized in a mutually exclusive pattern in WT synapses, which is altered in *Fmr1*$^{-/-}$ synapses. In parallel, a bimodal distribution of fast and slow mEPSCs in immature *Fmr1*$^{-/-}$ synapses phenocopies that of mature WT synapses. Basal mEPSC frequency was also altered and less sensitive to an elevation of extracellular Ca^{2+} in *Fmr1*$^{-/-}$ synapses, indicating a phenotypic difference in presynaptic remodeling. These findings indicate that a loss of FMRP accelerates the developmental organization of both pre-and postsynaptic elements underlying quantal transmission.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.12/B1

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIAAA - U10AA08401

Title: Investigating NMDA-R dependent neuronal activity in human NGN2 induced glutamate neurons

Authors: *A. TENGOLICS[1], I. GAMEIRO-ROS[1], I. PRYTKOVA[2,1], K. JACOBS[1], Z.-P. PANG[3], R. P. HART[4], A. M. GOATE[2], P. A. SLESINGER[1];
[1]Dept. of Neurosci., [2]Dept. of Genet. and Genomic Sci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [3]Child Hlth. Inst. of New Jersey, Rutgers Univ., New Brunswick, NJ; [4]Cell Biol. & Neurosci., Rutgers, The State Univ. of New Jersey, Piscataway, NJ

Abstract: N-methyl-D-aspartate receptors (NMDA-Rs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-Rs) comprise the major ionotropic glutamatergic receptors in the brain. NMDA receptors are unique because they are blocked by Mg^{2+} ions under physiological conditions and require binding of glutamate and membrane depolarization to remove the blockade and allow conduction through the open channel. This "coincidence detection" of NMDA receptors makes them important for long term potentiation, and involvement in reward circuits underlying conditions like alcohol use disorder (AUD). NMDA-R dependent activity has not been fully explored in human neurons. Our goal was to develop a model system based on NMDA-R dependent activity, which can be used later to discover new therapeutics for those disorders. Induced pluripotent stem cells (iPSC) from three healthy individuals were selected from COGA (Collaborative Studies on the Genetics of Alcoholism) participants and differentiated into glutamatergic neurons using the Neurogenin-2 protocol/NGN2 and then co-cultured with glial cells. After 11-12 weeks, we measured population neuronal activity (using Ca^{2+}-imaging and GCaMP8f expression) and single-cell activity (patch clamp electrophysiology) under different buffer conditions. In Ca^{2+}-imaging experiments, a dramatic increase in Ca^{2+} spike activity was observed in Mg^{2+}-free aCSF, which was further increased with glycine, a NMDA-R positive allosteric modulator. Blocking AMPA-Rs with NBQX decreased the activity, implicating AMPA-Rs in the high firing rate. Blocking NMDA-Rs with APV completely eliminated the activity. We observed high synchrony in firing between neurons in Mg^{2+}-free conditions. Patch-clamp experiments corroborated Ca^{2+} imaging experiments, and also revealed different proportion of firing types (e.g. bursts vs tonic) between lines. We observed differences in the general increase in activity in Mg^{2+}-free aCSF between the three lines, and these differences were also consistent between the different experimental approaches. Our model using stem cell-derived human neurons shows robust NMDA-R dependent neuronal activity. The key features of this activity remained unchanged between the different methodological approaches. These findings suggest our model can be use the further understanding of the NMDA-R related disorders like AUD.

Disclosures: A. Tengolics: None. **I. Gameiro-Ros:** None. **I. Prytkova:** None. **K. Jacobs:** None. **Z. Pang:** None. **R.P. Hart:** None. **A.M. Goate:** None. **P.A. Slesinger:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.13/B2

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR

Title: Excitatory glycine receptors and synaptic dysfunction in neurodegenerative disease

Authors: *E. HURLEY, L. FANG, L. AU, F. NAFAR, M. HIRASAWA, M. PARSONS; Fac. of Med., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: N-methyl-D-aspartate receptors (NMDARs) assemble as functionally diverse heterotetramers with essential roles in synaptic structure and plasticity. GluN3A is an unusual NMDAR subunit that when incorporated into NMDARs alters conventional properties. GluN3A expression peaks during the first 1-2 weeks of postnatal life, then progressively declines and remains low in most adult brain regions. GluN3A can assemble with GluN1 to form glutamate-insensitive GluN1/GluN3A excitatory glycine receptors (eGlyRs). Work from our lab and others has shown that eGlyR expression can influence tonic membrane currents, prevent synapse maturation, alter the localization and/or expression of conventional NMDARs, and reduce the magnitude of NMDAR-dependent synaptic plasticity. We demonstrate that GluN3A is abnormally elevated at synaptic and extrasynaptic locations in the hippocampus of a mouse model of Huntington Disease (HD). HD is an inherited neurodegenerative disorder characterized by involuntary motor function, cognitive decline, and psychiatric symptoms. Electrophysiological experiments demonstrate that abnormal GluN3A expression in the HD hippocampus results in an increase in eGlyR expression. In separate electrophysiological recordings, we show that Long-Term Potentiation (LTP) is impaired in HD mice. We conducted a super-resolution synaptic analysis that revealed multiple synaptic alterations, including synapse loss in the HD hippocampus. To determine whether abnormal GluN3A contributes to these synaptic deficits, GluN3A levels were successfully lowered with a shRNA injection. GluN3A reduction did not restore LTP or restore dendritic spine loss in HD. Our preliminary data suggest a shift in immature NMDAR subunit composition in CA1 pyramidal cells in the HD hippocampus; current experiments aim to determine if the altered expression of conventional NMDAR subunits is driven by an increase in eGlyR expression. While the hippocampus is heavily impacted in HD, so are other brain regions, including the striatum, where GluN3A is also elevated. Current experiments also investigate eGlyRs in the HD striatum. Together, we aim to gain a better understanding of the role that GluN3A has on synaptic properties to help uncover the potential mechanisms underlying cognitive decline and motor impairment in HD.

Disclosures: E. Hurley: None. **L. Fang:** None. **L. Au:** None. **F. Nafar:** None. **M. Hirasawa:** None. **M. Parsons:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.14/B3

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant F31MH133285
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UMN Undergraduate Career Opportunities in Neuroscience
UMN Undergraduate Research Opportunities Program

Title: Synaptic architecture and network function alterations following NMDA receptor ablation at medial prefrontal cortex synapses

Authors: *R. DICK[1], H. AHMED[2], A. J. SEDERBERG[3], P. E. ROTHWELL[4];
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Abstract: Decreased expression and function of N-methyl-D-aspartate (NMDA) receptors in the prefrontal cortex (PFC) is a biological abnormality associated with schizophrenia that may contribute to cognitive deficits in patients. NMDA receptors play an important role in working memory function by mediating slow excitation in neurons, thus enabling the reverberant neural activity that allows information to be retained in working memory. Previous studies have demonstrated reduced neural synchrony and increased working memory errors following acute systemic NMDA receptor blockade. Over time, NMDA receptor hypofunction may cause activity-dependent disconnection, a process by which synapses become progressively weakened due to loss of coordinated input timing. However, it is unknown how synaptic function and architecture change following chronic cortical NMDA receptor loss.
Our research investigates the effects of targeted NMDA receptor ablation upon excitatory synaptic strength and density in the mouse medial PFC, using clustered regularly interspaced short palindromic repeat (CRISPR) genome editing technology in transgenic mice. The ratio of NMDA receptor to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated current is significantly decreased in pyramidal neurons infected with a guide ribonucleic acid (RNA) virus to delete the Grin1 gene at 2, 4, and 6 weeks post-surgery ($p < 0.05$). Grin1 encodes the obligatory GluN1 NMDA receptor subunit. We are currently measuring the amplitude and frequency of miniature excitatory postsynaptic currents, which reflect spontaneous neurotransmission and provide information about synapse strength and density respectively. These functional measurements are being integrated with morphological analysis of dendritic spines in the same neurons. Following an examination of the synaptic effects of pan-neuronal NMDA receptor ablation, we will target Grin1 deletion to pyramidal neurons and interneurons separately to examine their relative contributions to excitatory synaptic function and architecture.
These electrophysiology and imaging datasets will be incorporated into a spiking network model that creates and tests predictions regarding brain activity stability and synchrony. Our model, based on Hass et al (2016), produces simulated spike trains that can be analyzed for properties such as firing rate variability and compared to real neural data obtained from in vivo mice. The goal of this computational work is to increase the translatability of our synapse-level experiments and provide insights into potential implications for network activity and working memory loss.

Disclosures: R. Dick: None. **H. Ahmed:** None. **A.J. Sederberg:** None. **P.E. Rothwell:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.15/B4

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant MH119347

Title: Functional characterization of endocytic signals in the proteins of the SynDIG/PRRT family

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Abstract: The transmembrane protein Synapse Differentiation Induced Gene 4 (SD4), also known as Proline-rich transmembrane protein 1 (PRRT1), has been identified as an auxiliary factor of the AMPA-type glutamate receptor (AMPAR) necessary for maintaining extra-synaptic pools of GluA1, a subunit of AMPARs, for synaptic plasticity. However, how SD4 establishes and maintains these pools is unclear. Previous studies suggested that endocytic machinery is important for maintaining a pool of mobile surface AMPARs, and that proteins associated with such cellular machinery are critical for proper protein trafficking and internalization. Additionally, SD4 co-localizes with GluA1 and resides in early and recycling endosomes in cultured neurons. Therefore, identifying the sorting signal targeting SD4 to these organelles is essential to elucidate the role of SD4 in GluA1 trafficking. In this study, we report that SD4 possesses a YxxΦ sorting motif, 178-YVPV-181, responsible for binding to the AP-2 complex cargo-sorting subunit μ2. This motif appears critical for proper SD4 internalization, as SD4 mutant 178-AVPA-181 (SD4 AVPA) induces aberrant SD4 accumulation at the plasma-membrane of heterologous cells and primary rat hippocampal neurons and does not bind to μ2. Previously, we have shown that co-expression of SD4 with GluA1 and GluA2 increases intracellular cluster size in heterologous cells. Here we show that SD4 mutants lacking an endocytic signal co-localize with GluA1 and GluA2 on the surface of heterologous cells. Furthermore, we have identified a non-canonical μ2 binding sequence in another family member, SynDIG1, which also induces aberrant accumulation at the plasma membrane of heterologous cells and primary rat hippocampal neurons. In conclusion, we identify a sorting signal in SD4 important for understanding the SD4-dependent regulatory mechanism of GluA1 trafficking.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.16/B5

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

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Ministry of Universities, FPU Fellowship / FPU2202071 to J.P.

Title: Functional Ca2+-Permeable AMPA Receptors Mediates Hippocampal Astrocytic Calcium Signaling.

Authors: *J. PICAÑOL[1], A. CASTELLANOS[2], E. GRATACÒS-BATLLE[3], X. GASULL[4], L. JIMENEZ-DIAZ[5], J. D. NAVARRO-LOPEZ[6], D. SOTO[7];
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Abstract: Calcium signaling is key to understanding the active involvement of astrocytes in the nervous system. Over the past few decades, emphasis has been placed on the role that ion channels play in this phenomenon. In this regard, AMPA receptors (AMPARs) play an important role in glutamatergic neurotransmission, being essential in learning and memory processes. However, they are also present in glia and, while the presence of GRIA genes in hippocampal astrocytes is well established, the functional expression of AMPARs in these astrocytes remains a subject of debate. Primary astrocytic cultures were prepared from hippocampal tissue and their purity was confirmed using specific astrocytic markers. Calcium-imaging experiments were performed by loading astrocytes with fura-2, a calcium-sensitive dye, to measure intracellular calcium changes upon astrocyte stimulation. Western blotting experiments were performed to confirm the protein expression of AMPAR subunits, and patch-clamp recordings were conducted in whole-cell configuration to investigate AMPAR functionality. Our results demonstrated significant changes in intracellular calcium levels upon AMPA stimulation, providing evidence for the functional presence of AMPARs in hippocampal astrocytes. Western blot analysis confirmed the expression of GluA1, GluA2, and GluA4 subunits of AMPARs in hippocampal astrocytes while patch-clamp recordings revealed distinct subpopulations with different kinetics and steady-state current, further supporting the presence of functional AMPARs. Additionally, we evidenced that calcium permeable AMPARs are present eliciting fast calcium signaling and

we observed that AMPA stimulation is capable to lead a cross-talking between astrocytes involving ATP release that augment the calcium response. In conclusion, our findings contribute to the ongoing discussion regarding the functional expression of AMPARs, establishing their presence and role in calcium signaling *in vitro*. Further investigation is warranted to fully understand the contributions of hippocampal astrocytic AMPARs to glutamatergic neurotransmission.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.17/B6

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant F31MH133285
NIH Grant T32MH115886
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UMN Undergraduate Career Opportunities in Neuroscience
UMN Undergraduate Research Opportunities Program

Title: Impact of NMDA receptor ablation upon synaptic architecture in mouse medial prefrontal cortex

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Abstract: Schizophrenia is a psychiatric disorder characterized by delusions, hallucinations, and cognitive impairments, including working memory deficits. A prominent neurobiological feature of schizophrenia is the dysregulated expression and function of N-methyl-D-aspartate (NMDA) receptors. These receptors play a vital role in sustaining neuronal activation and information processing through slow excitatory currents. Previous studies have demonstrated a decrease in the density of neuronal spines, indicative of excitatory synapses, within certain neuronal populations in schizophrenic patients with schizophrenia. This study investigates the effects of chronic NMDA hypofunction induced by targeted NMDA receptor ablation in medial prefrontal cortex (mPFC) pyramidal neurons. This study investigates which specific neuronal populations in the brain are susceptible to the impacts of NMDA receptor loss, and what is the threshold of NMDA receptor reduction required to induce downstream effects in the brain network, as evidenced by alterations in dendritic spine morphology and density. We hypothesize that NMDA receptor loss will cause decreased spine density and/or morphology changes indicative of

synaptic weakening. Clustered regularly interspaced short palindromic repeats (CRISPR) genome editing was used to target chronic NMDA hypofunction to a specific population of neurons. We first used a CaMKII virus promoter to target only pyramidal neurons, and then an hSyn promoter to target all neurons. Transgenic mice between 6-8 weeks old carrying the Cas9 gene were injected with a guide ribonucleic acid (RNA) adeno-associated virus (AAV) via bilateral mPFC stereotaxic injections mPFC. Pyramidal neurons were filled with neurobiotin dye during whole cell patch clamp electrophysiology experiments, preserved with 4% paraformaldehyde, and stained with a Cy5-streptavidin fluorophore. Imaging was conducted on a Leica Stellaris 8 using LasX software, and z-stacks of the apical dendrites were analyzed using Imaris software to measure spine morphology and density. There were no significant differences in spine density or morphology in infected with the CaMKII promoter viruses (three-way ANOVA for time, sex, and virus, $p > 0.05$). Imaging and analysis of cells infected with the hSyn promoter virus is ongoing, and early results from electrophysiology experiments indicate a stronger effect of the hSyn virus upon NMDA/AMPA ratio in recordings from pyramidal neurons. These experiments will provide important insights into how NMDA receptor loss in different neuronal populations leads to synaptic alterations and potential downstream working memory deficits.

Disclosures: H. Ahmed: None. **R. Dick:** None. **A.J. Sederberg:** None. **P.E. Rothwell:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.18/B7

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: K25AG086674
R01NS111749

Title: Group II mGluR Liberated Gβγ Inhibits AMPA Receptor Insertion in the Postsynaptic Membrane

Authors: Z. ZURAWSKI[1], S. HUYNH[2], M. POTCOAVA[1], C. DELBOVE[1], C. PETERS[1], H. E. HAMM[3], ***S. ALFORD**[4];
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Abstract: Vesicle fusion is mediated by SNARE complexes and is triggered by synaptotagmins in the presence of Ca^{2+}. This fusion can be directly modulated by heterotrimeric G proteins utilizing $Ga_{i/o}$, which interact with the c-terminal region of the SNARE protein, SNAP25 via membrane delimited effects of Gβγ. This provides a fast mechanism by which G protein coupled

receptors alter synaptic transmission. We show that group II mGluRs which couple via $Ga_{i/o}$, inhibit glutamatergic neurotransmission in the hippocampus act via Gβγ directly at the SNARE complex to cause this inhibition. These receptors also act to reduce miniature excitatory postsynaptic current amplitudes, but not frequency directly at SNARE complexes, however, they do not alter evoked glutamate release measured directly from CA3 synaptic terminals using genetically engineered sensors. We show that group II mGluRs act via a direct SNARE dependent interaction with Gβγ to increase rectification of AMPA receptor mediated EPSCs recorded in CA1 pyramidal neurons and inhibit Ca^{2+} signaling mediated by Ca^{2+} permeable AMPAR. These effects are consistent with a receptor mediated loss of AMPA receptor trafficking to the postsynaptic membrane that we hypothesize is mediated by SNAP25 containing SNARE complexes. To test this directly, we expressed halo-tagged GluA1 receptors in hippocampus and by labeling these receptors selectively in the intracellular space, while quenching receptors in the extracellular space. Using Lattice Light Sheet microsopy in hippocampal slices, we showed that group II mGluR activation caused their intracellular accumulation. We tested the effects of this inhibition of AMPAR trafficking on synaptic plasticity. We show that activation of group II mGluRs markedly inhibits LTP induction, but not in animals lacking Gβγ SNARE interactions. Measurement of mEPSC amplitudes and frequency before and after LTP induction demonstrates that this effect is mediated by a change in event amplitude, consistent with changes in AMPA receptor activity in the CA1 neuron. We conclude that AMPA receptor trafficking into the postsynaptic membrane is controlled by mGluRs via a direct effect of Gβγ on fusion of receptor bearing vesicles, providing an entirely novel mechanism of control of synaptic plasticity.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.19/B8

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR PJT-178372
CIHR
SFARI
CureGRIN
Schmidt Science Fellows

Title: Functional context is critical: Impaired patient-variant GluN1 NMDA receptors prolong dendritic excitation to increase seizure vulnerability

Authors: *S. VENKATESAN[1], D. NAZARKINA[1], M. SULLIVAN[2], Y.-F. TAN[1], S. QU[1], A. J. RAMSEY[3], E. K. LAMBE[1];

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Abstract: Mutations in the GRIN1 gene encoding the obligate GluN1 subunit of N-methyl-D-aspartate receptors (NMDARs) cause epileptic encephalopathy that is challenging to treat due to largely unknown neurophysiological consequences in the developing brain. Here, we use a novel mouse model with the Grin1 Y647S+/- patient variant in the highly conserved transmembrane region of the receptor to decipher and treat NMDAR dysfunction occurring in the patient. We conducted multi-scale neurophysiological assessment of NMDAR function at the level of isolated receptors, integrated dendritic signaling, and cortical circuits in age and sex-matched wildtype and Grin1 Y647S+/- mice. Electrophysiological recordings in prefrontal layer 5 neurons revealed significantly reduced isolated NMDAR responses at glutamatergic synapses in Y647S+/- mice ($t_{(192)} = 3.11$, *P = 0.017). However, further examination revealed paradoxically enhanced integrated NMDAR signaling characterized by significantly prolonged dendritic excitation ($t_{(17)} = 2.69$, P = 0.015) and large whole-cell NMDA currents in Y647S+/- mice. At the circuit level, widefield calcium imaging of neural population activity revealed epileptiform events in Y647S+/- brain slices as a consequence of protracted dendritic excitation. We identified a cell-autonomous mechanism where insufficient calcium-dependent negative feedback of NMDARs in Y647S+/- neurons causes prolonged dendritic excitation and seizures. Potentiating small conductance Ca2+ activated potassium channels (SK) restored normal dendritic excitation by promoting NMDAR Mg2+ block, while lowering extracellular Mg2+ levels caused disproportionate NMDAR hyperexcitation. To boost NMDAR negative feedback in vivo, we treated Y647S+/- mice with magnesium L-threonate (MgT) or normal drinking water and observed profound reduction in seizure occurrence and severity in MgT treated mice ($t_{(11)} = 3.04$, P = 0.011). Untreated Y647S+/- mice with severe seizures also showed an immediate improvement within a few weeks after being switched to MgT treatment. Our work reveals the paradoxical loss and gain-of-function consequences of an NMDAR mutation at different scales of neural organization and emphasizes the need for functional context to predict effective treatment for seizures. Our multi-scale analysis of NMDAR function reveals a novel mechanism behind seizures and identifies a promising treatment for GRIN disorder.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

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Program #/Poster #: PSTR204.20/B9

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR PJT-178372
CIHR
SFARI

Title: Machine learning approach to quantify behavioral differences in GRIN1-related disorder and evaluate the effectiveness of a candidate treatment

Authors: *D. NAZARKINA[1], S. QU[1], Y.-F. TAN[1], M. T. SULLIVAN[2], A. J. RAMSEY[2], S. VENKATESAN[1], E. K. LAMBE[1];
[1]Dept. of Physiol., [2]Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada

Abstract: GRIN1-related disorder is a rare neurodevelopmental condition caused by NMDA receptor mutations, characterized by seizures, hyperactivity, cognitive deficits, and developmental delay. The Y647S$^{+/-}$ patient variant in the GluN1 NMDA receptor subunit causes severe intractable epilepsy with no known effective treatments. Neurophysiological investigation in the Grin1 Y647S$^{+/-}$ mouse model identified a novel candidate treatment - Magnesium Threonate (MgT), but testing its efficacy required new methods to quantitatively assess behavioral consequences of seizures. This exploratory work aims to develop automated pipelines to assess treatments and discover behavioral biomarkers that characterize the disease in a novel mouse model. We focused on two behaviors: seizures induced by handling, and naturalistic exploration in an open field arena. Behavioral tests with video monitoring were conducted on two cohorts of age- and sex-matched mice expressing the patient variant (n=21) and wildtype (n=19) controls receiving ad libitum access to MgT treatment or normal drinking water. Seizures were assessed using a modified Racine scale, and movement patterns were analyzed using DeepLabCut (DLC), a machine learning pose estimation software, and DLCAnalyzer R code to identify patterns of movement such as darting, rearing, locomotion, and center avoidance. Preliminary findings revealed hyperlocomotion in untreated mutant mice and a strong correlation between total distance traveled in the open field and seizure severity, with greater distance traveled in the open field predicting more convulsions (R^2=0.952). Both treated and untreated mutant mice also exhibited center-avoidant behavior. Seizure metrics, including severity, duration, and percent of mice seizing revealed that mutant mice receiving treatment did not develop seizures until week 10, while untreated mutant mice had recurring, severe seizures from the beginning. Finally, switching untreated mice to treatment reduced seizure incidence as well as hyperactivity, whereas discontinuing treatment for treated mice had the opposite effect. Ongoing work is pursuing behavioral biomarkers that predict responsiveness to treatment and relapse of breakthrough seizures upon prolonged treatment in mutant mice. Our work supports MgT intervention as a promising treatment for patients with intractable epilepsy in GRIN disorder. We highlight the benefits of automated behavior assessment in identifying subtle behavioral patterns with clinical significance for evaluating treatments in mouse models of GRIN disorder.

Disclosures: D. Nazarkina: None. **S. Qu:** None. **Y. Tan:** None. **M.T. Sullivan:** None. **A.J. Ramsey:** None. **S. Venkatesan:** None. **E.K. Lambe:** None.

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.21/B10

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR Grant PJT-178372
CIHR
SFARI

Title: Morphological examination of prefrontal pyramidal neurons in GRIN1 Y647S+/- patient-variant mice

Authors: *S. QU[1], A. J. RAMSEY[2], S. VENKATESAN[1], E. K. LAMBE[1];
[1]Physiol., [2]Pharmacol. and Toxicology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Mutations in genes encoding N-methyl-D-aspartate receptor (NMDAR) subunits cause GRIN disorder, a rare neurodevelopmental disorder characterized by intellectual disability and epilepsy. NMDARs are essential for synaptic plasticity, learning and memory. Notably, they are critical for normal morphological development of neurons, including dendritic branching and spine formation. Treating GRIN disorder has been challenging due to the unknown neurophysiological and morphological consequences of NMDAR mutations in the developing and adult brain. Neurophysiological assessment in a recent mouse model with the Grin1 Y647S variant in the obligate GluN1 subunit that causes severe epilepsy in patients revealed major changes in dendritic and synaptic excitation in cortical neurons. Here, we examine whether there are equivalent morphological changes in cortical output neurons of male and female wildtype and Grin1 Y647S+/- mice (n=5/genotype). Layer V pyramidal neurons of the prelimbic cortex were filled with Neurobiotin during whole-cell patch clamp recording, slices were fixed, immunostained, and mounted for two-photon microscopy. Full neurons were analyzed in three-dimensions using Neurolucida360 to detect and quantify key structures such as the soma, dendrites and spines. Sholl and branch order analyses showed subtle differences in basal branching (Sholl: 2-way repeated measures ANOVA: Genotype $\times$ Radius: $F_{(16,176)} = 1.983$, $p = 0.0165$, Branch order: Genotype $\times$ Order: $F_{(10,70)} = 2.78$, $p = 0.006$) but not differences in apical branching between wild-type and Y647S-variant mice. The subtle difference in basal dendrites was recapitulated in overall spine density as well as mushroom spine density (Mushroom Spines Sholl: Genotype $\times$ Radius: $F_{(17,119)} = 1.91$, $p = 0.0234$, Genotype $\times$ Mushroom Spine Density by Branch Order: $F_{(10,70)} = 4.07$, $p = 0.002$). Overall, we report only subtle morphological changes in pyramidal neurons in Grin1 Y647S+/- mice, an unexpected finding given their greatly reduced isolated NMDAR currents. These results point to a possible compensation between the gain and loss of function effects of the Y647S+/- variant in the prefrontal cortex, especially in the apical dendritic field. Our work probes the underlying pathology of GRIN1 Y647S+/- in the native prefrontal cortex. We identify a small subset of vulnerable morphological domains and a subset that are more resilient. The degree of preservation of necessary morphological structures suggests promise for future treatments.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

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Program #/Poster #: PSTR204.22/B11

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant R01AG083090
NSF Grant 2015276

Title: Kynurenic acid inflammatory signaling expands in primates prefrontal cortex and impairs cognition

Authors: *S. YANG[1], D. DATTA[1], F. M. KRIENEN[2], E. K. WOO[3], V. C. GALVIN[4], A. C. NAIRN[5], A. F. ARNSTEN[6], M. WANG[7];
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Abstract: Kynurenine signaling is increased under conditions of inflammation, when tryptophan is metabolized by indoleamine 2,3-dioxygenase (IDO) or tryptophan 2,3-dioxygenase (TDO) to generate kynurenine instead of serotonin. Kynurenine can be further metabolized to either kynurenic acid (KYNA, also known as kynurenate) by KAT II, encoded by the gene *AADAT* or to quinolinic acid in a parallel pathway. Although extensive research has examined kynurenine's roles in the immune response, and apoptotic cell death, recent research suggests that it also plays a major role in the cognitive deficits caused by many inflammatory disorders, schizophrenia and Alzheimer's disease. The pattern of cognitive deficits in both long-COVID and schizophrenia fits with preferential dysfunction of the dorsolateral prefrontal cortex (dlPFC), the recently evolved cortical region that subserves working memory and higher cognition in primates. Thus, it is important to learn how KYNA impacts dlPFC physiology and function.The current study found a large expansion of KAT II/KYNA signaling in the primate dlPFC, with expression in both neurons and glia, where KYNA produced a marked loss of neuronal firing needed for working memory and higher cognition. The loss of firing arose from KYNA blocking both NMDAR and nic-a7R, the receptors essential to dlPFC neurotransmission. Systemic administration of agents that inhibited KAT II or kynurenine synthesis improved working memory in aged macaques with naturally occurring KYNA expression, encouraging the development of IDO or KAT II inhibitors for the treatment of inflammatory cognitive disorders such as long-COVID and schizophrenia.

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Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

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Program #/Poster #: PSTR204.23/B12

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: R35-NS111619
RO1-HD082373

Title: Enhancement of hippocampal interneuron excitability by a novel NMDA receptor positive allosteric modulator

Authors: *H. XING[1], T. G. BANKE[2], L. ZHANG[2], K. YU[3], R. FRITZEMEIER[4], N. AKINS[4], S. PALADUGU[4], E. Z. ULLMAN[5], D. C. LIOTTA[4], H. YUAN[2], S. F. TRAYNELIS[2,6]; [2]Dept. of Pharmacol. and Chem. Biol., [3]Dept. of Cell Biol., [4]Dept. of Chem., [5]Pharmacol. and Chem. Biol., [6]Ctr. for Neurodegenerative Dis., [1]Emory Univ., Atlanta, GA

Abstract: NMDA receptors mediate a slow component of excitatory synaptic transmission, and have been shown to play important roles in multiple brain functions, as well as in a wide range of neurological diseases. Here we describe the actions of a novel positive allosteric modulator (PAM), EU1622-A, that has multiple effects on NMDARs. EU1622-A is a pan- PAM that potentiates NMDAR function with sub-micromolar potency, with strongest effects on GluN2B-, GluN2C-, and GluN2D-containing NMDARs. We evaluated the effects of this PAM on both CA1 pyramidal cells and CA1 interneurons in whole cell current clamp and voltage clamp recordings from acutely prepared P17-P22 C57BL/6J mouse hippocampal slices. This PAM potentiates evoked-NMDAR EPSCs amplitude on both CA1 pyramidal cell ($p<0.0001$, n=11) and interneurons ($p=0.00006$, n=12, paired t-test), and increases synaptic NMDAR-mediated charge transfer on interneuron ($p=0.0166$, n=12) and pyramidal cell ($p=0.0313$, n=11, paired t-test.). We show that the PAM preferentially enhances interneuron excitability over principal cell excitability by increasing the spontaneous firing rate of interneurons at concentration of 3 μM ($p=0.0073$, n=11, one-way ANOVA with Dunnett's multiple comparisons test). This results from a depolarization of interneurons, secondary to enhanced NMDAR-mediated current charge transfer in interneurons. By contrast, multiple concentrations of EU1622-A did not detectably depolarize CA1 pyramidal cells nor significantly alter their firing rate (n=10, $p=0.26$ by one-way ANOVA with Dunnett's multiple comparisons test). However, EU1622-A did have significant effects on pyramidal cell properties when bicuculline was used to block GABA receptor signaling that arise from EU1622-A-induced enhanced interneuron activity in the slice. In the presence of bicuculline, EU1622-A increased spontaneous CA1 pyramidal firing frequency (n=10, $p=0.0019$ by repeated measures ANOVA with Tukey's multiple comparisons test). The data supports the idea that the NMDAR PAM EU1622-A can enhance interneuron function with modest effects on the CA1 pyramidal cells, and this may provide therapeutically beneficial effects.

Disclosures: H. Xing: None. **T.G. Banke:** None. **L. Zhang:** None. **K. Yu:** None. **R. Fritzemeier:** None. **N. Akins:** None. **S. Paladugu:** None. **E.Z. Ullman:** None. **D.C. Liotta:** 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.; member of the Board of Directors for NeurOp Inc., coinventor on Emory-owned Intellectual Property that includes positive allosteric modulators of NMDA receptor function. **H. Yuan:** 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 on research grants from Sage Therapeutics and GRIN Therapeutics to Emory, co-inventors on Emory-owned Intellectual Property that includes positive allosteric modulators of NMDA receptor function. **S.F. Traynelis:** Other; member of the SAB for Eumentis Therapeutics, Sage Therapeutics, Neurocrine, and Combined Brain; member of the Medical Advisory Board for the GRIN2B Foundation and the CureGRIN Foundation, advisor to GRIN Therapeutics; co-founder of NeurOp Inc. and AgriThera Inc., and member of the Board of Directors of NeurOp Inc..

Poster

PSTR204: Glutamate Receptor Function and Role in Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR204.24/B13

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: DoD Grant W81XWH1910353
NIH grant R01AG065611
NIH grant R01MH121102
NIH grant RF1AG079256
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NIH grant RF1AG064909

Title: Pathogenic TSC2 variants disrupt gene networks linking to neuron-astrocyte crosstalk, synaptic transmission and mitochondrial integrity

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Abstract: Tuberous Sclerosis Complex (TSC), an autosomal dominant condition, is caused by heterozygous mutations in either the *TSC1* or *TSC2* genes, manifesting in systemic growth of benign tumors. While many organ systems are affected, the neurological consequences lead to the greatest morbidity and mortality in TSC patients. Investigations utilizing *TSC1/2*-knockout animal or human stem cell models suggest that TSC deficiency-causing hyper-activation of mTOR signaling might precipitate anomalous neurodevelopmental processes. However, how the pathogenic variants of *TSC1/2* genes affect the trajectory of human brain development and neurological manifestations remain largely unexplored. Here, we employed 3-dimensional cortical organoids derived from induced pluripotent stem cells (iPSCs) from TSC patients harboring *TSC2* mutations, alongside organoids from age- and sex-matched healthy individuals as controls. Through comprehensively longitudinal molecular and cellular analysis of TSC organoids, including transcriptomics and single cell transcriptomics, we found that *TSC2*

pathogenic variants led to dysregulated neurogenesis, synaptogenesis, and gliogenesis, particularly for reactive astrogliosis. The altered developmental trajectory of TSC organoids significantly resembles the molecular signatures of neuropsychiatric disorders, including autism spectrum disorders, epilepsy, and intellectual disability. Single-cell transcriptomic analyses further identified that *TSC2* pathogenic variants disrupted the neuron-reactive astrocyte crosstalk within the NLGN-NRXN signaling network. Moreover, cellular and electrophysiological assessments of TSC cortical organoids, along with proteomics analyses of synaptosomes, revealed that the *TSC2* variants precipitate perturbations in mitochondrial translational integrity, synaptic transmission, and neuronal network activity. Collectively, our study illustrates that disease-associated *TSC2* variants disrupt the neurodevelopmental trajectories through perturbations of gene regulatory networks during early cortical development, leading to mitochondrial dysfunction, impaired synaptic formation, and hyperactive neuronal network activity.

Disclosures: W. Niu: None. **S. Yu:** None. **X. Li:** None. **Z. Wang:** None. **C. Michalski:** None. **M. Gambello:** None. **J. Peng:** None. **Z. Wen:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.01/B14

Topic: B.08. Epilepsy

Support: Duke-Coulter Translational Partnership

Title: Intraoperative heart rate and laryngeal muscle responses across vagus nerve stimulation parameters

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Abstract: Background: Vagus nerve stimulation (VNS) for epilepsy therapy targets small diameter myelinated fibers that project to the brainstem; it is limited by off-target activation of large myelinated fibers, which produces hoarseness and voice changes. In preclinical studies, change in heart rate (HR) is used as a proxy for activation of small myelinated fibers in the vagus nerve, but clinical stimulation parameters that cause a change in HR have not been reported. We varied stimulation amplitude and pulse width while recording HR and laryngeal electromyography (EMG) intraoperatively. **Methods**: We recruited adult epilepsy patients undergoing VNS implantation (1F, 1M) or VNS battery replacement (3F, 1M). Using the implanted left VNS lead, we delivered 20Hz bipolar symmetric pulses at variable pulse widths (50, 250, 1000 μs) and amplitudes (0.05-28 mA). Each pulse width-amplitude pair was delivered

for 25 s ON + 25 s OFF at higher amplitudes (targeting changes in HR) or for 3 s ON + 2 s OFF at lower amplitudes (targeting EMG). We recorded HR by electrocardiogram (EKG); EMG was recorded using two subdermal needles and an endotracheal (ET) tube with four contacts. HR and EMG were recorded in 5/6 and 6/6 participants, respectively. **Results**: HR decreased during stimulation in 5/5 participants; it decreased by 10% at ~10 and 3.5 mA for pulse widths of 50 and 250 μs, respectively. When stimulation was turned off, we observed rebound tachycardia. Two participants experienced asystole which was immediately reversed upon cessation of stimulation; these participants did not have lower thresholds for recruitment of EMG. EMG recorded from the ET tube had higher signal amplitude than from the subdermal needles, but the recruitment curves were strongly correlated. Stimulation amplitudes to recruit 50% of maximum EMG from the subdermal needles were ~1.2, 0.5, and 0.3 mA for pulse widths of 50, 250, and 1000 μs, respectively. Overall, thresholds for EMG recruitment and changes in HR were not correlated. There were no apparent differences in new implant cases compared to battery change in EMG recruitment or thresholds, HR changes or threshold, or lead impedance. **Conclusions**: The high degree of correlation between ET and subdermal EMGs indicates that the surface recording is a suitable, non-invasive proxy for traditional ET recording. EMG threshold was not a predictor of HR threshold. Rebound tachycardia is a confounding factor to this type of data collection and should be considered in study design.

Disclosures: K. Turk: None. **J. Peters:** None. **Y. Lin:** None. **D.G. Southwell:** None. **W.M. Grill:** None. **N.A. Pelot:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.02/B15

Topic: B.08. Epilepsy

Support: NIH/NIMH (R01-MH120194)
NIH/NIBIB (P41-EB018783)
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NIH/NINDS (U01-NS108916)
NIH/NINDS (U01-NS128612)
McDonnell Center for Systems Neuroscience
Fondazione Neurone
U54AT012307

Title: Acute Effects of Cervical Vagus Nerve Stimulation on Thalamic Local Field Potential and Scalp EEG During Deep Brain Stimulation Lead Placement in Humans

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Abstract: While cervical vagus nerve stimulation (VNS) is used to treat epilepsy, the underlying mechanism of action remains elusive. VNS may act via direct vagal projections to brainstem nuclei including the nucleus tractus solitarius, which in turn modulate subcortical and cortical structures. Previous functional imaging studies suggest that the therapeutic benefit may be related to VNS-induced changes in thalamocortical connectivity. Deep brain stimulation of the centromedian nucleus of the thalamus (CMT) is also used to treat epilepsy, and the CMT reciprocally connects with the reticular formation in the brainstem and may serve as a proxy of brainstem activity during VNS. We hypothesized that VNS modulates both thalamic local field potentials (LFPs) and thalamocortical connectivity. This study included three patients who already had implanted VNS devices, and who subsequently underwent bilateral CMT-DBS. During the DBS lead implantation surgery under general anesthesia, VNS was delivered at each patient's therapeutic stimulation amplitude (predetermined by clinical team) as well as at low and high stimulation amplitudes of 0.5 and 3.5 mA, respectively, while simultaneously recording LFPs from bilateral CMT via DBS electrodes and bilateral scalp EEG. Other stimulation parameters were held constant (25 Hz frequency, cycle pattern of 7 seconds on and 30 seconds off). Skin electrodes overlying the VNS implanted pulse generator in the neck were used to mark stimulation onsets and offsets. We estimated the Welch's power spectral density of thalamic LFPs between 1 to 20 Hz to avoid contamination from the stimulation frequency (25 Hz). To investigate thalamus and cortical (on scalp EEG) connectivity, we used a weighted phase lag index (ranged from -1 to 1, where 1 indicates maximal desynchronization) between CMT and EEG signals. We found a reduction of power in the theta to alpha (6-9 Hz) frequency range in the CM during stimulation compared to baseline. We observed no clear relationship between power reduction and stimulation amplitude; however, effects appeared more prominent as the stimulation session progressed, indicating a temporal relationship. Phase lag analysis suggested that VNS induces desynchronization of thalamus and cortical responses in the theta (4-8 Hz) frequency band. The results were consistent across three subjects. Thus, acute VNS is associated with spectral changes in thalamic LFPs and desynchronization between thalamus LFPs and scalp EEG in epilepsy patients undergoing DBS surgery. Insights gained here suggest the role of the thalamus in driving the therapeutic effects of VNS deserves further investigation.

Disclosures: X. Liu: None. **K.M. Donovan:** None. **G. Tan:** None. **J.L. Roland:** None. **T.J. Foutz:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aurenar, LLC. **P. Brunner:** None. **J.T. Willie:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.03/B16

Topic: B.08. Epilepsy

Support: NIH Grant U54 NS108874
Philanthropic Donations from KCNB1

Title: Functional Characterization of Variants Associated with KCNB1 Encephalopathy

Authors: *E. GIBES[1], L. BARSE[2], C. VANOYE[2], T. ABRAMOVA[2], A. L. GEORGE, JR[3], J. A. KEARNEY[4];
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Abstract: *KCNB1* encodes the α-subunit of the $K_V2.1$ voltage-gated potassium channel that is responsible for modulating neuronal excitability. Pathogenic variants in *KCNB1* have been associated with Developmental and Epileptic Encephalopathy (DEE26). Previously, our group found that clinically significant *KCNB1* missense variants led to predominantly loss-of-function. In this study, we used a combination of immunocytochemistry-flow cytometry and electrophysiology to determine the effects of a series of functionally ambiguous *KCNB1* variants. To determine whether a variant affected protein trafficking, wild-type (WT) or variant $K_V2.1$ was heterologously expressed in CHO-K1 cells and analyzed for total and cell surface expression by flow cytometry. We then used automated whole cell patch clamp electrophysiology to determine the functional properties of $K_V2.1$ variants that had evidence of cell surface expression. These two approaches allowed us to examine the relationship between the level of cell surface expression and K+ current density. We analyzed 58 pathogenic or likely pathogenic *KCNB1* missense variants and found impaired cell surface expression as the predominant cause for the loss-of-function phenotype, with 69% (40/58) of variants having cell surface expression <20% of WT. Eighteen missense variants reached the cell surface, with half (9/18) inducing peak K+ current densities ≥ 75% of WT. The remaining half exhibited mild to severe loss-of-function effects, including 17% (3/18) that had current densities 50-75% of WT and 33% (6/18) with very low or no measurable K+ current. Taken together, our results implicate deficits in protein expression and/or trafficking as a major cause of lower $K_V2.1$-mediated current leading to loss-of-function phenotype. Variants with the strongest impact on cell surface expression most densely populated the pore and voltage-sensor regions, suggesting an association between intensity of pathogenic phenotype and location of variant. Furthermore, while decreased K+ current density correlated with lower cell surface expression, presence at the cell surface did not necessarily indicate measurable current. Functional annotations of variant effects will enhance our understanding of disease mechanisms and facilitate the development of therapies targeted towards relieving deficiencies in expression at the cell surface and restoration of $K_V2.1$ channel functionality.

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Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.04/B17

Topic: B.08. Epilepsy

Support: NS105602
NS111022
NS117568
NS123378
P50HD105353

Title: Whole brain atrophy and amygdala subnuclear enlargement in temporal lobe epilepsy

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Abstract: Studies across the epilepsies reveal widespread reductions in gray matter volume, including amygdala. By contrast, amygdala enlargement (AE) has been described as a subtype of temporal lobe epilepsy (TLE) in patients without hippocampal sclerosis and is associated with later onset age and effective response to anti-epilepsy drugs (AEDs). Amygdala volume reduction in this subtype is also associated with improved prognosis. However, less is known about differences in amygdala subnuclei across disease subtypes and associated clinical outcomes. In this study, we test TLE subtype differences in amygdala subnuclear volume as a proportion of total brain volume (TBV) to understand AE in the context of whole brain atrophy and their correlates. Anatomical scans were acquired from 87 patients (TLE; 52F, age=34+/-10) and 45 typical controls (TC; 35F, age=45+/-12). A subgroup of TLE patients was evaluated and subdivided into patients with hippocampal sclerosis (HS) (N=14) and no HS (N=26). Estimated total intracranial volume and amygdala subnuclear volumes were extracted using FreeSurfer. Amygdala subnuclei were joined into 3 regions of interest (ROIs) and normalized by TBV: basolateral (BLA), centromedial (CM), and cortical (Co). Psychiatric outcomes of anxious depression and perceived stress were obtained. Group differences in TBV and in each ROI were tested with respect to the ipsilateral side of seizure or from the bilateral mean (TC and TLE with bilateral seizures). Psychiatric outcomes were regressed on the interaction between brain measures and group. Participants missing laterality information or self-report measures were excluded from those analyses. All analyses included covariates for age and sex. Outliers were excluded based on Cook's D > 4/(N-P). Compared to TC, TBV was significantly reduced in TLE ($t(126)=-3.21$, $p<.01$), HS ($t(75)=-3.03$, $p<.01$), and no HS ($t(75)= -3.85$, $p<.01$). Whole amygdala volume was significantly increased in TLE versus TC ($t(107)=2.25$, $p<.05$). ROI

analyses revealed significantly reduced volume in HS across all ROIs ($p<.01$) and significantly increased CM and Co volumes in no HS ($t(62)= 2.42$, $p<.05$; $t(60)=2.13$, $p<.05$). Reduced TBV was associated with increased perceived stress ($t(99)=-2.24$, $p<.05$). Results demonstrate reduced total brain volume in TLE. Despite significantly reduced whole brain volume in both HS and no HS compared to TC, findings further illustrate AE specifically in CM and Co amygdala of no HS compared to HS and HC. A better understanding of the mechanisms underlying amygdala subnuclear volume differences may help predict treatment response and facilitate more targeted interventions.

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Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.05/B18

Topic: B.08. Epilepsy

Title: Inhibitory interneuron firing as a biomarker for epileptogenic zone localization in patients with drug-resistant epilepsy candidates to surgery

Authors: *A. NASIMBERA[1], S. CORDISCO GONZALEZ[2], D. BRIZEE[3], N. E. CAMPORA[2], J. PRINCICH[2], P. SEOANE[2], S. KOCHEN[4];
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Abstract: Epilepsy is a brain disorder characterized by recurring epileptic seizures. Surgery can benefit patients with focal drug-resistant epilepsy if the site of origin of seizures, the epileptogenic zone (EZ) is identified. In some cases, invasive studies using intracerebral electrodes are required to identify the EZ, a procedure known as stereo encephalography (SEEG). Our objective was to identify epileptogenic biomarkers to improve surgical outcomes through more precise EZ mapping using single neuron recordings. To do so, we recorded neuronal population activity as well as single neuron activity using macro-micro intracerebral electrodes in patients undergoing SEEG. Microelectrodes (AD-TECH) were inserted into the lumen of the macroelectrodes implanted in the mesial temporal regions. Electrode placement and number was based on clinical criteria and verified with computed tomography imaging. EZ and propagation areas (PA) were then visually identified by certified neurologists. Signal from the microelectrodes was filtered between 1-9000 Hz and sampled at 30 kHz. Neuronal spikes were detected and clustered using the Wave_clus algorithm and manually inspected. Units were then

classified into putative inhibitory and excitatory based on waveform morphology. Firing rates before and during seizures were computed, using LOESS (Local regression) for peak firing rate estimation. Changes in mean firing rates relative to seizure onset were analyzed using mixed linear models. The study was approved by the institutional ethics committee and patients provided informed consent to participate. We isolated 512 single units from 18 patients and 180 seizures. Of the isolated units 210 (41.01%) were found in the EZ, and 115 (22.5%) were classified as putative inhibitory interneurons. In the EZ, inhibitory interneurons exhibited a significant increase in firing rate 7 seconds prior to seizure onset ($p<0,01$), while excitatory neuron firing rates peaked 4 seconds after seizure onset ($p<0,01$). In PA, inhibitory interneurons and excitatory principal neurons showed no significant change during seizure onset. Instead, their peak activity occurred approximately 12 seconds after seizure onset, during spread. Together, our data show a neuron-specific activation pattern around seizure onset, indicating that interneuron firing rates could be a potential biomarker for EZ localization. Ultimately, this finding may improve patient outcomes by enabling more accurate identification and targeted treatment of the EZ.

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Poster

PSTR205: Epilepsy: Human Studies

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Program #/Poster #: PSTR205.06/B19

Topic: B.08. Epilepsy

Support: NINDS K23NS112339
NINDS R01NS129622

Title: Noninvasive Brain Stimulation, Interictal Discharges, and Seizure Reduction

Authors: *B. LUNDSTROM[1], K. ISLAM[2], K. STARNES[1], T. J. RICHNER[2], N. GREGG[2], G. A. WORRELL[1];
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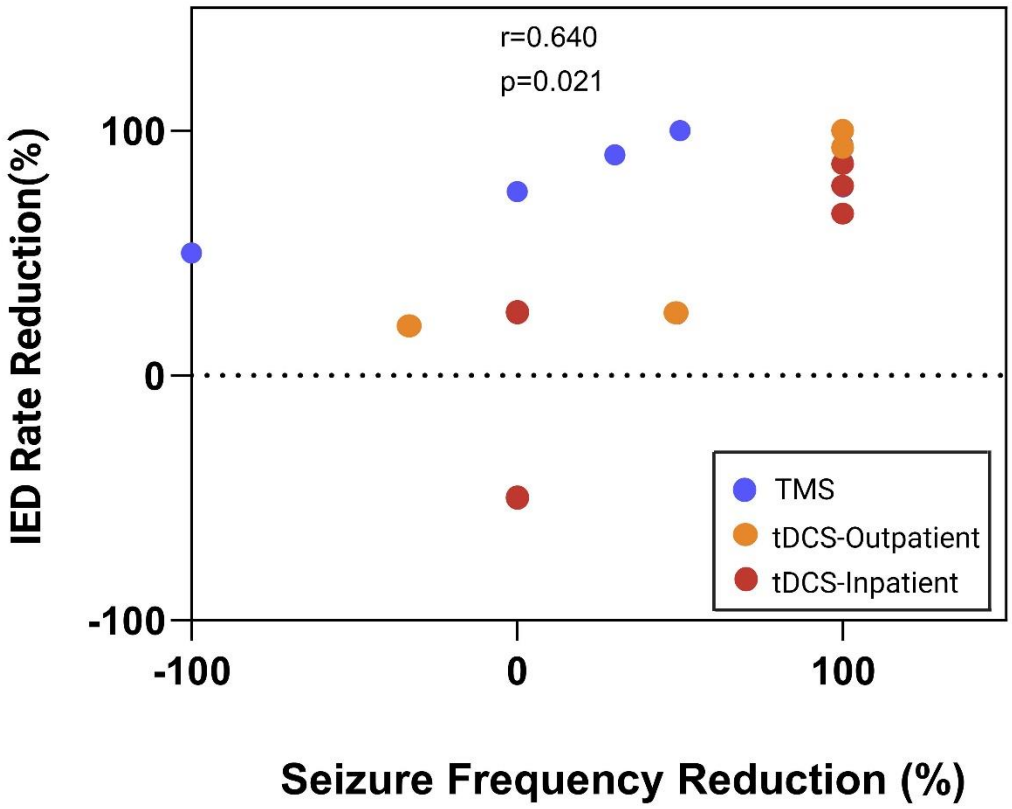
Abstract: Non-invasive brain stimulation (NIBS) is a treatment option for epilepsy in a variety of settings from the hospital to home. We retrospectively assessed 24 patients who received transcranial magnetic stimulation (TMS) or transcranial direct current stimulation (tDCS) to reduce seizure frequency. TMS was applied in the outpatient clinic (n=10), while tDCS was delivered as an outpatient (n=9) or inpatient (n=5). Five subjects treated as an outpatient continued treatment at home. Overall, median seizure reduction was 50% (IQR = 14.5%-100%, p=0.0014) with a 58% responder rate. For all patients with available data, EEG before and after treatment were correlated with changes in clinical seizure rates. Interictal epileptiform discharge (IED) rates decreased for 12 of 13 patients. For these patients, median IED rate reduction was

75% (IQR = 26%-90%, p=0.0042) and median seizure reduction was 49% (IQR= 0%-100%, P=0.06). Spearman's rank correlation (r=0.640, p=0.02) showed that the IED rate was correlated with seizure frequency reduction (Figure 1), although in a non-linear fashion as the Pearson correlation was not significant p=0.08). These results suggest that in some cases short-term reduction in IEDs may correlate with clinical seizure reduction.

Figure 1: The reduction of IED rate correlated with seizure frequency reduction following treatment of focal epilepsy with non-invasive brain stimulation approaches. The significant Spearman's rank correlation reflects a monotonic but not necessarily linear relationship.



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royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cadence Neuroscience, NeuroOne.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.07/B20

Topic: B.08. Epilepsy

Support: FACES
CTSI
NIH BRAIN initiative 1R01NS109994

Title: Cellular and Circuit Mechanism of Epilepsy in Acute Slices from Resected Human Brain Tissue

Authors: *T. BUTOLA[1], V. ROBERT[2], W. K. DOYLE[3], D. FRIEDMAN[4], S. DEVORE[5], R. TOMER[6], O. DEVINSKY[7], J. BASU[8];
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Abstract: Nearly one-third of epilepsy patients suffer ongoing seizures despite multiple medication trials. In humans, the hippocampus and the entorhinal cortex are central to the pathophysiology of temporal lobe epilepsy, the most common drug-resistant epilepsy. However, most of what we know about this cortico-hippocampal circuit is from rodent studies. Our study fills this gap by characterizing the structure and function of human cortico-hippocampal circuit. Our aim is to identify better targets for future therapies in a model system based on the human brain. We use freshly resected human brain tissue from patients undergoing surgery for refractory epilepsy to characterize neuronal circuits and mechanisms governing the origin and progression of seizures in the epileptogenic human brain. At the level of individual neurons, we observed that both hippocampal CA1 and CA3 neurons in epileptic human brain tissue demonstrate comparable excitability. However, at the circuit level, cortical inputs synapsing onto CA3 neurons have a higher release probability than those at CA1 neurons. Our data suggest that the increased excitability in CA3 observed in epilepsy might be due to an increased circuit excitability rather than changes in the excitability of individual neurons. Additionally, upon comparing our human tissue data with mouse electrophysiology, we found that synaptic strength between human entorhinal cortex and hippocampal CA1 is tenfold higher than its mouse counterpart. However, both mouse and human CA3 to CA1 synapses have similar synaptic strengths. We have identified substantial differences between human and mouse tissue neurons, which may stem from species differences or could be due to epilepsy pathology. To address this,

we are currently comparing recordings obtained from human epileptic brain tissue with non-epileptic human brain tissue obtained from tumor resection surgeries. Our study depicts the fundamental principles of synaptic transmission, input-output transformation and short-term plasticity dynamics in cortico-hippocampal circuit in the human brain and how these key processes may predispose a brain area to be more excitable and prone to seizures.

Disclosures: T. Butola: None. **V. Robert:** None. **W.K. Doyle:** None. **D. Friedman:** None. **S. Devore:** None. **R. Tomer:** None. **O. Devinsky:** None. **J. Basu:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.08/B21

Topic: B.08. Epilepsy

Title: Pathological Neural Circuits Initiate Seizure Events in Hippocampal Slices from Epilepsy Patients

Authors: *M. ELLIOTT[1], T. SHARF[2], J. GENG[3];
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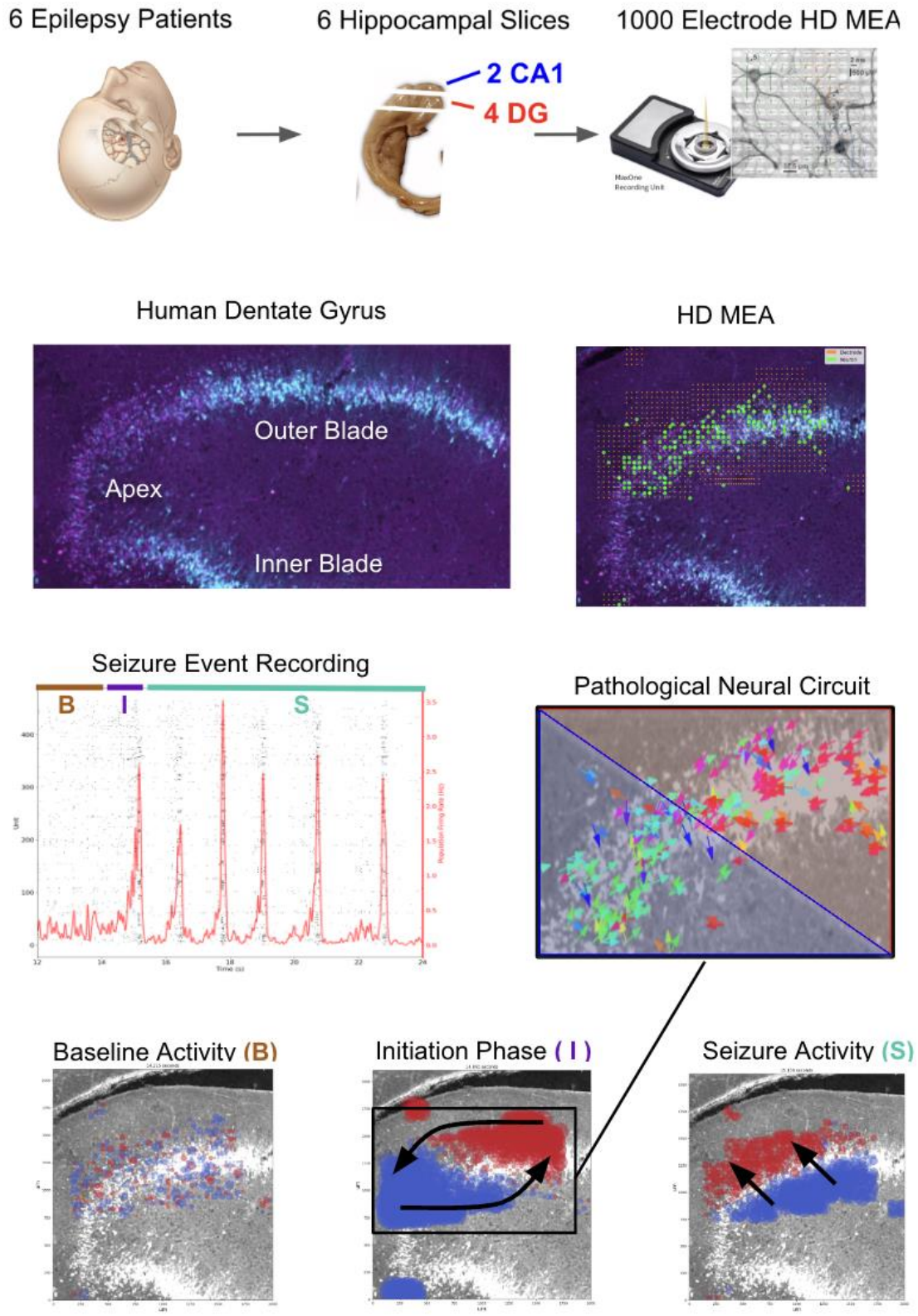
Abstract: Epilepsy is one of the most common life threatening neurological diseases. Of this, hippocampal sclerosis is the most common etiology to require surgical intervention. While the disease is readily identifiable by sclerotic tissue, it is not yet known why this initiates seizure events. From histological studies, one of the prevailing hypotheses of epileptogenesis is that the sprouting of mossy fibers in the dentate gyrus leads to hyperexcitable recurrent circuits that initiate epileptic activity. This hypothesis has, so far, remained unvalidated due to our inability to measure the precise behavior of neural circuits on a microscopic scale. We performed a study on 6 hippocampal slices surgically extracted from human epilepsy patients. Spontaneous extracellular activity was recorded using a high-density CMOS microelectrode array, a device capable of measuring the behavioral interactions between hundreds of neurons in high resolution. Limbic status epilepticus was induced in 2 dentate gyrus slices through the administration of kainic acid. Using original computational methods, we found the slices that exhibited ictal events had circuits with recurrent excitation, consistent with the mossy fiber sprouting hypotheses of epileptogenesis. Both recordings came from the same subregion of the dentate gyrus. This pathologic circuit is unique from the circuitry of all the other slices that did not express seizure activity. More generally, the methods we performed on the high resolution electrophysiology recordings provide a fascinating glimpse into the neural behavior of subregions of the hippocampus. This work provides a deeper understanding of what causes seizures and may prove useful in future diagnostic and therapeutic approaches.



Disclosures: M. Elliott: None. **T. Sharf:** None. **J. Geng:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.09/B22

Topic: B.08. Epilepsy

Support: JSPS KAKENHI Grant JP23K03875

Title: Comparison of Time Variation of Mean Correlation Values between ECoG Channels in Patients with Generalized and Focal Epilepsy

Authors: *R. TANIGUCHI[1], T. KOHAMA[2], H. YOSHIDA[3], N. NAKANO[4];
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Abstract: Epilepsy is a neurological disease with a prevalence of approximately 1% of the population, and it is said that seizures can be suppressed in approximately 75% of patients through medication. However, approximately 25% of patients have intractable epilepsy, and surgical treatment is considered effective. In surgical treatment, it is important to accurately identify the focal point of epilepsy and the propagation path of abnormally excited electroencephalograms. We have proposed a method to identify the propagation pathway by defining the connectivity between electrocorticogram (ECoG) channels and constructing a minimum spanning tree from them, and a method to overview the temporal structural changes of epileptiform activity by tracking the average variation of the top 1% of the maximum correlation function between channels. In this report, we present the results of a comparison of temporal structural changes in epileptiform activity in patients with generalized epilepsy and patients with focal epilepsy, tracking the temporal changes in epileptiform activity using the mean correlation tracking method.First, in patients with generalized epilepsy, the measured ECoG showed abnormal excitatory electroencephalograms propagating from a specific channel, and then epileptiform activity continued for about 110 seconds. On the other hand, the mean correlation value increased rapidly in the early phase of the seizure but decreased rapidly after approximately 10 seconds. This indicates that epileptiform activity occurred independently in each region of the brain after the abnormal brain discharges were transmitted from the focal point to each region of the brain. The mean correlation value increased again 40 seconds before the end of the seizure, indicating that the brain regions synchronized toward the end of the seizure. On the other hand, in patients with focal epilepsy, the mean correlation value gradually increased from 100 seconds or more before epileptiform activity was observed in the measured ECoG, and the mean correlation value remained relatively high between seizures. This indicates that epileptiform activity continued locally. Furthermore, because the seizures were local epileptiform activities, unlike generalized epilepsy, there was no synchronous convergence of seizures in different parts of the brain.

Disclosures: R. Taniguchi: None. **T. Kohama:** None. **H. Yoshida:** None. **N. Nakano:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.10/B23

Topic: B.08. Epilepsy

Support: DFG SCHW866

Title: Characterization of novel pathogenic GOSR2 variants and potential therapeutics

Authors: *M. SCHWAKE;
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Abstract: SNARE proteins facilitate membrane fusion in the secretory pathway through the formation of a specific complex involving four SNARE domains from at least three SNARE proteins. This complex forms a heterooligomeric parallel four-helix bundle, with 16 tightly packed layers of mainly hydrophobic amino acid residues, excluding the central "0-layer". Fusion processes between the ER, ER-Golgi intermediate compartment (ERGIC), and Golgi apparatus are mediated by a SNARE complex consisting of syntaxin-5 (STX5), GOSR2, BET1, and SEC22b. Loss-of-function mutations in STX5, GOSR2, and BET1 have been associated with severe and early-onset diseases, such as North Sea Progressive Myoclonus Epilepsy (PME). During the last few years, novel pathogenic GOSR2 variants have been identified which, in addition to the symptoms of PME, also show signs of congenital muscular dystrophy, likely related to altered glycosylation of α-Dystroglycan. Recently, two siblings were identified with congenital microcephaly, brain atrophy as well as severe neuromuscular symptoms carrying the novel pathogenic combined heterozygous GOSR2 variants c.1A>G, p.Met1? and c.448G>A, p.Gly150Arg. The patients exhibit severe clinical features suggesting strong loss-of-function variants. Functional analysis of novel GOSR2 variants in patient fibroblasts revealed hypomorphic GOSR2 expression and the translation of different molecular weight GOSR2 proteins. The c.1A>G mutation likely leads to alternative translational start, resulting in an unstable variant detected only after proteasomal inhibition. The c.448G>A mutation results in an alternative splice variant missing exon 5, leading to a GOSR2 variant lacking part of the SNARE domain required for function. Yeast growth and pC4 transport assays showed impaired ER-Golgi transport for the exon 5 deleted GOSR2 variant. Brefeldin A assay confirmed impaired ER-Golgi transport in patient fibroblasts due to hypomorphic expression combined with loss-of-function GOSR2 Variant. Since the p.Gly150Arg substitution appears functional in *in vitro* experiments, a potential therapeutic approach with antisense oligonucleotides was initiated. An 18 bp long 2′-O-methoxyethyl (MOE) with a phosphorothioate backbone modified oligonucleotide sequence significantly rescued alternative splicing in patient-derived fibroblasts, resulting in an increase of functional GOSR2 *in vitro* and offering a therapeutic option for these severely affected patients.

Disclosures: M. Schwake: None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.11/B24

Topic: B.08. Epilepsy

Support: Departmental Research fund

Title: Neuropsychiatric comorbidities and sleep dysfunction in idiopathic generalized epilepsy of childhood

Authors: *V. MEHTA;
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Abstract: Table of Contents

- Neuropsychiatric comorbidities and sleep dysfunction in idiopathic generalized epilepsy of childhood

Neuropsychiatric comorbidities and sleep dysfunction in idiopathic generalized epilepsy of childhood
Vinod Kumar Mehta, Ayushi Jain, Jitendra Jeenger, Maheep Bhatnagar, Abbas Ali Saifee, Anis Jukkarwala.Aim: Neuropsychiatric disorders are complex, multifactorial and underscored by heterogeneous symptomology. To better understand the neuropsychology of Idiopathic generalized epilepsy (IGE), this study evaluated the cognitive profiles, anxiety, sleep dysfunction in children with IGE, uniformly treated with valproic acid with well-controlled seizures.Methods: We performed a comprehensive cognitive and neuropsychiatric evaluation of children with IGE. (n=84, mean age=09.5±2 years). We compared results between patients (IGE, sub-phenotype) and control group (n=80) children age-matched, similar socio-economic and education. The p-values were adjusted for multiple testing. Children's Sleep Habits Questionnaire (CSHQ) scores were used to evaluate sleep.Results: Children with IGE performed significantly poorer in cognitive function tests (non-verbal and verbal attention, verbal learning and memory, word fluency, and controlled sequential fine motor responses) excluding nonverbal memory. Cognitive profiling with the type of seizure, revealed that generalized tonic-clonic seizures (GTCS) and absence seizure (AS) group had an attention deficit, whereas only children with AS showed deficits in speech, verbal learning and memory. IGE-GTCS showed the high anxiety index as compared to IGE sub-phenotypes. Most tests were not influenced by valproate intake, and the dose did not correlate with cognitive performances. Children with IGE had higher sleep dysfunction. (total CSHQ score, $p < 0.05$).Conclusion: Our results provide insights into a long-term risk of cognitive impairment for children with IGE, even if they have normal intelligence and their seizures are well controlled. Our findings suggest that patients with IGE have significantly lower abilities in executive functions and acquired knowledge when compared to controls. Furthermore, the results indicate that IGE increase the susceptibility of sleep and

cognitive dysfunction in childhood and highlights the importance of early interventions in neuropsychiatric comorbidities of IGE.

Disclosures: V. mehta: None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.12/B25

Topic: B.08. Epilepsy

Support: NIH-NINDS K23
NIH NIMH R01

Title: Naturalistic film paradigm captures episodic memory performance in temporal lobe epilepsy patients.

Authors: *H. ZHANG[1], F. VASHEGHANI FARAHANI[2], H. LEE[3], J. CHEN[4], A. A. LIU[5]; [1]NYU Langone Hlth., New York, NY; [2]New York Univ. Grossman Sch. of Med., New York, NY; [3]Dept. of Psychological Sci., Purdue Univ., West Lafayette, IN; [4]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [5]New York Univ. Sch. of Med., New York, NY

Abstract: Patients with epilepsy (PWE), particularly those with seizures from the temporal lobe, often experience episodic memory impairment. Existing clinical memory assessments are criticized for their artificial nature and data-poor estimation of episodic memory in the real world. To address this gap, we showed a set of short audiovisual films to patients with temporal lobe epilepsy (TLE) and healthy controls (HC) and asked them to spontaneously recall film events. Eligible subjects were between the ages of 18-60; healthy controls (HCs) had to score >=27 (out of 30) on the Montreal Cognitive Assessment (MOCA), while TLE patients had to score >=22. Patients had a diagnosis of probable or definite TLE through a combination MRI Brain, interictal EEG, and seizure semiology. We recruited 47 participants (34 F, 13 M) with a mean age of 29.6 (±7.6) between the years of 2019-2023 at a single center, including 24 HCs and 23 TLE patients. Subjects watched a series of six films from 2 to 5 minutes in length. Following film viewing, participants were instructed to freely recall as many details as possible. Audio responses were recorded, transcribed through a combination of human and automated processes, segmented to align with predetermined event segmentation, and scored for recollection at the film and event level. Overall, TLE patients performed similarly to HCs in recall of specific films (84.2% vs 89.6%, p = 0.87) and events (39.3% vs 42.9%, p = 0.41) but with a wider range of variability. There was no significant correlation between MOCA scores and recall performance (p=0.24), but a trend was observed for Left TLE patients (p=0.11). These results reflect the cognitive heterogeneity in the TLE population, with some patients demonstrating normal cognition and others reporting memory impairment. These findings suggest that spontaneous

film recall may be used to probe episodic memory for continuous, naturalistic events, independently from memory for unstructured information.

Disclosures: H. Zhang: None. **F. Vasheghani Farahani:** None. **H. Lee:** None. **J. Chen:** None. **A.A. Liu:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

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Program #/Poster #: PSTR205.13/B26

Topic: B.08. Epilepsy

Support: NIH Grant R01NS031718
NIH Grant R37NS115439
NIH Grant R01NS101156
NIH NeuroBioBank

Title: Chronic activation of oxidative stress response pathways in human temporal lobe epilepsy

Authors: *N. L. SZABO, M. BAUMGARTNER, F. E. JENSEN, D. M. TALOS;
Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Temporal lobe epilepsy (TLE) is a major cause of therapy-resistant epilepsy. A better understanding of the mechanisms involved in seizure generation and therapy resistance may lead to improved diagnostic and therapeutic strategies. While animal models point towards a causal role for mitochondrial dysfunction and oxidative stress in epileptogenesis, little is known about their relationship to epileptogenesis in humans. Impaired mitochondria function can result in increased reactive oxygen species production, oxidative damage and decreased ATP production. In turn, energetic and oxidative stress may trigger neuroprotective defense mechanisms, including induction of AMP-activated protein kinase (AMPK) pathway, upregulation of antioxidant enzymes and the formation of cytosolic protein/RNA condensates known as stress granules. In this study, we hypothesized that cortical tissue resected from patients with TLE would exhibit alterations in markers of mitochondrial function and antioxidant stress response. The TLE samples, prospectively collected following brain surgeries performed at the Hospital of the University of Pennsylvania (n=16), were compared with matched control autopsy samples (n=8) obtained from the NIH NeuroBioBank. The study was approved by the local Institutional Review Board. The expression of mitochondrial oxidative phosphorylation complexes (OXPHOS I, II, III, and V), along with energetic and oxidative stress response markers AMPK, phospho-AMPK Thr172 (pAMPK), glutathion peroxidases 1 (GPX1) and 4 (GPX4), and the stress granule marker Ras-GTPase-activating protein binding protein 1 (G3BP1) were quantified by western blotting. TLE patient samples demonstrated a selective deficiency of OXPHOS V (72.46% of control, $p<0.01$), accompanied by a significant increase in pAMPK/AMPK ratio (661% of control, $p<0.01$), consistent with diminished mitochondrial ATP production. We also

found an adaptive oxidative stress response via increased GPX4 (139% of control, $p<0.001$) and G3BP1 levels (334% of control, $p<0.0001$). These data collectively point to an increase in energetic and oxidative stress in TLE due to mitochondria dysfunction and a chronic increase in the activity of endogenous antioxidant defense mechanisms. While these activities might be an effective response in acute settings, their chronic activation may contribute to epilepsy pathology. These studies further suggest a potential avenue for treatment of epilepsy with redox-based agents.

Disclosures: N.L. Szabo: None. **M. Baumgartner:** None. **F.E. Jensen:** None. **D.M. Talos:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.14/B27

Topic: B.08. Epilepsy

Title: Enhancing the pseudo-prospective performance of preictal classifiers: Leveraging seizure prediction dynamics

Authors: *G. ACHARYA[1], E. CONRAD[2], K. A. DAVIS[2], E. NOZARI[3];
[1]Univ. of California, Riverside, Riverside, CA; [2]Hosp. of the Univ. of Pennsylvania, Philadelphia, PA; [3]Mechanical Engin., Univ. of California, Riverside, Riverside, CA

Abstract: Extensive research in the past decade has focused on investigating the presence and identification of a discernable preictal period. Despite the effectiveness of seizure forecasting algorithms trained on labeled interictal and preictal intracranial electroencephalography (iEEG) data, as evidenced by machine learning metrics (>80% test accuracy and >0.8 AUC), their practical utility remains limited when evaluated against (pseudo) prospective metrics such as warning time duration. Leveraging the dynamics inherent in seizure prediction features may offer a pathway toward a more pragmatic seizure forecaster.
In this study, we utilized data from the Hospital of the University of Pennsylvania (HUP), comprising observations from n=5 subjects suffering from epilepsy, each with an average of c=99 iEEG channels and k=10±2 lead seizure events. Employing independent autoregressive (AR) models, we investigated the dynamics of features extracted from iEEG data for preictal classification. Our analysis demonstrated consistent predictability of seizure forecasting features across subjects over an extended time horizon, with a mean R-squared of 0.9±0.1 for 2-second ahead predictions and 0.5±0.2 for 10-minute ahead predictions. In addition, our study revealed that integrating current features with power-band features extracted from longer horizons (>15 minutes) of historical iEEG data led to a 11±6.5% increase in classification accuracy. Substituting observed features with those predicted from the trained AR model resulted in a lower classification performance (10% decrease in AUC), while concurrently enhancing the pseudo-prospective performance (PPP) by 5±3%. Furthermore, fine-tuning the predictor using a

second-level dynamical classifier trained over lags of predicted probability boosted the PPP by another 5±2%. Additionally, we model the ground truth transition into and out of the preictal state close to seizure onset and describe a moving window algorithm to forecast these transitions. Utilizing feature dynamics offers a promising path towards improving real-time seizure forecasting, providing valuable insights into the evolving patterns of epileptic activity and enabling preemptive seizure management, thus enhancing the quality of life for individuals with drug-resistant epilepsy.

Disclosures: G. Acharya: None. **E. Conrad:** None. **K.A. Davis:** None. **E. Nozari:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.15/B28

Topic: B.08. Epilepsy

Support: AMED JP23wm0425005

Title: Transcriptional features of low grade neuroepithelial tumors associated with epileptogenicity

Authors: *S. MIYASHITA[1], M. HOSHINO[2];
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Abstract: Epilepsy is a common neurological disorder that affects one out of every hundred individuals in the population. About 30% of all patients with epilepsy have a refractory epilepsy in which seizures are difficult to control with drugs. In many cases, the cellular and molecular mechanisms underlying refractory epilepsy remain elusive. Thus, an understanding of the pathogenesis is essential for effective treatment. Low-grade neuroepithelial tumors (LGNET) are major causes of drug-resistant focal epilepsy. The BRAF V600E mutation is frequently observed in LGNET and linked to poor seizure outcomes. However, its molecular role in epileptogenicity remains elusive. To understand the molecular mechanism underlying the epileptogenicity inLGNET with the BRAF V600E genetic mutation (BRAF-LGNET), we conducted comprehensive transcriptome analysis using surgical specimens of BRAF-LGNET obtained and stored at a single institute. Bioinformatic analysis using these dataset identified 2,134 differentially expressed genes between BRAF-LGNET and control. Additionally, gene set enrichment analysis provided novel insights into the association between estrogen response-related pathways and the epileptogenicity of BRAF-LGNET patients. Our datasets and findings will contribute toward the understanding of the pathology of epilepsy caused by low-grade neuroepithelial tumor and the identification of new therapeutic targets.

Disclosures: S. Miyashita: None. **M. Hoshino:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

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Program #/Poster #: PSTR205.16/B29

Topic: B.08. Epilepsy

Support: University of Kentucky College of Medicine Alliance Initiative
University of Kentucky Neuroscience Research Priority Area
University of Kentucky Department of Neurosurgery NEUSTAR Awards

Title: Delta focused ictal electrical source imaging for seizure onset localization in focal refractory epilepsy

Authors: *J. RYBARCZYK[1], J. CLAY[2], F. RASLAU[4,2,3], M. KHALID[3], F. MIRZA[3], J. BAE[1]; [1]Electrical and Computer Engin., [2]Neurol., [3]Neurosurg., Univ. of Kentucky, Lexington, KY; [4]Radiology, Univ. of Kentucky Chandler Med. Ctr., Lexington, KY

Abstract: Background: Up to 40% of active epilepsy cases are refractory to anti-seizure medications. Many refractory cases are due to focal epilepsy, in which seizure onset occurs in a discrete location in the brain, the seizure onset zone. Electrical source imaging (ESI) techniques provide an objective and accurate estimation of the source localization based on scalp electroencephalogram (EEG). Conventionally, ESI has been focused largely on interictal epileptiform discharges (IEDs) to localize seizure onsets. However, there has been a great emphasis on ESI based on ictal EEG (ictal ESI) as seizure is the main characteristic of epilepsy. Objective: This study investigates how specific frequency component influences ictal ESI for the localization of seizure onsets in focal refractory epilepsy, specifically focusing on the slow waves (delta oscillation of frequency less than 4 Hz). Ictal ESI strategies on inverse solutions and ictal EEG processing were evaluated. Methods: Structural magnetic resonance imaging (MRI) and 32 channel scalp EEG obtained from 33 patients (15 females and 18 males, age ranges from 20 to 62) with focal refractory epilepsy, admitted to the University of Kentucky Healthcare's Epilepsy Monitoring Unit (EMU) were used to perform the delta focused ictal ESI. 3 well known source estimation algorithms, including Minimum Norms Estimate (MNE), standardized Low Resolution Electromagnetic Tomographic Analysis (sLORETA), and Equivalent Current Dipoles (ECD), were implemented on both clinically (n = 33) and electrographically (n = 41) defined seizures. 2 different ranges of delta components, 0.3-4 and 1-4 Hz, were examined on various sizes and locations of EEG time window. The estimated source location was compared to the clinical onset, defined by an epileptologist. Results: A lateralization and partially concordant localization accuracy of 83.61% and 80.33% were obtained when 1-4 Hz delta component from the entire duration of electrographically defined seizures were applied to ECD method. On the same data, exact concordance was obtained 24.59% accuracy when MNE and sLORETA were implemented. When accuracy was separately counted on seizures containing dominant delta components during seizure initiation, both lateralization and partially concordant localization accuracies reached to 85.71% in a case when 0.3-4 Hz delta component from the first 2 seconds

of clinically defined seizures were applied to ECD method. Conclusion: These results support that delta focused ictal ESI provides clinically useful laterization and localization information in focal refractory epilepsy.

Disclosures: J. Rybarczyk: None. **J. Clay:** None. **F. Raslau:** None. **M. Khalid:** None. **F. Mirza:** None. **J. Bae:** None.

Poster

PSTR205: Epilepsy: Human Studies

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.18/B30

Topic: C.03. Parkinson's Disease

Support: NSTC 112-2321-B-002-022 -

Title: Neural correlates of inhibitory control in patients with multiple system atrophy (MSA) using counting Stroop functional MRI

Authors: *L.-Y. FAN[1], M.-C. J. KUO[2,3], W.-S. LAI[4], T.-L. CHOU[4];
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Abstract: Multiple system atrophy (MSA) is a progressive neurodegenerative disorder associated with potential cognitive impairments, particularly in executive function. While structural atrophy in regions such as the putamen, middle cerebellar peduncle, pons, and cerebellum has been observed in MSA patients through neuroimaging studies, limited research has explored executive function using functional magnetic resonance imaging (fMRI). This study aimed to investigate inhibitory control neural substrates in MSA patients and healthy controls using the counting-Stroop task with fMRI and Cambridge Neuropsychological Test Automated Battery (CANTAB). Thirteen MSA patients (mean age: 60.5 ± 5.2) and 13 age- and gender-matched healthy controls (mean age: 61.0 ± 5.3) underwent assessment with the counting-Stroop task during fMRI scanning, supplemented by CANTAB assessments outside the scanner. Group comparisons of brain activation and correlations between brain activity and inhibitory control performance were conducted. MSA patients exhibited longer reaction times and reduced accuracy compared to healthy controls under incongruent conditions. Additionally, MSA patients demonstrated increased activation in the anterior cingulate cortex (ACC, BA32) compared to healthy controls in incongruent versus congruent conditions. These findings suggest a heightened reliance on frontal cortical inhibitory control mechanisms in MSA patients to manage interference between numerical and semantic information, potentially indicative of compensatory neural activity patterns in response to executive function deficits.

Disclosures: L. Fan: None. **M.J. Kuo:** None. **W. Lai:** None. **T. Chou:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.19/B31

Topic: B.08. Epilepsy

Support: NIH NIDCD Grant R01-DC-016364
NIH NIDCD Grant R01-DC-018539
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NIH NIDCD Grant R01-DC-021663
NIH NINDS Grant T32 NS047987

Title: Interictal epileptiform activity couples to respiration during NREM sleep in humans

Authors: *A. SHERIFF[1], S. WOODWARD[1], G. ZHOU[1], J. ROSENOW[2], G. LANE[1], M. Z. KOUBEISSI[3], S. SCHUELE[1], C. ZELANO[1];
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in patients with epilepsy. SUDEP is characterized by a cascade of symptoms including apnea, cardiac arrythmia and asystole, with peri-ictal apnea always occurring first. Converging evidence implies that the amygdala may be involved in SUDEP (Dlouhy et al., 2015; Lacuey et al., 2017), as both electrical stimulation of (Nobis et al., 2018) and seizure spread to (Nobis et al., 2020) the amygdala are linked with cessation of breathing in epilepsy patients. Mortality following a seizure often occurs during sleep, which warrants further investigation into the relationship between epileptic activity in the amygdala and breathing during sleep. Here, we investigated interictal epileptiform activity in relation to ongoing respiratory activity during sleep in 6 patients undergoing intracranial monitoring for medically intractable epilepsy. Sleep stages were scored using scalp EEG recordings following traditional methods, and one hour of N2 sleep segments for each patient was obtained. Intracranial electrodes covering the amygdala, piriform cortex, and hippocampus were analyzed for each patient. Interictal epileptiform activity was detected using a semi-automated approach. Specifically, when amplitude at 25-50 Hz increased to 3.5 standard deviations relative to background activity for each channel, timepoints of interictal epileptiform activity that exceeded this amplitude threshold and included less than 3 cycles were collected for further analysis following manual validation of each event. Preliminary findings indicate that interictal epileptiform activity in the amygdala tends to occur at a preferred phase of breathing for all patients, with most occurring near the end of exhalation and the start of inhalation ($p < 0.05$, Rayleigh's test). Respiratory coupling with interictal spiking was also found in piriform cortex and hippocampus. Further analysis will involve a deeper investigation into the seizure networks for each individual to assess whether the epileptogenic zone is linked to greater

respiratory coupling of interictal epileptiform activity during sleep. Future studies will investigate effects on breathing before and after seizures during sleep. Altogether, findings will advance understanding of seizure networks implicated in SUDEP and how their interaction with respiration is related to epileptic activity.

Disclosures: A. Sheriff: None. **S. Woodward:** None. **G. Zhou:** None. **J. Rosenow:** None. **G. Lane:** None. **M.Z. Koubeissi:** None. **S. Schuele:** None. **C. Zelano:** None.

Poster

PSTR205: Epilepsy: Human Studies

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.20/B32

Topic: B.08. Epilepsy

Epilepsy Research Institute UK
UK Research and Innovation
Wellcome Trust

Title: Combining superficial white matter and adjacent grey matter abnormalities to identify epileptogenic tissue

Authors: *C. KOZMA[1], J. HORSLEY[2], G. HALL[2], C. SIMPSON[2], A. MCEVOY[3], J. DE TISI[4], G. P. WINSTON[5], Y. WANG[2], J. DUNCAN[4,3], P. TAYLOR[2];
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Abstract: Resective surgery offers relief for drug-resistant temporal lobe epilepsy (TLE). Achieving post-surgical seizure freedom hinges on effectively removing the epileptogenic zone (EZ). However, long-term seizure freedom is achieved in only about half of cases, highlighting the challenge in EZ localization. While diffusion-weighted MRI (dMRI) is not commonly used for pre-surgical localization, recent studies suggest its potential in identifying epileptogenic regions. Here, we use dMRI measures of white matter (WM) and volumetry of grey matter (GM) to localize epileptogenic tissue, validated through individual resection masks and post-surgical outcomes. We retrospectively analysed 138 TLE patients, examining adjacent WM and GM abnormalities. Using mean diffusivity (MD) from 97 healthy controls, we generated normative maps. Abnormal WM voxels were identified by z-scoring MD values against controls, with those exceeding a threshold classified as abnormal. We calculated the proportion of abnormal WM voxels within 5 mm of each GM region. Additionally, we determined GM abnormalities by deriving regional volumes from T1-weighted MRI and z-scoring patients against controls. Using WM abnormality proportions as weights, we combined them with GM region z-scores to create patient-specific abnormality maps. Assessing resected versus spared regions for each patient, we computed distinguishability score (D_{RS}) and correlated them with post-surgical outcome.

Seizure-free patients tended to have larger abnormalities surgically resected. Specifically, in the left TLE cohort, robust differentiation between seizure free and non-seizure free outcome patients was achieved (AUC=0.71, p=0.01), with even stronger results observed in right TLE cohort (AUC=0.77, p<0.001). WM abnormalities predominantly drove this combined abnormality detection in both LTLE (AUC=0.71, p=0.01) and RTLE (AUC=0.77, p<0.001) cohorts, while GM abnormalities exhibited lesser contributions (LTLE: AUC=0.58, p=0.31; RTLE: AUC=0.66, p=0.04). Our study shows the benefits of integrating WM and GM abnormalities for localizing epileptogenic tissue and distinguishing patient outcomes. Voxel-level dMRI emerges as a promising tool for pinpointing epileptogenic foci. Incorporating this approach into pre-surgical assessments may improve seizure control, especially for challenging cases. Furthermore, the integration of multiple modalities allows for comprehensive exploration and localization of epileptogenic abnormalities, offering potential for robust prognostic methods.

Disclosures: C. Kozma: None. **J. Horsley:** None. **G. Hall:** None. **C. Simpson:** None. **A. McEvoy:** None. **J. de Tisi:** None. **G.P. Winston:** None. **Y. Wang:** None. **J. Duncan:** None. **P. Taylor:** None.

Poster

PSTR205: Epilepsy: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR205.21/B33

Topic: B.08. Epilepsy

Title: Development and Clinical Modeling of Kv7 Channel Opener Prodrug for Treatment of Focal Onset Seizures and Status Epilepticus

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Abstract: Current 1st line treatment for status epilepticus (SE) are benzodiazepines. Ezogabine (EZG), a first-in-class Kv7 channel opener, was approved in 2011 for focal onset seizures (FOS). However, it was subsequently withdrawn from the market because of its large PK swing leading to TID dosing, poor tolerability due to PK, and AEs associated with skin discoloration. EZG's poor aqueous solubility and photo-instability was overcome by development of a soluble prodrug suitable for QD PO dosing and parenteral administration for SE. A prodrug strategy with a water soluble promoiety, that is generally recognized as safe (GRAS), was developed and has demonstrated 20,000 fold increase in solubility and is stable in acidic and neutral pH solutions for at least 3 months. It's oral, IM and SC pharmacokinetics in mouse, rat and dog were determined. The PK data was allometrically scaled to human for predictive exposure. Its efficacy after IM dosing in maximal-electroshock was determined in rat and potential for DDI with IM midazolam was assessed. The prodrug administered IM in rat exhibited linear PK for ezogabine from 5-75 mg/kg. No statistical difference in midazolam Cmax and AUClast when coadministered by itself or with Kv7 prodrug. The prodrug demonstrated increasing protection to

MES from 0.25 h through 1 h and full protection thereafter through 8 h where ezogabine drug levels were > 2µM in plasma. Allometrically scaled to human, a 200 mg dose is > 2uM at 100 minutes and is sustained for 20 h. The half-life of ezogabine from the administration of the prodrug is simulated to be 31 h by the IM route from the Kv7 prodrug compared to 6-8 h by the oral route from the tablet for Potiga®. These data together support that the Kv7 prodrug of ezogabine has suitable PK/PD profile for adjunct therapy to 1st line SE treatments that would allow for transition to oral post exposure treatment with the prodrug. As a prodrug of ezogabine, this prodrug is patent pending and can be developed under the 505(b)(2) regulatory pathway for FOS and SE.

Disclosures: G. Lee: None. **C. Crean:** None. **S. Thrasher:** None. **P. Danshina:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.01/B34

Topic: C.01. Brain Wellness and Aging

Title: Alteration of cGAS-STING signaling pathway components in the mouse cortex and hippocampus during healthy brain aging

Authors: *S. PASSARELLA[1], S. KETHISWARAN[2], P. CAVALLI[3], A. KRÖGER[4], D. C. DIETERICH[5], P. LANDGRAF[2];
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Abstract: The cGAS-STING pathway is a pivotal element of the innate immune system, recognizing cytosolic DNA to initiate the production of type I interferons and pro-inflammatory cytokines. This study investigates the age-dependent alterations of the cGAS-STING signaling components in the cortex and hippocampus of mice aged 24 and 108 weeks. In the cortex of 108-week-old mice, a significant increase in the dsDNA sensor protein cGAS and its product 2’3’-cGAMP was observed, without corresponding activation of downstream signaling, suggesting an uncoupling of cGAS activity from STING activation. This phenomenon may be attributed to increased dsDNA concentrations in the entorhinal cortex (EC) neurons, potentially arising from nuclear DNA damage and the formation of micronuclei. Contrastingly, the hippocampus did not exhibit increased cGAS activity with aging, but there was a notable elevation in STING levels, particularly in microglia and other cell types like neurons and astrocytes. This increase in STING did not correlate with enhanced IRF3-target gene expression, indicating that brain inflammation induce by the cGAS-STING pathway may manifest extremely late in the aging process. The

study also highlights the role of autophagy and its interplay with the cGAS-STING pathway, with evidence of autophagy dysfunction in aged hippocampal neurons leading to STING accumulation. Our findings underscore the complexity of the cGAS-STING pathway's involvement in brain aging, with regional variations in activity and potential implications for neurodegenerative diseases. Hence, our study provides a descriptive overview of the alterations in the cGAS-STING pathway during normal aging in the EC and hippocampus, suggesting that further focused research could elucidate the pathway's function in aging and its potential manipulation to delay senescence or treat neurodegenerative diseases.

Disclosures: S. Passarella: None. **S. Kethiswaran:** None. **P. Cavalli:** None. **A. Kröger:** None. **D.C. Dieterich:** None. **P. Landgraf:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.02/B35

Topic: C.01. Brain Wellness and Aging

Support: William and Ella Owens Medical Research Foundation
NIH R01 NS132778

Title: LRP1 knockout in adult neural stem cells in age-related hippocampal dysfunction and its potential link to psychiatric disorders

Authors: *N. MARION[1], K. DIETERT[1], S. SPRAGUE[1], P. REED[1], E. KOKOVAY[2], N. L. SAYRE[1];
[1]Neurosurg., [2]Dept. of Cell. and Structural Biol., Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX

Abstract: Adult neurogenesis in the dentate gyrus of the hippocampus influences learning, memory, and mood regulation. However, our comprehension of the mechanisms linking adult neurogenesis with depression and other psychiatric conditions remains limited. Dysfunctions in the hippocampus have been associated with various psychiatric disorders such as depression, anxiety, post-traumatic stress disorder, and schizophrenia (Goncalves et al., 2016; Sala et al., 2004). Studies suggest that antidepressant treatments enhance neurogenesis, potentially contributing to their therapeutic effects (David et al., 2009; Santarelli et al., 2003). Additionally, neurogenesis appears to mitigate neuroendocrine responses and depressive symptoms induced by stress in animal models (Eliwa et al., 2021; Hill et al., 2015). We believe better understanding factors regulating adult neurogenesis will improve our pathobiological understanding of psychiatric disorders and advance treatment options. Our laboratory identified low-density lipoprotein receptor-related protein 1 (LRP1) as a novel modulator of adult neurogenesis. We found that knocking out LRP1 in adult neural stem cells (NSCs) leads to hippocampal-dependent memory impairments. Using inducible Nestin-Cre, we selectively targeted LRP1 knockout in

adult NSCs of 3-month-old floxed mice, while using age-match Nestin-Cre control mice that lacked floxed LRP1. By 9 months of age, KO mice exhibited elevated anxiety in the elevated plus maze, while the Barnes maze revealed impaired hippocampal memory compared to controls. Histological assessments revealed altered NSC migration and increased maturation in LRP1 knockout mice, indicating potential disruption of hippocampal neural circuitry. We observed NSCs migrating farther from the stem cell niche towards the dorsal/suprapyramidal blade of the dentate gyrus in LRP1 knockout mice. Additionally, there was an increase in NeuN-positive, td-tomato-positive neurons in 9-month-old LRP1 KO mice. Overall, our findings suggest that LRP1 knockout affects NSC migration and maturation which is potentially disrupting hippocampal neural circuitry. Our goal is to investigate whether LRP1 in NSCs contributes to psychiatric disorders by examining depressive-like or other psychiatric symptoms in our mouse models and elucidating underlying mechanisms.

Disclosures: N. Marion: None. **K. Dietert:** None. **S. Sprague:** None. **P. Reed:** None. **E. Kokovay:** None. **N.L. Sayre:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.03/B36

Topic: C.01. Brain Wellness and Aging

Support: R35CA283926
R35CA197289
NIH R01 AG067258

Title: Chemotherapy increases Ascl1 expression in the dentate gyrus

Authors: *C. NG[1], G. FRANCO QUARESMA DE MOURA[2], L. F. KROMER[2], J. MANDELBLATT[4], G. W. REBECK[3];
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Abstract: Cancer-related cognitive impairment (CRCI) is a common side effect of cancer and its treatments. Cancer chemotherapy may affect brain function through effects on neuroinflammation, neurotoxicity, damage to the blood brain barrier, or impaired neurogenesis. Here, we investigated the effects of chemotherapy treatment on neural stem cell proliferation, using a tamoxifen inducible mouse model designed to express tdTomato in response to Ascl1 induction; Ascl1 is a factor active in adult neurogenesis. Five to six-month-old Ascl1-CreERT2 mice (control n= 5 male, 4 female; treated n= 5 male, 4 female) were treated with either doxorubicin (10mg/kg), a common chemotherapeutic drug, or DMSO vehicle. One week later, mice were treated with 4 daily doses of tamoxifen (60mg/kg). Four days later mice were

euthanized, and brains were perfused then fixed for immunohistochemistry. The brains were sliced into 30um sections and immunostained with antibodies for tdTomato. As expected, there was strong staining of cells in the dentate gyrus subgranular layer. Doxorubicin-treated mice had 93% higher levels of tdTomato compared to control, in both male and female mice ($p<0.05$). We are analyzing the effects of doxorubicin on other neurogenesis genes (such as Sox2) and on Ascl1-positive cell morphologies. Our current results suggest that doxorubicin treatment increased Ascl1 cell lineages of the dentate gyrus within two weeks of exposure.

Disclosures: C. Ng: None. **G. Franco Quaresma de Moura:** None. **L.F. Kromer:** None. **J. mandelblatt:** None. **G.W. Rebeck:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.04/B37

Topic: C.01. Brain Wellness and Aging

Title: Necroptosis is reduced after voluntary exercise in hippocampal subfields during aging

Authors: *F. VELIZ[1,2], M. S. ARRAZOLA[1,2], F. A. COURT[1,2];
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Abstract: Abstract: We have previously demonstrated the role of necroptosis in the progression of axonal degeneration in the hippocampus during aging, showing that inhibition of necroptosis reduces age-related cognitive impairment. In the present study, we investigated whether necroptosis is reduced following voluntary exercise along aging. Here, we show that aged mice exposed to voluntary exercise exhibit a decrease in necroptosis activation (RIPK3, MLKL) in hippocampal subfields. In vitro, we demonstrate that serum from aged sedentary mice, but not from exercised ones, increases necroptosis activation. Conversely, shRNA against RIPK3 could reverse this effect. We propose voluntary exercise as a non-pharmacological intervention to mitigate necroptosis activation during normal aging, thus improving cognitive performance.
Materials and methods: Wild-type (WT) C57BL/6J male mice of 18 months were exposed to voluntary exercise protocol on running wheels for 2 months. After training and behavioural evaluations (Morris Water maze, Open Field, Rotarod, Y Maze), 20-month-old mice were perfused and brains were dissected. Serial cutting of 20 μm thickness sagittal brain sections was performed. Neurons were visualized by fluorescence microscopy and axonal degeneration was determined in the hippocampus. To evaluate axonal degeneration and necroptosis markers *in vitro*, primary culture of hippocampal neurons were prepared from WT mice (E16) and cultures were maintained for 28 days. AAV expressing shRNA for RIPK3 or a scramble control sequence was added at day 7. Serum administration (2%) was added to cell cultures at day 14 until day 21.**Results:** Voluntary exercise attenuated age-related hippocampal neuroinflammation, the expression of necroptosis markers in hippocampal subfields and ameliorated age-related

cognitive impairments in aged mice. Also, we found that aged serum from runner mice could attenuate the expression of RIPK3 and MLKL. Furthermore, we show that exposure of hippocampal neurons to serum from aged mice caused a significant increase in necroptosis markers, neurodegeneration and changes in astrocyte morphology, and the downregulation of RIPK3 could reverse this effect. **Conclusions:**Voluntary exercise attenuated necroptosis activation in hippocampus of aged mice, providing a non-pharmacological treatment to prevent age-related impairment in cognitive capacity.

Disclosures: F. veliz: None. **M.S. Arrazola:** None. **F.A. Court:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.05/B38

Topic: C.01. Brain Wellness and Aging

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Centro Ciencia & Vida, FB210008, Financiamiento Basal para Centros Científicos y Tecnológicos de Excelencia de ANID to CTR
FONDECYT No. 11241376 to CJ

Title: Mitochondrial Dysfunction in the Aging Hippocampus: Accumulation of Phosphorylated Tau PHF-1 and its relationship with Lonp1 Proteolytic Activity

Authors: *K. CICALI[1], J. LLANQUINAO[1], B. RIVERA[1], A. TORRES[1], C. JARA[1], A. SCHÜLLER[2], C. TAPIA-ROJAS[1];
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Abstract: Brain aging is a natural process characterized by cognitive decline linked to mitochondrial dysfunction and the accumulation of abnormal proteins in the hippocampus. Among these abnormal proteins is the pathological form of tau PHF-1 (phosphorylated at Ser396 and 404), which dissociates from microtubules and accumulates in neurons, triggering mitochondrial dysfunction, synaptic failure, and ultimately neurodegenerative diseases. Previous studies from our laboratory have demonstrated increased levels of tau PHF-1 in the hippocampus of aged mice, specifically inside synaptic mitochondria in aging. However, how and why tau PHF-1 localizes and accumulates within mitochondria is unknown. Lonp1 is the main matrix mitochondrial protease, responsible for degrading the mitochondrial proteome and, interestingly, also can degrade abnormal cytosolic proteins imported into mitochondria. Through bioinformatics and biochemical assays, we analyzed whether tau PHF-1 is imported inside mitochondria for degradation by Lonp1, and the changes of Lonp1 in the mice hippocampus during aging and its impact on tau PHF-1 accumulation, and consequently on mitochondrial

function. Our results show that tau PHF-1 is imported to mitochondria by a conventional import mechanism. In addition, bioinformatics analysis and an in vitro degradation assay revealed that tau PHF-1 is a new substrate of Lonp1. We also observed that Lonp1 protein and proteolytic activity decreased in the aged hippocampus, explaining the mitochondrial tau PHF-1 accumulation in aging. Ultimately, we demonstrate that tau PHF-1 accumulation induced mitochondrial dysfunction. Taken together, these results suggest that phosphorylated tau PHF-1 could be imported into mitochondria to be degraded by Lonp1. However, this degradation mechanism could be compromised in the aged hippocampus, thus promoting mitochondrial dysfunction in normal aging.

Disclosures: K. Cicali: None. **J. Llanquinao:** None. **B. Rivera:** None. **A. Torres:** None. **C. Jara:** None. **A. Schüller:** None. **C. Tapia-Rojas:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.06/B39

Topic: C.01. Brain Wellness and Aging

Support: Canadian Institutes of Health Research (CIHR) (PJT- 173540)

Title: Synaptic density in healthy aging measured by [^{18}F]SynVesT-1 PET

Authors: *S. L. MARTIN[1], E. CARMONA[2], C. URIBE[2], K. L. DESMOND[2], I. BOILEAU[2], A. GRAFF-GUERRERO[3], N. VASDEV[2], A. P. STRAFELLA[4];
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Abstract: Background: In vivo quantification of synaptic density is possible due to novel radiotracers that target the ubiquitously expressed pre-synaptic vesicle protein SV2A. An early version of the SV2A tracer [^{11}C-UCB-J], indicated that synaptic density did not substantially change during aging [1]. However, post-mortem synaptic density quantified using synaptophysin, a presynaptic vesicle protein, has shown age-related changes in the neocortex of cognitively normal aging controls [2]. Hence, further investigation is warranted. The new [^{18}F]SynVesT-1 radiotracer has optimal pharmacokinetics and a longer half-life. Here we investigate synaptic density in healthy aging using [^{18}F]SynVesT-1 PET. Understanding how [^{18}F]SynVesT-1 PET changes in healthy aging will help guide the research conducted in development, accelerated aging, and patient populations [3] [4]. **Methods:** 26 subjects were included in the analyses [23 - 73 years old]. Subjects were divided into Younger (< 40 years, N = 14, 31.82±5.53 years, 5F) and Older (> 40 years, N = 12, 55.5±14.06, 9F) cohorts. All subjects had a [^{18}F]SynVesT-1 PET scan on a GE Discovery MI PET-CT system. Images underwent attenuation correction using CT scan and aligned to the subject's T1-weighted MRI. Using

PMOD (v4.203), the standardized uptake value ratio (SUVR) and non-displaceable binding potential (BPnd) were calculated. SUVR was calculated for the 60-90 minute time window [5], and the BPnd was calculated using the simplified reference tissue model (SRTM2) for the 0-90 minute time window. Due to low specific binding of SV2A, the centrum semiovale (CS) was used as the reference region. 14 volumes of interest (VOI) relevant to healthy brain aging were generated using the Hammers AAL atlas [1]. Statistical analyses were performed in SPM with FWE $p < .05$ and cluster threshold $k > 50$. For total and each VOI, a t-test was used to compare cohorts, and linear regressions between BPnd/SUVR and age. **Results:** No significant difference was observed in total brain BPnd (Younger: 322.96±25.76 vs Older: 326.56±43.61) and SUVR (Younger: 413.91±28.61 vs Older: 426.1±44.39) or VOIs. Linear regression analyses between BPnd/SUVR and age did not show significant relationships. Our results are based on the assumption that age does not influence the number of SV2A vesicles per synapse. **Conclusion:** Pre-synaptic density assessed via [^{18}F]SynVesT-1 did not show age-dependent changes. This is in line with results reported using [^{11}C]UCB-J. These analyses are preliminary and larger cohorts of diverse populations are essential to determine age-related synaptic density and understand discrepancies between in-vivo and post-mortem findings.

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Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.07/B40

Topic: C.01. Brain Wellness and Aging

Support: NIEHS R35-S030523

Title: Impact of Long-Term Partial Drp1 Knockout in Aging Brain

Authors: *S. S. SALEHE[1], R. Z. FAN[1], Y. LAI[1], H. GU[2], K. TIEU[1];
[1]Envrn. Hlth. Sci., Florida Intl. Univ., Miami, FL; [2]Col. of Hlth. Solutions, Arizona State Univ., Phoenix, AZ

Abstract: Aging is a major risk factor for neurodegenerative disorders, and shares overlapping hallmarks. Our group investigates the benefit of inhibiting dynamin-related protein 1 (Drp1), a master regulator of mitochondrial fission, in models of Parkinson's disease. Previous studies show that partial Drp1 inhibition is protective against mitochondrial dysfunction, synaptic dysfunction, autophagic impairment, and protein aggregation. These mechanisms are also

implicated in aging and progeria syndromes. Therefore, we hypothesized that partial Drp1 knockout (*Dnm1l*$^{+/-}$) would be protective against age-related brain alterations. We generated and aged *Dnm1l*$^{+/+}$ and *Dnm1l*$^{+/-}$ for this study (young ~ 3m and old ~ 18-24m).Since motor impairment and memory deficits are common in aging, we determined the impact of *Dnm1l*$^{+/-}$ in aging mice using activity chambers and object recognition test. *Dnm1l*$^{+/-}$ improved mice's locomotor performance by increasing distance traveled (~40%, p=0.003), travel speed (~40%, p=0.002), and less resting time (~30%, p=0.007) [n= 9-12]. On the learning and memory test, *Dnm1l*$^{+/-}$ attenuated age-related learning and memory impairment (p=0.0003) [n=16-20]. From these behavioral studies, we investigated synaptic changes as they play major roles in brain functions including locomotion and learning. We determined the levels of synaptic proteins by immunoblotting. *Dnm1l*$^{+/-}$ significantly increased cortical (p=0.02) and hippocampal (p=0.001) postsynaptic density [n=8]. In aging, postsynaptic density peaks around 1 month and gradually declines with age. On the other hand, *Dnm1l*$^{+/-}$ significantly increased presynaptic density in the cortex but not in the hippocampus. To identify the potential protective mechanism of *Dnm1l*$^{+/-}$ in the aging brain, we used untargeted metabolomics and lipidomics [n=10]. These omics identified several significant features and pathway analysis identified the enrichment of lipid metabolism pathways – biosynthesis of unsaturated fatty acids, alpha-linolenic acid, sphingolipids, and glycerophospholipids metabolism. Immunohistochemistry revealed that *Dnm1l*$^{+/-}$ reduced lipid droplet accumulation in the aging microglia. Lipid-laden microglia are known to be pro-inflammatory and by using qPCR we determined that *Dnm1l*$^{+/-}$ reduced *Tnf-α*, *Il-6*, and *Lcn2* gene transcripts in different brain regions of aging mice. Our results demonstrate the benefits of Drp1 inhibition in reducing the hallmarks of aging. Furthermore, these data show that long-term Drp1 inhibition as a therapeutic strategy does not produce detectable negative impact. The mechanisms of *Dnm1l*$^{+/-}$-mediated protection in aging are being investigated.

Disclosures: S.S. Salehe: None. **R.Z. Fan:** None. **Y. Lai:** None. **H. Gu:** None. **K. Tieu:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.08/Web Only

Topic: C.01. Brain Wellness and Aging

Support: Qingdao University Excellent Second Scholarship
Qingdao University Excellent Third Scholarship

Title: Transcriptome analysis of brain aging identifies genes involving immune and neurological diseases

Authors: *H. XUE;
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Abstract: Aging profoundly affects individual health, which is frequently compromised by aging-associated disorders that are highly prevalent among the elderly. Although neurodegenerative diseases are closely linked to aging, the underlying connections between brain aging and the onset of these diseases remain poorly understood. We analyzed brain transcriptome data from 302 individuals across 13 different brain regions (2,181 samples), sourced from the GTEx database. We employed robust regression analysis to adjust for sex and other confounding factors and identify genes whose transcript levels altered with age. A random effects model in a meta-analysis summarized the aging effects across all brain tissues. Gene ontology enrichment analysis was utilized to explore the biological processes linked with these aging-associated genes. Our analysis identified an average number of 16,761 genes (mean TPM ≥ 1) expressed across the 13 brain regions. Among these, 7,931 genes showed significant associations with aging (meta_FDR < 0.05), including 271 up-regulated and 7,660 down-regulated genes. Gene ontology analysis results indicated that up-regulated genes were primarily involved in immune processes such as antigen processing and autoimmune diseases (e.g., Type I diabetes, autoimmune thyroid disease). Conversely, down-regulated genes were associated with axonogenesis, synaptic signaling, longevity regulating pathway, and were linked to neurological disorders such as amyotrophic lateral sclerosis. Overall, our study indicates significant systemic changes in the brain transcriptome with aging. These changes are closely related to pathways associated with both immune and neurological diseases, highlighting potential targets for understanding and treating aging-related conditions.

Disclosures: H. Xue: None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.09/B41

Topic: C.01. Brain Wellness and Aging

Support: NSFC Grant 81930030, 82230036, 31771109, and 31722023
the Science and Technology Innovation 2030-Major Project 2021ZD0203501
CAMS Innovation Fund for Medical Sciences 2019-I2M-5-057

Title: Molecular control of biological age and frailty in midlife females

Authors: *J. QU[1], H. MA[2];
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Abstract: Despite women's longer lifespan, they paradoxically experience increased frailty in later life. Here, we unveil a causal link between this and the calcium-responsive kinase γCaMKII during biological aging. Regulated by microRNA miR-X-5p, it induced a midlife-specific reduction in γCaMKII expression exclusive to females. Loss of γCaMKII in mice heightened

frailty, impaired mitochondrial function, and worsened cellular senescence, signifying accelerated biological aging. Brain-targeted delivery of a miR-X-5p antagomir elevated γCaMKII, reducing cellular senescence and improving cognitive performance in middle-aged female mice. Under pathological conditions resembling female-biased Alzheimer's disease, manipulation of the γCaMKII/miR-X-5p axis ameliorated AD-like deterioration in patient-derived neurons and AD model mice. Collectively, these findings suggest that midlife-programmed γCaMKII loss accelerates biological aging in females, providing a promising therapeutic avenue for addressing sex-specific aging-associated frailty.

Disclosures: J. Qu: None. **H. Ma:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.10/B42

Topic: C.01. Brain Wellness and Aging

Support: Wakunaga Pharmaceutical Co.
MU-SOM research funds

Title: Aged Garlic Extract Attenuates Age-Associated Cognitive and Memory Decline Through Altered Multi-Functional Clusters in Cortex and Hippocampus

Authors: *T. MONY[1], W. YU[2], M. JACKSON[2], A. ZUCKERMAN[2], R. LI[2], A. BALDERRAMA[2], C. M. GREENLIEF[2], G. Y. SUN[2], J. CUI[2], Z. GU[2,3];
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Abstract: Garlic (Allium sativum) is an ancient medicinal plant with over 5000 years of history found in Egypt, Israel,Greece, China, and India. Aged garlic extract (AGE) is recognized as a nutraceutical for health maintenanceespecially among older adults with decline in synaptic plasticity and increase in innate immunity. With theincrease in aged population, the prevalence of cognitive decline and affected neurodegenerative diseases isestimated to double every 20 years. In recent studies, there are evidence for AGE supplementation to displaybeneficial effects on cardiovascular and neuropsychological functions. Our recent studies also showed abilityfor AGE and its bioactive components to suppress microglial activation and enhance antioxidant ability. Giventhese insights, this study is to investigate dietary supplement of AGE in aging mice to confer cellular resiliencefor brain health and to elucidate its underlying mechanisms on cognition-related molecular networks andfunctions. C57BL/6 male mice at the age of 42 weeks were fed AGE diet for 40 weeks (10 months). A batteryof behavior tests was carried out to examine effects of AGE on various aspects of cognition and learningdomains; This was followed by label-free global proteomics and machine-learning driven bioinformaticsanalysis focusing on molecular alternations in the cortex and hippocampus in order to identify signaling driversin

dietary AGE fed mice for altered behavioral phenomes. Aging mice fed the AGE diet showed no change inbody weight, but improvements in cognitive and learning abilities, including short-term memory and spatiallearning ability. Among close to 6,000 proteins identified by 4D label-free quantitative proteomics and withgene ontology enrichment analysis, results revealed multi-functional clusters from over 200 proteins withstatistically significant changes in the AGE-fed aging mice. Taken together, these findings demonstrate dietaryAGE to confer beneficial effects and improvement on cognitive and memory function in the aging brain.Results also suggest AGE to serve as a valuable supplement for preventing aging-related neurologicalcomorbidities.

Disclosures: T. Mony: None. **W. Yu:** None. **M. Jackson:** None. **A. Zuckerman:** None. **R. Li:** None. **A. Balderrama:** None. **C.M. Greenlief:** None. **G.Y. Sun:** None. **J. Cui:** None. **Z. Gu:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.11/B43

Topic: C.01. Brain Wellness and Aging

Support: R01 AG066018-04

Title: Single-cell Multi-omic view of the Aging Mouse Brain

Authors: *R. ZENG[1], W. TIAN[2], J. R. ECKER[3], B. REN[4], M. BEHRENS[5];
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Abstract: Aging is recognized as a primary risk factor for numerous neurodegenerative diseases, including Alzheimer's Disease and Parkinson's Disease, underscoring the complex interplay between aging and cellular degeneration. The past decade has witnessed significant advancements in our understanding of aging at the molecular level, particularly regarding the progressive alterations to cellular epigenomes. These changes, encompassing histone post-transcriptional modifications, DNA methylation, and chromatin conformation have been closely linked to aging processes. Despite these advances, previous genomic studies mainly use bulk-level tissues to identify potential epigenetic changes during aging. However, different cell types within the same organism can mature along highly distinctive aging trajectories. Here, we employ enhanced single-nucleus methylation sequencing (snmC-seq3) and snm3C-seq technologies to analyze DNA methylomes and the 3D genome with unprecedented detail. We collected 132,551 methylomes for the male brain samples and 72,666 m3C joint profiles from the female brain samples. For both genders, we dissected 8 brain regions including anterior hippocampus (AHC), posterior hippocampus(PHC), frontal cortex (FC), amygdala (AMY), nucleus accumbens (NACB), PAG/PCG, entorhinal cortex(ENT) and caudate putamen(CP) from2-month, 9-month, 18 month-old mice. We constructed a methylation-based cell taxonomy

54 cross-modality-annotated cell types.
Highlight:
Here we systematically evaluated aging effects on different the cell types: 1. Non-neuronal cells exhibit more significant age-related methylation changes compared to neurons.2. Aging is associated with hypermethylation of ribosomal genes, leading to decreased gene expression.3. Motif Enrichment Analysis reveals AP1 motifs are hypomethylated in various glutamatergic neurons; Sox family motifs are hypermethylated in oligodendrocyte progenitor cells.
Transposon Regions as Methylation Aging Hotspots: LTR-ERV1-MuRRs-int retrotransposons on chromosome 13 are hypomethylated in glutamatergic neurons from diverse brain regions, including the hippocampus, entorhinal cortex, amygdala, and frontal cortex, and also show increased chromatin accessibility.
Increase of domain boundary strength leads to decreased gene expression: An increase in domain boundary strength during aging leads to more insulated domains, with genes within these domains showing decreased expression due to diminished enhancer-promoter interactions.
Recurrent increase in compartment strength: We found a recurrent increase in compartment strength across most cell types.

Disclosures: R. zeng: None. **W. Tian:** None. **J.R. Ecker:** None. **B. Ren:** None. **M. Behrens:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.12/B44

Topic: C.01. Brain Wellness and Aging

Support: NIH AG083078-01

Title: Transmitter release site changes at aged NMJs are correlated with weakened synaptic strength

Authors: *N. LI[1], M. GANDHI[2,3], Y. BADAWI[2], S. D. MERINEY[2];
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Abstract: Neuromuscular junctions (NMJs) age over a biphasic time course in male mice, with an early enhancement in function at 20-22 months that coincides with alterations to the postsynaptic receptor distribution, and a later weakening at 25-30 months. Interestingly, individual NMJs within one muscle show such aging changes independent of one another and in these later ages, there is significant variability in structure and function. Here, we have stratified individual NMJs into strong, medium, and weak synapses at a later aging stage (26 months) and explored the potential presynaptic mechanisms that contribute to neuromuscular weakness. Combining data acquired using electrophysiological recordings and immunohistochemistry, we

investigated the relationship between structure and function within single aged NMJs at 26 months of age. In addition to comparing weak vs. strong NMJs at 26 months of age, we also compared NMJs between 4 and 26 months of age. First, we found that weak NMJs (low quantal content) at 26 months of age had significantly reduced active zone density, and this was not observed in weak NMJs from 4-month-old mice. We also found that NMJs with low quantal content at 26 months of age showed increased facilitation during a train stimulation (hinting at a significantly lower probability of release at each active zone), unlike strong synapses from the same muscle, or any strength synapse in 4-month-old mice. Paradoxically, these low synaptic strength NMJs from 26-month-old mice showed a significantly increased readily releasable pool (RRP) size. Thus, we hypothesize that this increase in RRP may be a compensatory mechanism in these weak NMJs to increase transmitter release. This data led us to conclude that as NMJs become weak during the aging process, this weakness manifests as the result of altered presynaptic release properties (reduced AZ density and probability of release). The results from this work highlight the underlying heterogeneity between aged NMJs and narrow the scope for future research that targets factors that render these aged NMJs weak.

Disclosures: N. Li: None. **M. Gandhi:** None. **Y. Badawi:** None. **S.D. Meriney:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.13/B45

Topic: C.01. Brain Wellness and Aging

Support: NIH AG044486
NIH AG015379

Title: Age-associated changes in the expression and maturation of GLT-1 and its interaction with PS1/gamma-secretase

Authors: *M. SADEK, S. MITCHELL, F. PERRIN, P. SINHA, B. LUNDIN, G. ARMAGAN, N. WIECKIEWICZ, M. MAESAKO, O. BEREZOVSKA;
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Abstract: Glutamate transporter 1 (GLT-1, EAAT2 in the human), the predominant glutamate transporter in the brain, interacts with Presenilin 1 (PS1), the catalytic component of γ-secretase responsible for producing amyloid-beta (Aβ) peptides (Zoltowska et al., *Sci Rep*, 2018). Our group has previously shown that this interaction potentiates GLT-1 cell surface expression, multimerization, and glutamate uptake *in vitro* (Perrin et al., *Res Sq preprint*, 2023). Furthermore, the GLT-1/PS1 interaction is reduced in sporadic Alzheimer's Disease (AD) brains, which provides a link between Aβ pathology and glutamate dysregulation in AD. To better elucidate the impairment in GLT-1/PS1 interaction in pathological conditions, it is crucial to examine how these two proteins interact during normal aging. In the present study, we aim to

investigate the age-associated changes in GLT-1 expression, maturation, and its interaction with PS1, *ex vivo* in the C57BL/6 mouse cortex at age 2, 8 and 22.5-months (mo), as well as in CD1 mouse primary neurons cultured to 7, 14 and 21 days *in vitro* (DIV). We have measured the expression, homo-multimer formation, and cell surface trafficking of GLT-1 by Western Blot, Blue Native PAGE, and flow cytometry, respectively, and have assessed its interaction with PS1 using two methods: GLT-1/PS1 complex co-immunoprecipitation and fluorescence lifetime imaging microscopy. In the mouse cortex (n=4/group), we find that the GLT-1/PS1 interaction is concomitant with the age-associated changes in GLT-1 expression and multimerization, increasing from 2-mo to 8-mo but decreasing in the 22.5-mo mouse cortex. In primary neurons (n=3), we find that, although GLT-1 expression, multimerization, and cell surface delivery all increase progressively with age, the GLT-1/PS1 interaction is downregulated in 21DIV neurons. To reveal the consequences of the reduced GLT-1/PS1 interaction in advanced age, we have used a cell-permeable peptide (CPP) recently designed by our group to inhibit the GLT-1/PS1 interaction. We demonstrate that treatment of 14DIV neurons with the CPP increases GLT-1 cell surface delivery (n=5) and aggregation (n=3), and our preliminary data suggest that CPP treatment impairs GLT-1-dependent glutamate uptake. Together, these findings suggest that the age-related impairment in GLT-1/PS1 interaction may drive dysregulations in GLT-1 maturation and function in the old brain. Hence, with age being a major risk factor for neurodegenerative disease, the further downregulation of GLT-1/PS1 interaction in AD could contribute to the transition from normal to pathological aging.

Disclosures: M. Sadek: None. **S. Mitchell:** None. **F. Perrin:** None. **P. Sinha:** None. **B. Lundin:** None. **G. Armagan:** None. **N. Wieckiewicz:** None. **M. Maesako:** None. **O. Berezovska:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.14/B46

Topic: C.01. Brain Wellness and Aging

Support: CHDI Foundation

Title: Insights into biological ageing in human brain obtained from the methylome and hydroxymethylome of human striatal neurons

Authors: *H. CHETIA, M. BAFFUTO, K. MATLIK, L. KUS, N. HEINTZ;
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Abstract: Aging is a complex physiological process associated with many intrinsic and extrinsic factors. One of these factors, DNA methylation (DNAm), is fundamental to the identity and function of a cell. DNAm stably exists in Cytosines (C) in two forms: 5-methylC (5mC) and 5-hydroxymethylC (5hmC). Both 5mC and 5hmC play critical roles in regulation of gene

expression and have been studied using microarray and bisulfite sequencing methods. However, these techniques do not provide a distinction between 5mC and 5hmC signals. So, the total of 5mC+5hmC signals have been studied as a single entity, leading to development of the 'epigenetic' or DNAm clocks (Hannum et al., 2013; Horvath, 2013). These clocks are recognized as highly accurate molecular correlates of chronological age in humans and seem to provide a quantitative estimation of the biological age of the cell, tissue, or subject. However, the question remains as to how 5mC or 5hmC signals relate to the biological age as independent entities. In contrast to most peripheral cell types, neurons do not divide and must be maintained throughout life. Neurons have also been shown to have 10-fold higher levels of 5hmC than peripheral cell types, which has been proven to be required for active DNA demethylation generated by Tet-mediated demethylation pathways (Kriaucionis and Heintz, 2009; Stoyanova et al., 2021). In this study, we have generated paired WGBS-oxBS data from medium spiny neurons (MSNs) (n=21) of human striatum from 9 control donors. Using these data, we demonstrate that hydroxymethylation is a distinct and necessary metric to predict the rate of cellular aging. We also illustrate that 5mC and 5hmC levels differ in gene bodies and regulatory regions of the genome with respect to age and how these results can lead to an improved understanding of aging in the human striatum.
Acknowledgement: Tissue donors and family members, Miami Brain Bank, T.Carroll and lab members.

Disclosures: H. Chetia: None. **M. Baffuto:** None. **K. Matlik:** None. **L. Kus:** None. **N. Heintz:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.15/B47

Topic: C.01. Brain Wellness and Aging

Support: R01AG079989-01
5T32AG020506-22

Title: The Effect of Physiological Aging on Behavior and Epigenetics in Mice

Authors: *S. B. SCHEINMAN, H. DONG;
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Abstract: Background: Aging is a time-dependent deterioration of physiological functions that occurs in both humans and animals. Within the brain, aging cells gradually become dysfunctional through a complex interplay of intrinsic and extrinsic factors, ultimately leading to behavioral deficits and enhanced risk of neurodegenerative diseases such as Alzheimer's disease (AD). The characteristics of physiological aging are distinct from those associated with age-related diseases and it is important to understand the processes that contribute to this pathological

divergence. The identification of behavioral and epigenetic biomarkers associated with normal aging is key in determining the mechanisms of underlying pathological aging and how these impact AD. **Methods:** We conducted a comprehensive behavioral assessment of young (3-month-old) and aged (18-month-old) C57/BL6 mice including locomotion, memory-relevant, and anxiety-like behavior to elucidate the cognitive and behavioral phenotypes of aging. We then biochemically evaluated histone markers and histone modifying enzymes, and conducted CUT&RUN sequencing to analyze genome-wide abundancy and distribution of H3K27ac, a histone marker that associates with promoters and enhancers of active genes, to determine age-related changes in epigenomic profiles. **Results**: Compared to young mice, aged mice displayed decreased locomotion in the open field ($p<0.0001$), enhanced anxiety in the elevated plus maze ($p=0.0003$), and deficits in the novel object recognition task ($p=0.0011$) and novel arm entry task ($p=0.0155$). CUT&RUN analysis also revealed significant age-related alterations in H3K27ac genome occupancy, including sites associated with transcriptional regulation, synaptic plasticity, and neuron function. **Conclusions:** These data suggest that physiological aging induces a unique behavioral phenotype that's associated with alterations in the epigenome. Future studies will be focused on disentangling epigenetic and behavioral alterations that occur during normal aging from those that occur during pathological aging and aging-related diseases such as AD.

Disclosures: S.B. Scheinman: None. **H. Dong:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.16/B48

Topic: C.01. Brain Wellness and Aging

Support: NIH RF1AG076653
NIH R01AG074248

Title: Ultrastructural studies in human iPSC derived neurons reveal a more systemic role for MAPK8IP3 in regulating neuronal lysosomal maturation

Authors: *M. KROUT[1], S. GOWRISHANKAR[2];
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Abstract: Optimal axonal lysosome transport is critical for neuronal health and function. This involves specialized adaptors that link lysosomes to motor proteins for efficient microtubule-based transport. Disruptions in axonal lysosome transport homeostasis have been linked to both neurodevelopmental and neurodegenerative diseases. Recently, JIP3/MAPK8IP3, a neuronally enriched lysosome adaptor, was identified as a critical regulator of axonal lysosome abundance. Loss of JIP3 leads to massive accumulation of lysosome-and related organelles within axon swellings, highly reminiscent of the lysosome pathology observed in Alzheimer's disease.

However, the precise nature of the accumulating organelles, including the identity of cargo that build up in them, are not fully understood. Here we take advantage of the MAPK8IP3 knockout model and utilize transmission electron microscopy (TEM) to reveal the complexity of accumulating axonal organelles at the ultrastructural level. Furthermore, using coincidence with endocytosed BSA-conjugated gold beads we reveal a stunning expansion in the spectrum of endo-lysosomal intermediates, even in the soma of MAPK8IP3 KO neurons. Thus, we now identify a new role for MAPK8IP3 in regulating lysosome maturation in the neuronal cell body. Our study also highlights the strength of TEM in shedding new insight into the cell biology of endolysosomal organelles when used to complement traditional immuno-staining and live imaging techniques.

Disclosures: M. Krout: None. **S. Gowrishankar:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.17/B49

Topic: C.01. Brain Wellness and Aging

Title: Role of neuronal autophagy on memory and synaptic maintenance

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Abstract: Autophagy, one of the conserved cytoplasmic component degradation pathways has been widely studied in non-neuronal cells. However, neurons have some unique features distinguishing them from other cells like compartmentalization which may make autophagy to function differently in neurons relative to other cells. Given that impaired autophagy has been implicated in various neurodegenerative diseases, differences in autophagic function in various cell types may be relevance for these conditions. We have previously showed that whole-body autophagy deactivation leads to memory impairment and accumulation of some synaptic proteins at the synapse. However, the brain has a diverse cell population, and thus it is still unclear which specific cell type drives these observed effects. Here, we aim to show if neuronal autophagy is the main driver of the effects observed in whole-body autophagy deactivation. We generated an inducible knock out of the essential autophagy gene, Atg7, by mating an Atg7 floxed mouse with a line having tamoxifen (tam)-inducible Cre under beta-actin or a neuron-specific promoter (SLICK-H). At 19 weeks after birth, the mice were injected with tamoxifen daily for 1 week to deactivate autophagy in the whole-body (beta-actin) or in neurons (SLICK-H). They were then tested on the novel object recognition (NOR) test and Barnes maze at 8 weeks post-autophagy deactivation. After testing, mice were euthanized, and their brains processed to assess the levels of synaptic proteins, brain trophic factors and neuronal integrity. Both autophagy deactivated mice had significant impairment in NOR test and performance on Barnes maze, suggesting

impaired learning, although the specific pattern of deficits differed. Synaptosomes analysis from both lines showed accumulation of a subset of synaptic proteins, suggesting disrupted protein homeostasis at the synapse. However, the specific proteins affected differed between the lines. Whereas SLICK-H iKO mice had decreased levels of a subset of proteins in synaptosomes and a trend toward a decrease in BDNF and an increase in c-Fos levels compared to control animals. Autophagy deactivation results in memory impairment and alterations in the levels of synaptic proteins and brain trophic factors at the synapse in both conditions. However, neuronal autophagy deactivation showed a less severe phenotype, which points to the importance of glia autophagy in memory function and synaptic maintenance. This research adds to the fact of the relationships of autophagy in diverse cell populations in the brain for synaptic protein dynamics, adding potential insights into the pathophysiology of neurodegenerative disorders.

Disclosures: A.O. Ishola: None. **H. Grosso Jasutkar:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.18/B50

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant OD-011092

Title: Characterizing aquaporin-1 + astrocyte clusters in oldest-old rhesus macaque cortex

Authors: O. E. STAYER-WILBURN[1], D. L. ROSENE[2], P. L. SHULTZ[2], A. VITANTONIO[2], C. DIMOVASILI[2], J. MATTISON[3], K. VAUGHAN[3], H. F. URBANSKI[1], *S. KOHAMA[1]; [1]ONPRC, Beaverton, OR; [2]Boston Univ. Sch. of Med., Boston, MA; [3]Translational Gerontology Br., NIA, Baltimore, MD

Abstract: Aquaporin-1 (AQP1) is a conserved water channel protein, expressed in human and non-human primate brains in the choroid plexus, white matter, glial limitans and around cerebral blood vessels. With overt neuropathology, cortical AQP1 increases in astrocytes and has been found associated with amyloid beta and glial fibrillary acidic protein (GFAP). Previously we also found AQP1 increased with advanced age (late 20s+) in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC) of male and female rhesus macaques (22 to 44 years old, n= 36). Using the same tissue source (NIA longitudinal study on caloric restriction) we corroborated the appearance of AQP1 with advanced age and now explore astrocytic involvement with AQP1. To do so, we used multiple immunofluorescence labeling with the astrocytic cytoskeletal markers, GFAP and vimentin, with DAPI as a nuclear marker. Following labeling, sections were digitized for analysis. We found that the percent area coverage of the ACC with AQP1 was significantly correlated ($p<0.05$) with advanced age, in agreement with our prior study. Moreover, vimentin was also significantly correlated with advanced age ($p<0.05$). Although the area covered by GFAP was high in aged animals, the levels did not correlate with age, corroborating past studies

that established GFAP expression is already very high in old animals. The distribution pattern of AQP1 with aging begins with sparse positive cells at the white matter interface and glia limitans, progressing into involvement of perivascular astrocytes. Scattered bushy astrocytes are observed across cortical layers, later developing into large, multicellular clusters of AQP1+ labelling in the oldest-old. In the same sections, adjacent cortical labeling follows a similar pattern, while the subcortical striatum has a unique aging pattern of AQP1+ labelling. Phenotypically, we saw AQP1 surrounding and overlapping with GFAP+/vimentin+ astrocytes and labeling every branch and leaflet of the astrocyte's cell domains beginning in old animals. This indicates co-expression of vimentin and AQP1 increases in connection with advanced aging, whereas astrogliosis marked by GFAP begins earlier in the aging process. Because the late appearance of aberrant AQP1 expression in the oldest-old parallels similar reactionary astrocyte behavior observed with cases of clinical neuropathology, similar mechanisms may be involved.

Disclosures: O.E. Stayer-Wilburn: None. **D.L. Rosene:** None. **P.L. Shultz:** None. **A. Vitantonio:** None. **C. Dimovasili:** None. **J. Mattison:** None. **K. Vaughan:** None. **H.F. Urbanski:** None. **S. Kohama:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

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Program #/Poster #: PSTR206.19/B51

Topic: C.01. Brain Wellness and Aging

Support: NIH Gant 1RF1AG062831
NIH Grant 2RF1AG043640
NIGMS Grant 5T32GM008541-25
NIA Intramural Research Program

Title: Long-term calorie restriction ameliorates age-associated, cell-type-specific gene expression changes within glia in the aging monkey brain

Authors: *A. VITANTONIO[1,2], C. DIMOVASILI[3], S. G. KOHAMA[4], J. MATTISON[5], D. L. ROSENE[3];
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Abstract: Normal brain aging in monkeys and humans, free of neurodegenerative disease, is accompanied by progressive, cognitive decline despite the preservation of neurons. However, in aging monkeys and humans, it has been shown that reduction in white matter volume and an accumulation of myelin pathology occurs and is correlated with cognitive decline. Our previous work in normal aging monkeys has shown significant, age-related dysfunction of glial cells

including increased inflammatory microglia expression, an accumulation of damaged oligodendrocytes, and T cell infiltration, which are correlated with cognitive decline. When considering the underlying cause of this cellular dysfunction, studies have revealed that glia demonstrate dramatic, age-related transcriptomic changes with aging. Interventions that slow or prevent such changes in gene expression are of interest to promote healthy brain aging in humans. Calorie restriction (CR) is a well-established intervention that has been shown to decelerate the biological process of aging, improve energy metabolism, and reduce age associated inflammation and oxidative stress. We recently published that CR effectively prevents age-associated, oxidative DNA damage accumulation in oligodendrocytes in the monkey brain, with implications in myelin integrity. In the present study, we asked if long-term CR attenuates age-associated gene expression alterations in multiple glial cell types in the monkey brain. To answer this we utilized brain tissue from 12 rhesus monkeys, 6 CR and 6 controls, ranging in age from 28 - 34 years old. CR monkeys had undergone more than 20 years of a 30% reduced calorie diet as part of the NIA Calorie Restriction study. Single nuclei RNA sequencing and in situ hybridization were performed to profile and characterize subpopulations of aged microglia, infiltrating immune cells, and oligodendrocytes in response to long-term CR. Gene enrichment and ontology analysis revealed that transcriptional hallmarks of aging such as reduced autophagy and protein quality control were improved in microglia by long-term CR. Additionally, oligodendrocyte-neuron cross talk was improved with CR treatment, possibly enhancing support systems for axons within aging white matter tracts. Overall, we found a transcriptomic shift in glial cells that suggests that long-term CR offers a protective effect against age-related white matter pathology.

Disclosures: A. Vitantonio: None. **C. Dimovasili:** None. **S.G. Kohama:** None. **J. Mattison:** None. **D.L. Rosene:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.20/B52

Topic: C.01. Brain Wellness and Aging

Title: Identification of specific EEG biomarkers in aged mice

Authors: *V. DUVEAU[1], N. VAUTRELLE[2], P. ANDREUX[3], C. ROUCARD[4];
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Abstract: The current challenge in neurodegenerative disease research is the identification of reliable biomarkers for early diagnosis, as existing methods often detect the disease only after significant neurological damage has occurred. Aging is well-known a risk factor for age-related pathologies such as Parkinson’s disease, Alzheimer’s disease, and cognitive impairment. It leads to deep changes in the brain such as a shrinkage of the cortex, the loss of brain plasticity and

reduced neuroplasticity of brain circuits. The question is whether specific biomarkers can be identified to predict an upcoming decline in brain function. In this work we took advantage of electroencephalography and related technologies to characterised aged mice at the physiological and functional levels using quantitative electroencephalography (QEEG) method. We also evaluated the impact of aging on the ability to process sensory (auditory) information. C57Bl6/J mice aged of 3- and 16- month-old, were implanted with cortical and depth electrodes to record resting EEG and auditory information processing by measuring the Auditory evoked potential (AEP) and 40Hz Auditory steady states response (40Hz-ASSR). Two independent recording sessions for resting EEG (3 hours) and AERP and 40Hz ASSR were performed two weeks apart. For QEEG analysis, Fast-Fourier Transform was applied and the power of specific frequency bands was calculated. The amplitudes of the AERP responses and the evoked power and intertrial coherence in response to 40Hz-ASSR were calculated by internally developed algorithm using Matlab software. Quantitative analysis of the resting EEG recordings showed a significant increase of the delta and theta power in aged mice vs young one in the frontal cortex. A significant increase of the epsilon power was observed in aged mice as compare to adult one in the parietal cortex. Using the AERP paradigm, we observed a significant decrease of the amplitude of the evoked response in aged mice. Finally, in aged we observed a significant decrease of the response to the 40Hz stimulation in both the evoked response as well as in the inter-trial coherence in aged vs young mice. Overall this project, showed that aged mice display a specific resting EEG modulation as well as overall decrease of the evoked response (AERP, 40Hz-ASSR). These biomarkers hold promise for streamlining drug development, providing a measurable endpoint to evaluate the efficacy of neuroprotective therapies. Thus, our study not only contributes to the fundamental knowledge of brain aging but also paves the way for advancements in clinical interventions and therapeutic strategies.

Disclosures: V. Duveau: A. Employment/Salary (full or part-time):; SynapCell SAS. **N. Vautrelle:** A. Employment/Salary (full or part-time):; SynapCell SAS. **P. Andreux:** A. Employment/Salary (full or part-time):; Vandria SA. **C. Roucard:** A. Employment/Salary (full or part-time):; SynapCell SAS.

Poster

PSTR206: Aging: Molecular and Cellular Changes

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Program #/Poster #: PSTR206.21/B53

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01NS108407-05

Title: Aging induces selective anatomical and molecular changes of nNOS neurons in mice

Authors: *D. SHIN, H.-J. PI, S. MANJILA, D. PARMAKSIZ, Y. KIM;
Penn State Col. of Med., Hershey, PA

Abstract: Aging is one of the primary risk factors for various neurodegenerative diseases associated with cerebrovascular alterations and neuronal impairment. Neuronal nitric oxide synthase (nNOS) neurons are a key cell type that mediate neurovascular coupling in the brain. Previous studies in selected brain areas suggest that nNOS neurons might be vulnerable in aging. Moreover, our prior research showed a selective vulnerability in cerebrovasculature upon aging, prompting us to investigate the impact of aging on nNOS populations across different brain regions. Here, we utilized whole brain mapping, including light-sheet fluorescence microscopy (LSFM) imaging with 3D immunolabeling followed by computational cell counting analysis, to determine nNOS density alterations between young-adult (2-month-old) and aged (24-month-old) C57/BL6 male and female mice. Our analysis showed a significant decrease in nNOS neuronal density in specific regions such as the cortical subplate and cerebral nuclei including the amygdala and the caudate putamen. Additionally, we applied multiplexed error-robust fluorescence in situ hybridization (MERFISH) to analyze aging-induced molecular changes of nNOS neurons. We found a significant reduction of Nos1 (nNOS gene) and other related gene transcripts in aged brains. In summary, these findings identified vulnerable brain regions with reduction of nNOS neurons that can results in impaired neurovascular coupling and lays a groundwork for future investigations to understand the relationship between aging-related cognitive decline and emotional regulation.

Disclosures: D. Shin: None. **H. Pi:** None. **S. Manjila:** None. **D. Parmaksiz:** None. **Y. Kim:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.22/B54

Topic: C.01. Brain Wellness and Aging

Support: NIH R01AG077783

Title: Endurance exercise restores aging-induced mitochondrial dysfunction in the hippocampal dentate gyrus

Authors: *X. MAO, R. MONTALVO, H. BHONSLE, Z. YAN;
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Abstract: Aging is a primary risk for various neurodegenerative conditions, including Alzheimer's disease. Exercise, particularly in the form of endurance training, has emerged to be a highly effective intervention to mitigate age-related dysfunction across bodily systems. Mitochondria, the power house of cells, have been prominently implicated in the exercise benefits in the brain with aging. However, direct evidence regarding how exercise modulates the mitochondria structural integrity and function in brain aging or aging-related decline in learning and memory is lacking. To address this knowledge gap, we conducted a study using young (2

months of age) and middle aged (14 months of age) male mice (C57BL/6) by subjecting them to voluntary wheel running for 8 weeks with age-match sedentary mice as controls. We found that middle aged mice exhibited reduced voluntary wheel running activity compared with young mice (5.36 ± 0.61 vs. 10.27 ± 0.72 km/day, $p<0.01$). Utilizing transmission electron microscope (TEM) image analysis, we have found that the cristae density (occupancy of cristae structure per mitochondria area) in the presynaptic area of adult neurons in dentate gyrus of hippocampus was significantly reduced in middle aged mice compared with young mice (4.56 ± 0.05 vs. 15.62 ± 1.07%, $p<0.05$), which was significantly restored by 8 weeks of voluntary wheel running (12.99 ± 2.91%). Neither aging nor voluntary wheel running changed the size and circularity of mitochondria. Furthermore, middle aged mice showed significant reduction of length of post-synaptic density (PSD) and number of synaptic vesicles, both of which were completely ameliorated by voluntary wheel running. Taken together, our findings suggest that endurance exercise training can reverse mitochondrial structural deficits within the presynaptic area of adult neurons in dentate gyrus, which may underlie the benefits for improving learning and memory in aging or aging-related disorders.

Disclosures: X. Mao: None. **R. Montalvo:** None. **H. Bhonsle:** None. **Z. Yan:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.23/B55

Topic: C.01. Brain Wellness and Aging

Title: Wnt/β-catenin signaling dynamics in the aging African turquoise killifish brain

Authors: *E. WHISENANT, A. LEKVEN;
Univ. of Houston, Houston, TX

Abstract: The dysregulation of the Wnt/β-catenin pathway is associated with human agingand several neurological disorders including Alzheimer's disease, Parkinson's disease,and multiple sclerosis. In the African turquoise killifish (*N.furzeri*) model, I amuncovering how changes in Wnt signaling relate to aging and the spontaneousneurodegeneration process observed in aging killifish. We have established afluorescent Wnt signaling reporter line in *N.furzeri* in which a 1.1 kb fragment of thezebrafish *sp5a* promoter, a known Wnt responsive element with conserved LEF/TCFbinding sites, drives expression of eGFP in areas where Wnt signaling is presumed tobe active. This reporter remains active from developmental stages to adulthood,including stages when neurodegeneration is observed. Transgenic founders of anadditional fluorescent Wnt reporter line, 7XTCF-Xla.Siam:mCherry, regulated by sevenmultimerized TCF elements upstream of the *Xenopus* siamois minimal promoter, have asimilar pattern of expression in embryonic stages when compared to the *sp5a:eGFP*reporter. These transgenic founders will be outcrossed and screened for germlinetransmission, followed by a comparative analysis of fluorescence in the adult brain. Tointerpret changes in fluorescence associated with aging, we

adapted the EZ-Cleartissue clearing method to adult fish brains to preserve endogenous fluorescence fromthe reporter line, while also enabling future immunostaining. Interestingly, eGFPexpression in the *sp5a:eGFP* reporter is strongly localized to the habenula, a structurein the brain responsible for many behavioral responses. *In situ* hybridization for Wntpathway mRNAs (*axin2*, *sp5*, *wnt1*) in the adult brain also shows expression in thehabenula with possible age-dependent changes in expression levels. When comparingmRNA expression of 15-week and 25-week-old brain sections, both *sp5* and *axin2*mRNAs were decreased in response to aging across multiple brain regions includingareas in the forebrain and in the midbrain optic tectum. Overall, my data suggests aprominent role for Wnt/β-catenin signaling in the adult killifish brain and suggestsobservable changes in Wnt signaling activity associated with aging.

Disclosures: E. Whisenant: None. **A. Lekven:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.24/B56

Topic: C.01. Brain Wellness and Aging

Support: NICHD R01HD107489

Title: Prenatal folate supply alters cerebral cortical interneuron distribution and connectivity of the offspring

Authors: *A. MUSTAFA[1], E. RODRIGUEZ-FORTI[2], N. CANNIZZARO[2], L. TAT[2], S. M. ALI[3], J. L. SILVERMAN[4], J. LASALLE[5], R. BEN-SHALOM[6], R. GREEN[2], K. ZARBALIS[7]; [1]Univ. of California, California, CA; [2]Univ. of California, Davis, Sacramento, CA; [3]Pathology and Lab. Med., Univ. of California, Davis, Sacramento, CA; [4]UC Davis MIND Inst., Sacramento, CA; [5]UC Davis Sch. Med., Davis, CA; [6]Neurol., UC Davis Med. Ctr., Sacramento, CA; [7]Dept. Pathology/IPRM, Sch. of Med., Sacramento, CA

Abstract: Folate is an essential micronutrient and prenatal folate deficiency is known to cause neural tube defects, prompting the implementation of folic acid (FA) fortification programs and recommendations for FA supplementation during pregnancy. Recently, it has been shown that both maternal folate deficiency and excess FA intake modulate prenatal cerebral cortical neurogenesis in favor of the generation of additional late born cortical projection neurons. However, the role of prenatal folate intake on interneuron development (IN) remains uncertain. Disturbances in IN numbers have been associated with many neurological conditions like autism spectrum disorder, schizophrenia, and epilepsy.In this study, C57BL/6N dams were provided during pregnancy and until weaning with five distinct diets, varying in folate content [1. Control - 2mg FA/kg chow; 2. FA (0) - 0 mg FA/kg chow; 3. FA (10) - 10 mg FA/kg chow; 4. FA (20) - 20 mg FA/kg chow; folinic acid (5-FTHF) (11.7) - 11.7 mg 5-FTHF/kg chow]. Offspring brains were collected at postnatal day 21 (P21) and subjected to histological analyses designed to assess

IN distribution and arborization. In addition, individualized neocortical cells were collected at P0 and used in network-level electrophysiological recordings using high-density microelectrode arrays (hd-MEAs). Also, at P0, neocortical tissue was collected to perform RNA-Seq and whole-genome bisulfite sequencing to assess DNA methylation states. Compared with controls, we found significant reductions in the number of INs in the cortex of mice that had been pre- and postnatally exposed to FA (0), FA (10) and FA (20) diets. Analyses of hd-MEA recordings revealed that neurons derived from FA (10) offspring were having less synchronous activity manifested in lower burst peaks and number of bursts. This may suggest that FA excess neurons are less capable of forming functional synapses and creating functional neuronal networks compared to the control and also deficient FA (0) neurons that did not display similar deficits. RNA seq analysis revealed that FA (10) changes transcriptional networks associated with mitochondrial and synaptic function. FA (10) also alters DNA methylation levels in regions associated with neurodevelopmental and synaptic processes.

Disclosures: A. Mustafa: None. **E. Rodriguez-Forti:** None. **N. Cannizzaro:** None. **L. Tat:** None. **S.M. Ali:** None. **J.L. Silverman:** None. **J. LaSalle:** None. **R. Ben-Shalom:** None. **R. Green:** None. **K. Zarbalis:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.25/B57

Topic: C.01. Brain Wellness and Aging

Support: NRF-2022R1A4A1018963
HU23C0199003648200142000 1

Title: The dysregulation of phagocytosis in senescent microglia: implications for age-related neurodegenerative diseases

Authors: *D. GUPTA[1], G. SONG[2];
[1]Catholic Kwandong Univ., South Korea, Incheon, Korea, Republic of; [2]Inst. for Transnational Neurosci., Dept. of Medial Sci., Catholic Kwandong Univ., Incheon, Korea, Republic of

Abstract: The brain immune cell, microglia, plays a vital role by protecting from toxic substances and invading agents by phagocytosis and clearing. Increasing evidence suggests that microglia, expressing ferritin with ferroxidase activity for iron storage, plays a significant role in brain aging, where inflammation is implicated due to iron accumulation. With aging, microglia accumulate iron and transforms into senescent cells, leading to inflammations. Additionally, iron dysregulation in microglia oxidative stress and neuronal damage causes dyshomeostasis and Alzheimer's diseases (AD) pathophysiology. Here, we found that the aged and AD brain with high number of senescence cells, specifically microglia show senescence characteristics and iron accumulation. Considering the iron accumulation in aged microglia, we hypothesize that

regulating the iron accumulation in the microglia could promote them to efficiently gain the senescence characteristics for an in-vitro primary senescence microglia model. First, we analyzed the senescence markers, β-galactosidase, p21, γH2Ax and p16 in iron-fed microglia. We found that expression levels of the senescence markers were increased in the senescent microglia along with enlarged cell body, DNA damage, lysosomal dysfunction and increased IL-1B. Further, the microglia from aged mice show increased levels of β-galactosidase, lysosomal dysfunction, lipid droplet accumulation, and mitochondrial dysfunction. The lysosomal dysfunction was observed in senescent microglia. Senescent microglia show uncontrolled phagocytosis up to 10 hours and fail to clear the zymosan particles leading to 55% of cell death. We propose that targeting senescence microglia can be a promising strategy for the treatment of neurodegenerating diseases due to brain aging and AD that involves changes in cognitive function and high susceptibility to neurodegeneration.

Disclosures: D. Gupta: None. **G. Song:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.26/B58

Topic: C.01. Brain Wellness and Aging

Title: NAD^+ biology is linked to ciliogenesis and lysosomal functions in hypothalamic neurons

Authors: *S.-E. PARK[1], M.-S. KIM[2];
[1]Ulsan Univ., Seoul, Korea, Republic of; [2]Univ. Ulsan Coll, Seoul, Korea, Republic of

Abstract: Nicotinamide adenine dinucleotide (NAD^+) is an essential cofactor that mediates numerous biological processes and mainly produced via Nampt-mediated salvage pathway. Primary cilia, tiny hair-like organelles on the plasma membrane, play a critical role in leptin signal transduction in hypothalamic neurons. Impaired ciliogenesis in hypothalamic neurons leads to the development of obesity. Similar to obesity, we found that primary cilia are shortened in hypothalamic neurons of 24-month-old aged mice (C57BL/6, n=5) compared to 3-month-old young mice(n=6). Hypothalamic NAD^+ level has also dropped by more than twofold in aged mice. Therefore, we investigated the causes and consequences of the short cilia phenotype in aged mice. As depletion of cellar NAD^+ levels have been suggested the pathological mechanisms of aging, we investigated the possible link between NAD^+ biology and ciliogenesis in hypothalamic neurons. We found that depletion of cellar NAD^+ levels with FK866 treatment in N1 hypothalamic neuron cells (n=3) markedly reduced cilia lengths which was reversed by NAD^+ precursor NMN cotreatment. As for the mechanism, we found that NAD^+-dependent deacetylase SIRT1 stimulated the transcription of cilia-related genes via SIRT1 overexpression in N1 cells. We next investigated the impacts of NAD^+ depletion-induced short cilia phenotype in hypothalamic neurons and systemic metabolism. We found that NAD^+ depletion significantly impaired lysosomal acidification through lysosensor, a pH indicator of lysosome lumen, in N1

cells. Furthermore, NAD^+ depleted cells lost capability to repair from lysosome membrane puncture which was verified by late recruitment of ESCRT proteins in live cells. Importantly, these impairments of lysosome due to NAD^+ depletion occurred in cilia-dependent manner, through a ciliogenesis gene (IFT88) knockdown process. In mice, hypothalamic NAD^+ depletion led to a reduction in exercise capacity, as verified by treadmill and grip strength tests conducted on young mice (n=4). These data suggest that reduced NAD^+ levels in hypothalamic neurons may contribute to the aging-related muscle dysfunction via impaired ciliogenesis and lysosomal dysfunction in hypothalamic neurons.

Disclosures: S. Park: None. **M. Kim:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.27/B59

Topic: C.01. Brain Wellness and Aging

Title: Effects of TiO_2 nanoparticles synthetsized and loaded with salvia rosmarinus extract over cellular senescence in astrocytes

Authors: *C. RODRÍGUEZ-PÉREZ[1], E. ORTIZ[1], V. CAMPOS-PEÑA[2], M. TORRES-RAMOS[3];
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Abstract: The astrocytes, star-shaped cells, and major glial cell type in the brain, are involved in many functions of nervous system. During senescence astrocytes no longer proliferative and not protect neurons. Also, playing an important role in neurodegenerative diseases losing their supportive attributes and lose neuroprotective. Pathological changes or ageing of astrocytes contribute to neurodegenerative diseases as Alzheimer. In recent years, the green synthesis of nanomaterials has gained interest as an efficient ecofriendly method for obtain metal oxide nanoparticles. The objetive of this study was observar and know de effect of TiO_2 bionanoparticles charged with biological extract of *salvia rosmarinus* over cellular senescence ageid astrocytes *in vitro*.

Disclosures: C. Rodríguez-Pérez: A. Employment/Salary (full or part-time):; INNNMVS. **E. Ortiz:** None. **V. Campos-Peña:** A. Employment/Salary (full or part-time):; INNNMVS. **M. Torres-Ramos:** A. Employment/Salary (full or part-time):; INNNMVS.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.28/B60

Topic: C.01. Brain Wellness and Aging

Support: F31AG079653
T32AG20506
R01AG078796
R21AG080248

Title: Derailed protein turnover in the aging mammalian brain.

Authors: *N. R. RAO, A. K. UPADHYAY, J. N. SAVAS;
Northwestern Univ., Chicago, IL

Abstract: Efficient protein turnover is essential for cellular homeostasis and organ function. Loss of proteostasis is a hallmark of aging culminating in severe dysfunction of protein turnover. To investigate protein turnover dynamics as a function of age, we performed continuous in vivo metabolic stable isotope labeling in mice along the aging continuum. First, we discovered that the brain proteome uniquely undergoes dynamic turnover fluctuations during aging compared to heart and liver tissue. Second, trends in protein turnover in the brain proteome during aging showed sex-specific differences that were tightly tied to cellular compartments. Next, parallel analyses of the insoluble proteome revealed that several cellular compartments experience hampered turnover, in part due to misfolding. Finally, we found that age-associated fluctuations in proteasome activity were associated with the turnover of core proteolytic subunits, which was recapitulated by pharmacological suppression of proteasome activity. Taken together, our study provides a proteome-wide atlas of protein turnover across the aging continuum and reveals a link between the turnover of individual proteasome subunits and the age-associated decline in proteasome activity.

Disclosures: N.R. Rao: None. **A.K. Upadhyay:** None. **J.N. Savas:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.29/B61

Topic: C.01. Brain Wellness and Aging

Support: K99NS126639

Title: Long-lived mitochondrial proteins as pillars of mitochondrial ultrastructure

Authors: *E. BOMBA-WARCZAK, J. N. SAVAS;
Northwestern Univ., Chicago, IL

Abstract: Continuous replenishment of mitochondria is essential for maintaining high-quality organelles throughout the lifespan of post-mitotic neurons. Age-related impairments in mitochondrial renewal contribute to progressive neuronal. Accordingly, average half-life of mitochondrial proteins is estimated at less than 3 weeks (1). However, we found that a discrete subset of the mitochondrial proteome persists for at least 4 months in mammalian brains, hearts and oocytes (2). These mitochondrial long-lived proteins, or mt-LLPs are enriched at cristae invaginations and include OxPhos, MICOS, mt-DNA proteins, chaperones, and cytochrome C. We performed cross-linking MS-based quantitative analysis on native OxPhos complexes which revealed a significant spatial restriction of mt-LLPS and co-preservation within same cristae. Since cristae stability is intimately coupled to mitochondrial function, we propose that mt-LLPs could play a previously unrecognized role in the long-term stabilization of mitochondrial ultrastructure, which in turn might be imperative for mitochondrial fitness. Our ongoing research efforts are centered on (1) the investigation of molecular quality-control mechanisms governing the exceptional persistence of mt-LLPs, (2) the spatio-temporal distribution of mt-LLP within neuronal mitochondrial networks, and (3) their persistence in the context of cristae stability and remodelling. Insights from this work will provide with a new understanding of the role of long-lived proteins in post-mitotic and long-lived cells, and could lead to novel targets for potential therapeutic interventions for neurological disorders.

Disclosures: E. Bomba-Warczak: None. **J.N. Savas:** None.

Poster

PSTR206: Aging: Molecular and Cellular Changes

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR206.30/B62

Topic: C.01. Brain Wellness and Aging

Support: R41 AG073144
NIH R21 AG073743
P20 GM103645

Title: Characterization of antisense oligonucleotides designed to target the MuSK-BMP pathway to enhance adult hippocampal neurogenesis

Authors: *A. SINGH[1], A. S. PAYNE[3], S. OH[4], C. XI[1], F. KELLER[5], A. WEBB[6], L.-A. HASSELL[1], J. PAGE[2], J. R. FALLON[1];
[1]Dept. of Neurosci., [2]The Warren Alpert Med. Sch., Brown Univ., Providence, RI; [3]Dept. of Dermatol., Columbia Univ. Vagelos Col. of Physicians and Surgeons, New York, NY; [4]Hallym Univ., Chuncheon, Korea, Republic of; [5]UMass Chan Med. Sch., Worcester, MA; [6]The Buck Inst. for Res. on Aging, Novato, CA

Abstract: Adult hippocampal neurogenesis (AHN), the process of generating newborn neurons from hippocampal neural stem cells (NSCs) in adulthood, is critical for learning, memory, and

emotional regulation. AHN persists well into adulthood in the healthy brain but is impaired from the earliest stages of Alzheimer's Disease as well as in Parkinson's Disease and Major Depressive Disorder. While BMP (bone morphogenetic protein) signaling has been established as a potent negative regulator of AHN, global perturbation of this pathway results in unwanted off-target effects. Our lab has shown that the transmembrane protein MuSK (muscle-associated receptor tyrosine kinase) is a tissue-specific BMP co-receptor that promotes and shapes BMP signaling, termed the 'MuSK-BMP Pathway' (Yilmaz et al., 2016). Notably, the MuSK Ig3 extracellular domain is necessary for binding BMP but is dispensable for neuromuscular junction formation (Jaime et al., 2024), making it an attractive target for therapeutic interventions to enhance AHN. Moreover, MuSK is expressed in NSCs but is only sparsely expressed in the body, strengthening the appeal of this approach. Knock-in mice engineered to constitutively express MuSK lacking the Ig3 domain have average lifespans, show a greater than two-fold increase in AHN, and improved hippocampus-dependent cognition. As a first step to developing drugs targeting this pathway, we identified splice-modifying antisense oligonucleotides (ASOs) that induce skipping of the MuSK exons encoding the Ig3 domain while having minimal impact on total MuSK transcript levels. Here, we characterized the translated MuSK protein product in ASO-treated myogenic cells using monoclonal antibodies specific for 1) the Ig3 domain or 2) the Ig2 domain ('total MuSK'). Immunostaining revealed that ASO treatment results in the selective reduction of MuSK containing the Ig3 domain, while the overall level of MuSK protein is unaffected. Importantly, MuSK lacking the Ig3 domain is expressed at the cell surface, indicating that the protein produced from the splice-modified transcript is folded and transported correctly. These results set the stage for functional studies in ASO-treated cells and animals, such as RNA sequencing, to assess BMP responsiveness.

Disclosures: A. Singh: None. **A.S. Payne:** None. **S. Oh:** None. **C. Xi:** None. **F. Keller:** None. **A. Webb:** A. Employment/Salary (full or part-time):; The Buck Institute for Research on Aging. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics. **L. Hassell:** None. **J. Page:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics. **J.R. Fallon:** A. Employment/Salary (full or part-time):; Brown University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bolden Therapeutics.

Poster

PSTR207: Alzheimer’s Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.01/B63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG014449
AG085572
AG072599

AG074004
AG077103
T32AG052909

Title: Frontal cortex pyramidal neuron expression profiles differentiate the prodromal stage from progressive degeneration across the Alzheimer's disease spectrum

Authors: *A. LABUZA[1], M. J. ALLDRED[2], H. PIDIKITI[3], M. MALEK-AHMADI[4], A. HEGUY[5], P. D. COLEMAN[6], G. CHIOSIS[7], E. J. MUFSON[8], S. D. GINSBERG[9]; [1]Nathan S Kline Inst., Orangeburg, NY; [2]Nathan Kline Inst., Orangeburg, NY; [3]Nathan S. Kline Inst., Orangeburg, NY; [4]Banner Alzheimer's Inst., Glendale, AZ; [5]New York Univ. Grossman Sch. of Med., New York, NY; [6]ASU-Banner Neurodegenerative Res. Ctr., Tempe, AZ; [7]Chem. Biol. and Med., Sloan Kettering Inst., new york, NY; [8]Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ; [9]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY

Abstract: Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative disorder affecting an estimated 6.7 million Americans. There is no current treatment to stop or prevent the onset or progression of dementia. Therefore, it is imperative to identify molecular and cellular mechanism(s) underlying AD. We employed laser capture microdissection (LCM) to isolate layer III pyramidal neurons from frontal cortex (BA9) postmortem human tissue. Single population RNA-sequencing was conducted using subjects with no cognitive impairment (NCI), mild cognitive impairment (MCI), and AD. Differentially expressed genes (DEGs) were compared and contrasted across the AD spectrum. The number of DEGs increased from Prodromal (MCI versus NCI; 1,306 DEGs) through Progression (AD versus MCI; 2,505 DEGs) to Frank AD (AD versus NCI; 3,259 DEGs). Bioinformatic inquiry revealed several pathways that were altered with disease onset and progression including oxidative phosphorylation, mitochondrial dysfunction, and immune responses, implicating a failure of bioenergetics in vulnerable corticocortical projection neurons. We found the majority of DEGs and pathways shared between Prodromal and the later stage Progression comparisons switched direction, which we term “directionally divergent”. In contrast, DEGs and pathways that were shared between the later stage Progression and Frank AD or Prodromal and Frank AD remained in the same direction, which we term “directionally convergent”. This data shows AD does not progress linearly in all pathways but may be divergent between early and late-stage disease. Additionally, we identify candidate genes and pathways that demarcate early-stage AD onset (Prodromal) that are clearly differentiated from AD progression (Progression and Frank AD), enabling a previously unknown roadmap to study vulnerability for mechanistic understanding of both onset and progressive degeneration as well as providing key targets for intervention at early stages.

Disclosures: A. Labuza: None. **M.J. Alldred:** None. **H. Pidikiti:** None. **M. Malek-Ahmadi:** None. **A. Heguy:** None. **P.D. Coleman:** None. **G. Chiosis:** None. **E.J. Mufson:** None. **S.D. Ginsberg:** None.

Poster

PSTR207: Alzheimer’s Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.02/B64

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: AG014449
AG107617
AG072599
AG074004
AG077103

Title: Profiling Layer III and Layer V pyramidal neurons using postmortem human FFPE tissue with the GeoMx digital spatial profiling platform

Authors: *A. STANISAVLJEVIC[1], P. H. STAVRIDES[1], C. BARE[1], H. PIDIKITI[1], K. IBRAHIM[1], M. J. ALLDRED[1,2], A. HEGUY[3], S. LEE[1], R. A. NIXON[1,2,4,5], S. D. GINSBERG[1,2,6,5];
[1]Ctr. for Dementia Res., Nathan Kline Inst., Orangeburg, NY; [2]Psychiatry, [3]Dept. of Pathology, [4]Cell Biol., [5]NYU Neurosci. Inst., [6]Neurosci. & Physiol., NYU Grossman Sch. of Med., New York City, NY

Abstract: Digital spatial profiling (DSP) is an emerging technique for RNA sequencing (RNA-seq) and quantification that reveals precise spatial information from small amounts of tissue, surpassing other currently available spatial profiling methods. The GeoMx Digital Spatial Profiling System by NanoString utilizes formalin fixed paraffin embedded (FFPE) tissue and employs various histochemical techniques. The procedure involves i) unmasking fixed RNA in tissue to enable access for system specific probes, ii) labelling targets (i.e., pyramidal neurons) for probe extraction, iii) extracting probes from selected regions of interest and iv) preparing probes as a library for next generation sequencing (NGS). This approach enables spatial RNA expression profiling by linking RNA-seq data to precise areas, utilizing the tissue bound probes. We hypothesize the GeoMx system will allow us to spatially characterize transcriptomic data from layer III (L3) and layer V (L5) pyramidal neurons in human postmortem brain tissue, which is an unmet need. We have adjusted the methodology of the NanoString protocol from mouse tissue to efficiently establish a robust and reproduceable method in human postmortem tissue. We specifically use L3 and L5 pyramidal neurons from Brodmann area 9 (BA9) frontal cortex identified with the neuronal marker Ca2+/calmodulin-dependent protein kinase II (CamKII). The GeoMx Human Whole Transcriptome Atlas (hWTA) is used in the present study to maximize the number of detected genes in pyramidal neurons. Validation of the hWTA probe kit provides a platform for later development and optimization of more disease specific probe sets. DSP by the GeoMx system significantly reduces the amount of tissue needed for RNA sequencing as only one 5 µm thick section is needed to provide RNA seq data in a group of ~20 cells. The present study is being conducted in (n=8) postmortem human nondemented control FFPE BA9 cortical tissues. Transcriptomic data is retrieved from 2 types of areas of interest, i) pyramidal neurons of L3 and L5 and ii) cortical ribbons containing L3 and L5 pyramidal neurons. To validate this revised DSP protocol, comparison of pyramidal neuron expression profiles will be conducted via Laser Capture Microscopy (LCM) and subsequent single population RNA-seq in adjacent FFPE BA9 tissue sections. We propose RNA-seq data produced via the GeoMx platform will provide high resolution genetic information elucidating the role of pyramidal neuron populations in the ageing and demented brain.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.03/B65

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: AG074004
AG072599
AG085572
AG014449
AG077103
AG081286

Title: Frontal cortex pyramidal neurons display laminar specific gene expression in Down syndrome

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Abstract: Down syndrome (DS) is caused by triplication of human chromosome 21 (HSA21), resulting in ~1/700 live births per year. Individuals with DS present with altered craniofacial features and deficits in learning, language, memory acquisition and consolidation. By early mid-life (mid-30's-40's), the brain of those with DS display extensive Alzheimer's disease (AD) pathology including amyloid plaques due to the triplication of the amyloid precursor protein (APP) gene, located on HSA21. Additionally, during development individuals with DS have reduced proliferation and disorganization of corticocortical pyramidal projections neurons located in laminar III and V in the frontal cortex {e.g., Brodmann area 9 (BA9)}. BA9 is a hub of the default mode connectome that plays a role in executive memory function. We postulate that trisomy alters unique differentially expressed genes (uDEGs) in layer III compared to layer V, which play a role in executive memory dysfunction in individuals with DS. Here, human frontal cortex (BA9) layer III and V pyramidal neuron gene expression was interrogated via laser capture microdissection combined with single population RNA-sequencing from aged individuals with DS with confirmed AD pathology compared to age- and sex-matched controls. Bioinformatic inquiry revealed that layer III pyramidal neurons displayed ~2,966 uDEGs, while layer V displayed ~1,999 uDEGs ($p<0.05$) in DS compared to controls. These uDEGs revealed

multiple pathways implicating functional neuronal differences, including metabolism and production of reactive oxygen species specifically dysregulated layer III DS pyramidal neurons, while layer V DS pyramidal neurons were linked to upregulated pathways including metabolism of phospholipids and sphingolipid metabolism. These findings indicate that trisomy differentially affects gene expression in layers III and V BA9 projection neurons in aged individuals with DS, which may inform the molecular and cellular pathogenesis of DS and AD.

Disclosures: M.J. Alldred: None. **K. Ibrahim:** None. **H. Pidikiti:** None. **S. Lee:** None. **A. Heguy:** None. **T.M. Wisniewski:** None. **E.J. Mufson:** None. **G.E. Stutzmann:** None. **S.D. Ginsberg:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.04/B66

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: P01AG14449
RF1AG061566
RF1AG081286
CA2018010
Barrow Neurological Foundation
Arizona Alzheimer's Consortium

Title: APOEε4 genotype alters myelin integrity in frontal cortex white matter during the progression of Alzheimer's disease

Authors: *M. MORENO-RODRIGUEZ, S. E. PEREZ, E. J. MUFSON;
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Abstract: APOEε4 allele is the strongest genetic risk factor for Alzheimer's disease (AD) and is associated with a decrease in myelin in the white matter (WM) and cognitive decline. APOE is a lipidic transport protein produced mainly by astrocytes involved in the maintenance of brain lipid levels. The effect that the ε4 allele has upon APOE in astrocytes and cellular components supporting myelination remains incompletely understood. Here, we used antibodies against APOE and Olig2 to quantitate the number of astrocytes and oligodendrocytes, respectively, in WM of the frontal cortex (FC). Myelin integrity in WM was determined by optical density (OD) measurements of the luxol fast blue (LFB) stain and myelin basic protein (MBP) immunoreactivity. FC tissue was obtained from APOEε4 carriers and non-carriers that died with an antemortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI), and mild to moderate Alzheimer's disease (AD) from the Rush Religious Orders Study. Qualitatively, APOE-labeled astrocytes were virtually absent in the WM of APOEε4 non-carriers, in both NCI and MCI cases, but were slightly more abundant in AD. However, APOE-

labeled astrocytes were observed in APOEε4 carriers in all clinical groups, showing significantly higher numbers in MCI compared to non-carriers. Olig2-positive cell numbers significantly decreased in AD compared to NCI in carriers and non-carriers. A within group analysis revealed significantly greater number of Olig2-positive in MCI non-carriers compared to carriers. MBP OD values were significantly higher in MCI APOEε4 non-carriers compared to both NCI non-carriers and MCI carriers. Like MBP, LFB OD values were significantly greater in MCI compared to NCI among APOEε4 non-carriers. By contrast, APOEε4 carriers displayed higher LFB OD values in NCI compared to AD, which were significantly lower than NCI non-carriers. LBF OD measurements positively correlated with global cognition, visuospatial, and episodic memory across groups. Additionally, MBP OD measures correlated with episodic memory and global cognition in the MCI and AD groups. The presence of APOE astrocytes in the WM of APOEε4 carriers suggests a defect in the ability of these cells to transport APOE required by oligodendrocytes to maintain myelin integrity. In summary, APOEε4 astrocytes in the WM are susceptible to degenerative changes in APOEε4 carriers compared to non-carriers during the progression of the disease.

Disclosures: M. Moreno-Rodriguez: None. **S.E. Perez:** None. **E.J. Mufson:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

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Program #/Poster #: PSTR207.05/B67

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIA R01 AG077103

Title: Differentially expressed genes and pathways in Down syndrome models manifest as defects in neuronal signallig, mitochondrial dysfunction, and protein mishandling

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Abstract: Individuals with Down Syndrome (DS) will develop Alzheimer's disease (AD) pathology by midlife along with progressive cognitive decline and dementia. Neuropathological hallmarks include beta-amyloid plaques, neurofibrillary tangles, endosomal-lysosomal dysfunction, synaptic deficits, and neurodegeneration. Basal forebrain cholinergic neurons, prefrontal cortical pyramidal neurons, and hippocampal CA1 pyramidal neurons in the septohippocampal and in the basocortical connectome are selectively vulnerable in DS

individuals and display differentially expressed gene pathways in key ontology categories, including synaptic plasticity, calcium signalling, and AD-associated neurodegeneration related to beta-amyloid peptide clearance. To assess functional deficits, we used whole cell patch clamp electrophysiology with 2-photon (2P) Ca^{2+} imaging within acute mouse brain slices from the Ts2 model of DS/AD to detect altered Ca^{2+} handling and the effects in neuronal signalling. Parallel studies in human induced neurons (HiN) from individuals with DS compared to HiN from age-matched normal controls measured autophagosome turnover and altered mitochondrial responses, as assessed via the genetically encoded Ca^{2+} indicator mitoGCaMP6m. The analysis of single population RNA-sequencing revealed significant downregulation of numerous GABAergic genes and GABAergic neurotransmission pathway in DS. Preliminary results from acute Ts2 mouse brain slices compared to normal disomic (2N) littermates show altered presynaptic release properties in spontaneous postsynaptic potentials, suggesting an increased presynaptic Ca^{2+} tone. Preliminary HiN results demonstrate increased beta-amyloid$_{42}$ and intracellular phospho-tau levels as well as increased resting Ca^{2+} levels and altered mitochondrial responses. Deficits in calcium handling and changes in cytosolic and mitochondrial Ca^{2+} have significant consequences in terms of bioenergetics and physiological function. We are beginning to see these dysfunctions in animal and cellular models of DS, providing mechanistic information regarding vulnerability of the septohippocampal and basocortical systems, which may lead to better outcomes in individuals with DS as well as inform on therapeutic potential for AD dementia subjects.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

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Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant P01AG14449
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Barrow Neurological Foundation
Arizona Alzheimer's Consortium

Title: Spliceosome, tau and amyloid pathology differentiate hubs of the default mode connectome during the progression of Alzheimer's disease

Authors: *E. MUFSON[1], J. MIGUEL[1], D. MORENO[1], M. MORENO-RODRIGUEZ[1], M. MALEK-AHMADI[2], C. HALES[3], S. E. PEREZ[1];

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Abstract: Recent evidence suggests that alterations in the splicing proteins U1-70K and U1A small nuclear ribonucleoprotein (RNP), serine/arginine splicing factor 2 (SRSF2), and the heterogeneous nuclear (hn) RNPA2B1 play a role in the onset of tau pathology in AD. Interestingly, the extent of tau pathology differs within cortical nodes of default mode network (DMN), a connectome underlying episodic memory function. Here, we examined nuclear spliceosome protein levels, size and counts of U1-70K, U1A, SRSF2 and hnRNPA2B1 and phosphorylated AT8 tau, 3Rtau and 4Rtau isoforms containing neurons within layers III and V-VI in the frontal cortex (FC), precuneus (PreC) and posterior cingulate cortex (PCC) hubs of the DMN obtained from subjects who came to autopsy with a premortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) and mild/moderate Alzheimer's disease (mAD) using immunocytochemistry combined with qualitative cellular morphometry and densitometry. Amyloid plaque load was evaluated across the same cases within each cognitive group and DMN hubs. We found SRSF2, U1A, U1-70K and hnRNPA2B1 immunoreactivity in neurons located in each cortical layer in each hub across clinical groups. Optical density (OD) measurements of nuclear SRSF2, U1-70K, U1A, and hnRNPA2B1 immunoreactivity, size and numbers were unchanged in layers III and V-VI across clinical groups in FC and PCC, while in the PreC, OD hnRNPA2B1 measures were significantly greater in layers III and V-VI in mAD. Mislocalized nuclear U1A and U1-70K reactivity displayed a granular or tangle-like appearance mainly in layers V-VI neurons in a few MCI and mAD cases in the PCC. Although the PCC MCI and mAD cases contained the highest average number of AT8, 3Rtau and 4Rtau positive NFTs, only the number of AT8 positive NFTs were significantly higher in FC layers V-VI in mAD compared to NCI. Double immunofluorescence revealed U1-70K positive tangle-like structures associated with AT8 and 4Rtau, while mislocalized U1A was primarily seen in AT8 positive NFTs. Correlational analysis revealed no associations between nuclear OD values for SRSF2, U1-70K, U1A and hnRNPA2B1, size, counts, 3Rtau, 4Rtau, AT8 cell numbers, plaque density, cognitive domains, demographics, and neuropathological criteria across clinical groups in the DMN hubs. Moreover, no association was found between U1-70K and U1A NFT-like structures and counts of NFTs and plaques. These data suggest that nuclear mislocalization of the spliceosomal U1s is regional-specific and independent of tau or plaque pathology within the DMN memory circuit during the onset of AD.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

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Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: NIH Grant RF1AG081286
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NIH Grant P01AG14449
BrightFocus Foundation CA2018010
Barrow Neurological Foundation
Arizona Alzheimer's Consortium

Title: Frontal cortex splicing protein U1A and tau pathogenesis in Down syndrome with and without Alzheimer's type dementia

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Abstract: Down syndrome (DS) people have an increased risk of developing Alzheimer's disease (AD) type dementia and by age forty exhibit significant tau containing neurofibrillary tangles (NFTs) and Aβ plaques. However, not everyone with DS develops dementia. We reported that DS with dementia (DSD+) display a greater number of NFTs consisting of a more advanced tau pathology compared to those without dementia (DSD-) in the frontal cortex (FC) suggesting differences in tau pathobiology. Recent evidence suggests that alterations in the splicing protein small nuclear ribonucleoprotein U1A play a role in tau pathogenesis in AD and DS. We examined U1A nuclear protein levels, size and counts as well as phosphorylated AT8 tau, 3Rtau and 4Rtau containing neurons and amyloid plaque load in layers III and V-VI in the FC of demented (n=22) and non-demented (n=12) people with DS. We observed intense nuclear U1A immunostaining in layers III and VI in all DSD-, while DSD+ cases also displayed pale immunostaining across layers. The number of intense U1A stained nuclei was significantly greater in layer III in DSD- compared to DSD+. By contrast, the number of nuclei displaying weak U1A labeling was significantly increased in layer III in DSD+ compared to DSD-. In DSD+, optical density (OD) measurements of lightly immunoreactive U1A nuclei were significantly reduced compared to more intensely reactive U1A labeled nuclei in layers III and V-VI. Nuclear area of lightly stained U1A was smaller than the more intensely immunostained in layer III in DSD+. Cytoplasmic mislocalized U1A reactivity had either a fine granular or tangle-like appearance in DSD+ cases, which was similar in number between layers. FC AT8, 3Rtau and 4Rtau positive NFT counts were significantly higher in DSD+ than in DSD- in layers III and V-VI, while plaque load was unchanged between groups. Correlational analysis revealed that weak nuclear U1A OD values, nuclear area, and counts were strongly associated with AT8 and 3Rtau NFTs, but only with layer V-VI 4Rtau NFTs and layer III plaque load across clinical groups. Mislocalized U1A tangles showed the strongest association with 4Rtau NFT counts in both layers across groups. Similar changes in U1A measures and associations with NFTs were not observed between intensely reactive nuclei across groups. These findings suggest that FC U1A nuclear pathogenesis is greater and show a stronger relationship with tau than plaque pathology which contributes to the onset of dementia in DS.

Disclosures: S. Perez: None. **J. Miguel:** None. **E.J. Mufson:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

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Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: P01AG14449
P30AG066468
P50AG005133
P01AG025204

Title: Unique neuroinflammatory molecular profiles in Default Mode Network regions contribute to resilience to Alzheimer's disease pathology

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Abstract: Regional differences in neuroinflammatory changes during the clinical and neuropathological progression of Alzheimer's disease (AD) are not well understood. Previous studies suggested that individuals resilient to AD pathology (high pathology-no cognitive impairment, HP-NCI) may have brain cytokine/chemokine profiles that distinguish them from controls and AD patients. To further investigate the neuroinflammation profile of individuals classified as HP-NCI, we used the Luminex platform and a multiplex ELISA to measure 27 cytokines/chemokines in brain tissue from individuals in clinical-pathological groups of low pathology-no cognitive impairment (LP-NCI), HP-NCI, mild/moderate AD, and severe AD. Brain regions included the hubs of the Default Mode Network (DMN: precuneus, PreC; frontal cortex, FC; posterior cingulate, PC) as well as cortical regions affected early (inferior temporal cortex, IT) or late in AD (primary visual cortex, PVC). We found regional differences in the groups of analytes that differentiated the HP-NCI group from the clinical-pathological groups of LP-NCI and severe AD. In all three DMN regions, IL-7 levels were higher in the HP-NCI group relative to AD. In addition, HP-NCI cases had higher PreC levels of IL-9, IL-1Ra, IFNgamma, and eotaxin, higher PC levels of IL-9 and VEGF, and higher FC levels of IL-1Ra relative to AD. PVC of HP-NCI cases also had higher IL-7 and IL-9, but also higher levels of IL-1beta, IL-2, IL-4, IL-12p70, IL-17, TNFalpha, MIP1beta, and PDGF BB when compared to the AD. In IT cortex, the HP-NCI group had higher MCP-1 and IP-10 compared to the LP-NCI group, while IL-1Ra were higher and MIP-1alpha lower than in the AD group. These results indicates regional differences in neuroinflammatory change across the AD clinical/pathological spectrum assessed by multiplex assay of cytokines/chemokines. Our findings suggest that upregulation of MCP-1 and IP-10, and resulting monocyte/macrophage recruitment and phagocytotic activity, is a robust pro-inflammatory change in IT cortex of HP-NCI cases when compared to LP-NCI. In contrast, greater levels of IL-7 and IL-9, which have anti-apoptotic functions, and the anti-inflammatory

interleukin receptor, IL-1Ra, were the most consistently observed changes in HP-NCI relative to AD and may have contributed to their resilience.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant: NIA-P30AG10133, P01AG014449, P30AG072931, NIA-R56AG072810, R21AG056007, R21AG074539, and R21AG076202.

Title: Human microRNA-153 associates with the risk of Alzheimer's disease and targets specific neuronal genes.

Authors: *D. K. LAHIRI[1,2], R. WANG[1], B. MALONEY[1], K. NHO[3], M. R. FARLOW[4], F. A. WHITE[5], J. T. ROGERS[6], A. G. KANTHASAMY[7], N. H. GREIG[8], K. SAMBAMURTI[9], S. E. COUNTS[10];
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Abstract: Alzheimer's disease (AD) is a progressive degenerative disease characterized by a significant loss of neurons and synapses in cognitive brain regions and is the leading cause of dementia worldwide. AD pathology includes brain deposition of extracellular amyloid plaques and intracellular neurofibrillary tangles. Our goal is to understand the regulation of the amyloid-β precursor protein (APP), microtubule-associated protein tau (MAPT), and α-synuclein (SNCA), which are involved in AD-related dementias. Also, the excess accumulation of APP C-terminal fragments and disruption of lysosomal pathways are gaining interest in the AD field. In addition, repressor element 1-silencing transcription/neuron-restrictive silencer factor (REST/NRSF), a transcription repressor of neuronal genes, is dysregulated during AD pathogenesis. How REST is dysregulated is still poorly understood, especially at the post-transcriptional level. MicroRNAs (miRNAs), a group of short non-coding RNAs that typically regulate protein expression by interacting with target mRNA transcript 3'-untranslated region (UTR)s, play essential roles in AD pathogenesis and may be involved in REST metabolism. Herein, we demonstrate that miR-

153 binds REST 3'-UTR, reduces REST mRNA and protein levels in multiple human cell lines, and also downregulates APP and SNCA. Our further work suggests that miR-153-3p elevation is associated with reduced AD probability, while conversely, elevated REST is associated with a higher AD probability. Altogether, these results suggest that a supplement of miR-153 would reduce levels of toxic protein aggregates by reducing the expression of APP, SNCA, and REST - all pointing towards a therapeutic and biomarker potential of miR-153 in AD-related dementias. Supported by NIH grants.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

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Program #/Poster #: PSTR207.10/B72

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: U01AG074960
AG066060
AG062378

Title: Htap2 mice exhibit object recognition deficits in an enhanced custom arena: implications for human microrna research

Authors: *K. SAMBAMURTI[1], D. CROWDER[2], D. K. LAHIRI[3], N. H. GREIG[4], R. D. PENROD[5], G. PHILLIPS[6], M. A. PAPPOLLA[7], A. G. KANTHASAMY[8];
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Abstract: We generated Alzheimer's disease (AD) model mice by combining 5xFAD with human Tau (hTau; MAPT) and named it HTAP2, which stands for transgenic MAPT, amyloid precursor protein (APP), and presenilin 1 (PS1) lacking mouse Mapt (AAIC2023). HTAP2 mice performed more poorly than the parental mice in the Barnes maze and in the radial arm water maze RAWM). However, differences in novel (NOR) and displaced (DOR) object recognition tasks were not detected. Object recognition tasks are particularly desirable as multiple memory pathways are evaluated without inducing motivation through aversive treatment. We enhanced mouse handling by cupping and cylinder and constructed custom arenas to improve object interaction. Methods: Mice of various genotypes, ages, and sexes were habituated in open chambers for 15 min twice on day 1, followed by familiarization with two identical objects

placed diagonally across from each other for 15 min twice on day 2. On day 3, testing commenced with either NOR/DOR for 15 min, followed by either DOR/NOR for 15 min after a 4 h gap. We compared three types of arenas: cuboid (34 x 24 cm), commercial (40 sq cm), and custom (25 sq cm). We optimized objects for detection on videos and mouse interactions by minimizing object preference and the tendency of mice to climb on or look past the objects. Any Maze live video software was used for data analysis. Additionally, the manual timing of interactions improved data precision over AnyMaze by focusing on relevant interactions determined by human judgment. Results: Mice tended to jump over the arena walls and escape when handled by their tail but were contained when handled by cupping through a cylinder. All systems were equally capable of discriminating between novel and familiar objects. However, differentiation between familiar and displaced objects was only achieved in the custom 25 sq cm arena. Moreover, manual data curation reduced standard errors within groups and increased the significance of differences between groups. We were able to detect impairment in HTAP2 mice compared to hTau. Conclusion: It is critical to handle mice using cupping via cylinder to ensure reduced stress and optimum object interaction. A smaller arena may promote object interaction, while taller objects reduce detection artifacts. This unique HTAP2 model could be valuable for understanding the role of a specific microRNA (miR298) reported to regulate APP and MAPT expression. We propose to use the model to evaluate drugs and novel biologic treatments.

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Poster

PSTR207: Alzheimer’s Disease: Genomics and Other Omics Approaches II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIAID Grant R21AI179434

Title: Discovery of dehydroamino acids and their crosslinks in the major aggregating proteins of Alzheimer's disease

Authors: *S. W. MARKOVICH, B. L. FREY, M. SCALF, L. M. SMITH;
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Abstract: Alzheimer’s disease (AD) is characterized by the accumulation of two types of protein aggregates, senile plaques (SPs) and neurofibrillary tangles (NFTs), which strongly contribute to the pathogenesis of the disease. SPs consist primarily of aggregated amyloid-ß, while NFTs commonly form via aggregation of the protein Tau, as well as other microtubule-associated proteins such as CRMP2. The spread of SPs and NFTs has been thoroughly investigated, yet it remains unclear how the constituent proteins begin aggregating. Many have hypothesized that

posttranslational modifications (PTMs) contribute to the initial aggregation events. For example, it is widely reported that Tau hyperphosphorylation promotes NFT formation. Dehydroamino acids (DHAAs) are PTMs rarely observed in humans and have not previously been reported in AD. DHAAs arise from the eliminylation of serine, threonine, or cysteine creating a double bond with distinct molecular geometry and unique reactivity. Their geometry can lead to secondary structure rearrangement in model peptides, such as that seen in SP/NFT formation. Their unique reactivity provides a site for protein-protein crosslinking, which may chemically bind two proteins together. The capacity of this modification to produce protein crosslinks has previously implicated DHAAs in protein aggregation in the lens of the human eye. We hypothesized that this modification might be present in protein aggregate-associated neurodegenerative disorders like AD. We performed mass spectrometry-based bottom-up proteomics on the sarkosyl-insoluble (protein aggregate-enriched) material from ten AD brains and three age-matched controls. We discovered 267 unique DHAAs in 82 proteins, including in major aggregating proteins such as Tau and CRMP2. Comparison with previous protein aggregate interactomics studies show that DHAA-modified proteins are more exclusively involved in NFTs and SPs than any other individual PTM. We further observed 11 protein crosslinks arising from DHAAs, including two from the Tau protein. Crosslinks were validated using an isotopic labeling strategy as well as spike-in mass spectrometry experiments with a synthesized crosslink standard. Similar DHAA-related modifications and protein crosslinks were identified in publicly available proteomic datasets obtained from AD brains. In total, this work details a novel and prevalent brain PTM that may contribute to the pathogenesis of AD.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CAPES 88887.687008/2022-00
ALZ-NAN-22-928381
Serra-1912-31365
AARGD-21-850670

Title: Combined bulk transcriptomics reveals a neurodevelopmental signature in the Alzheimer's disease postmortem brain

Authors: *G. CARELLO-COLLAR[1], M. A. DE BASTIANI[2], C. LIMBERGER[3], A. CRISTINO[4], D. O. SOUZA[5], E. R. ZIMMER[1,6,7];
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Abstract: PSEN1 mutations are the most common cause of autosomal dominant Alzheimer's disease (ADAD). Intriguingly, children carrying a PSEN1-ADAD variant presented high plasma amyloid-β_{1-42} levels and brain functional hyperconnectivity, indicating a dysregulation in the early developmental stages. However, whether there is a neurodevelopmental signature in the adult ADAD brain remains to be elucidated. Thus, we aimed to investigate neurodevelopment-related genes in the postmortem brain of adult PSEN1-ADAD carriers compared to sporadic Alzheimer's disease (AD) individuals. We searched RNA-seq and microarray AD datasets deposited in the GEO for postmortem brain regions vulnerable to AD pathology from PSEN1-ADAD and sporadic early- and late-onset AD (EOAD and LOAD, respectively). We downloaded processed RNA-seq data of developmental stages from the Brain Span Atlas of the Developing Human Brain. We obtained the differentially expressed genes (DEGs) [FDR adjusted p-value < 0.05] and DEG-containing gene ontology terms of biological processes (GOBP) in common between ADAD or sporadic AD and neurodevelopmental stages. We included 1,086 AD individuals from 26 datasets and 42 individuals across seven developmental stages (**Table 1**). Curiously, not only PSEN1-ADAD but also sporadic AD shared DEGs with neurodevelopment (**Figure 1**). Both AD forms had neurodevelopment-related GOBP clusters, such as "neurogenesis and gliogenesis" and "embryonic development". The GOBP cluster "neuronal migration" was evidenced in sporadic EOAD and LOAD. PSEN1-ADAD carriers presented GOBP terms related to "axon guidance" and "neuronal differentiation" (**Figure 2**). Here, we show a neurodevelopmental transcriptomic signature in the autosomal dominant and sporadic adult AD brain, with DEGs and GOBP clusters related to the developing stages. Sporadic LOAD showed the highest transcriptome similarity with developmental stages, mainly in the fetal period. Our results suggest a neurodevelopmental contribution to AD pathology in adulthood regardless of AD genetic inheritance or symptom onset.

Table 1 | Included datasets and demographics.

A) Alzheimer's disease

	ADAD	EOAD	LOAD	Control
Included datasets	01	06	19	-
Sample size	07	66	1,013	871
Age at death (mean)	53.9	65.8	84.9	72
Sex (% of women)	14.3	44.7	59.2	43.7

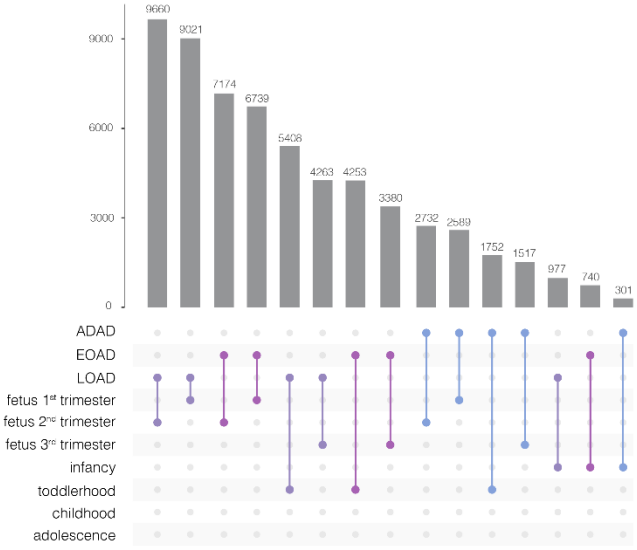
B) Developmental stages

	Fetus 1st tri	Fetus 2nd tri	Fetus 3rd tri	Infancy
Sample size	06	11	04	05
Age at death (mean)	11 PCW	17 PCW	31 PCW	7 mo
Sex (% of women)	60	36.4	75	20

	Toddlerhood	Childhood	Adolescence	Control
Sample size	03	04	03	07
Age at death (mean)	2.7 yo	7.6 yo	15.3 yo	29.4 yo
Sex (% of women)	66.7	25	33.3	67.1

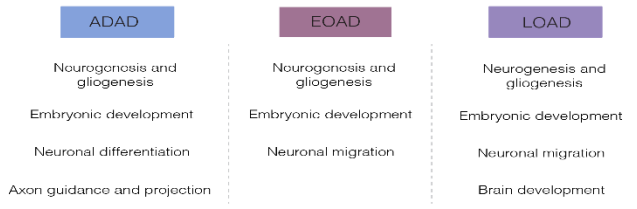
The prenatal phases were divided into trimesters. The postnatal stages were split into infancy (birth - 18 months-old), toddlerhood (18 months-old - 3 years-old), childhood (3 - 12 months-old), and adolescence (12 - 18 years-old). Young adults (18 - 40 years-old) served as controls in the developmental dataset. ADAD = autosomal dominant Alzheimer's disease. EOAD = early onset Alzheimer's disease. LOAD = late onset Alzheimer's disease.Tri = trimester. PCW = postconceptional week. mo = months old. yo = years old.

Figure 1 | Differentially-expressed genes in common between AD and developmental stages.



UpSet plot showing the intersection of differentially expressed-genes between AD subtypes and seven developmental stages. ADAD = autosomal dominant Alzheimer's disease. EOAD = early-onset Alzheimer's disease. LOAD = late-onset Alzheimer's disease

Figure 2 | Neurodevelopment-related clusters in AD.



ADAD = autosomal dominant Alzheimer's disease. EOAD = early-onset Alzheimer's disease. LOAD = late-onset Alzheimer's disease

Disclosures: G. Carello-Collar: None. **M.A. De Bastiani:** None. **C. Limberger:** None. **A. Cristino:** None. **D.O. Souza:** None. **E.R. Zimmer:** F. Consulting Fees (e.g., advisory boards); Novo Nordisk, Masima, Next Innovative Therapeutics.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.13/B75

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R03AG077406-01

Title: Pseudotime Analysis of Alzheimer's Disease: Identifying Key Genes of Molecular Progression in the brain and candidate RNA blood biomarkers.

Authors: *I. PIRAS[1], S. SONG[1], M. NAYMIK[1], F. ECCA[1], M. J. HUENTELMAN[2]; [1]TGen, Phoenix, AZ; [2]Neurogenomics, TGen, Phoenix, AZ

Abstract: Pseudotime (PT) methods are machine learning-based algorithms designed to extract latent temporal information from cross-sectional data. We applied PT analysis to publicly available bulk tissue RNA-profiling data obtained from post-mortem brain and blood samples of Alzheimer's disease (AD) and non-demented (ND) donors.We utilized data from the Accelerated Medicine Partnership-AD (AMP-AD) repository from eight brain regions: ACC, DLPFC, FP, IFG, PCC, PHG, STG, and TCX. We first extracted PT trajectories with the *phenoPath* method, investigating the correlation with clinical and neuropathological variables, including Clinical Dementia Rating, Braak stage, disease status, and amyloid plaque density. We detected a significant correlation in 83.8% of the variable/pseudotime comparisons. We identified 866 genes significantly correlated with pseudotime, with concordant directions across all eight brain regions ($|r| > 0.4$; Benjamini and Hochberg (BH) adj-$p < 0.05$). We observed consistent patterns at the cell-specific gene level across brain regions, with a significant increase in gene expression across PT for astrocyte, microglia, oligodendrocyte, and endothelial cell genes, and a significant decrease for both excitatory and inhibitory neuron genes. We conducted multi-brain coexpression network and key driver analysis across all datasets, identifying 358 significant key drivers, with 65 of them associated with PT in all the 8 brain regions. Notably, 9 genes were significant key drivers in 7 distinct brain regions: *CRYM*, *DRD1*, *GABRA4*, *KCNV1*, *LAMP5*, *PCP4*. *PCSK2*, *RASGRP1* and *ZCCHC12*. Finally, we extracted the pseudotime trajectory using the *DDRTree* method from two independent blood RNA profiling datasets from Gene Expression Omnibus (GSE63060 and GSE63061). After cross-referencing the results with the 866 genes significantly associated in brains, we identified an overlap of 112 genes also significantly associated in blood in both datasets ($|r| > 0.4$; BH adj-$p < 0.05$). In conclusion, our results highlighted key genes associated with AD, which might be useful targets for repurposed drugs or new molecule screening. Further in vitro and in vivo studies are warranted to validate the functional relevance of these genes. Additionally, we identified candidate blood-based RNA biomarkers that might predict AD onset before clinical symptoms appear. Validation in longitudinal studies will be a central step in assessing the applicability of these biomarkers in clinical practice.

Disclosures: I. Piras: None. **S. Song:** None. **M. Naymik:** None. **F. Ecca:** None. **M.J. Huentelman:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

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Program #/Poster #: PSTR207.14/B76

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R56 AG079857
Alzheimer's Association Research Fellowship
PRMRP Discovery Award W81XWH2010028
Edward R. and Anne G. Lefler Center Postdoctoral Fellowship
NIH T32 GM007753
NIH T32 GM144273
Suh Kyungbae Foundation
NIH DP2 AG072437
NIH R01 NS032457-20S1
NIH R01 AG070921
NIH R01 AG078929
F-Prime Foundation
Paul G. Allen Family Foundation
Howard Hughes Medical Institute

Title: Alzheimer's disease microglia exhibit an enrichment of somatic cancer driver mutations, correlating with disease-associated inflammatory states

Authors: *Y. HUANG[1,2], Z. ZHOU[1,2], M. TALUKDAR[1,2], S. G. MARRO[3], E. P. PAPAPETROU[3], E. LEE[1,2], C. A. WALSH[1,2];
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Abstract: Alzheimer's disease (AD), an age-associated neurodegenerative disorder, is characterized by progressive neuronal loss and the accumulation of misfolded proteins like amyloid-β and tau. Neuroinflammation, mediated by microglia and brain-resident macrophages, plays a pivotal role in AD pathogenesis, yet the intricate interactions among age, genes, and other risk factors remain elusive. Somatic mutations, known to accumulate with age, instigate clonal expansion across diverse cell types, impacting both cancer and non-cancerous conditions. Utilizing molecular-barcoded deep panel sequencing on 311 prefrontal cortex samples of AD patients and matched controls, our study unveiled an elevated occurrence of somatic mutations within cancer driver genes in AD brains. Recurrent somatic mutations, often multiple, were observed in genes associated with clonal hematopoiesis (CH). Remarkably, these somatic cancer driver mutations were specifically enriched in CSF1R+ microglia of AD brains and exhibited

signs of positive selection, suggesting mutation-driven microglial clonal expansion (MiCE). Single-nucleus RNA sequencing of the temporal neocortex samples from additional 62 AD patients and matched controls revealed a nominal increase in subchromosome-level somatic mutations associated with CH in AD microglia, with mutant microglia exhibiting upregulated pro-inflammatory and AD-associated genes. We further created three lines of induced pluripotent stem cell (iPSC)-derived microglia, each carrying driver mutations in one of the most mutated genes in AD brain. Through single-cell RNA sequencing, we verified that these mutant microglia consistently displayed an amplified inflammatory and disease-associated transcriptional profile compared to their wild-type counterparts. Our findings indicate that somatic cancer driver mutations in microglia are prevalent in normal aging but further enriched in AD, driving MiCE and promoting inflammatory, disease-related microglial signatures. This study provides crucial insights into microglial clonal dynamics in AD, potentially paving the way for novel approaches to AD diagnosis and therapy.

Disclosures: Y. Huang: None. **Z. Zhou:** None. **M. Talukdar:** None. **S.G. Marro:** None. **E.P. Papapetrou:** None. **E. Lee:** F. Consulting Fees (e.g., advisory boards); Inocras. **C.A. Walsh:** F. Consulting Fees (e.g., advisory boards); Third Rock Ventures, Flagship Pioneering, Maze Therapeutics.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.15/B77

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant R01AG061796 (Ertekin-Taner)
Fl DoH 22A06 (Allen)

Title: Alzheimers disease protective and risk signatures in microglia subtypes and states

Authors: *Ö. IS[1], J. BERGMAN[1], J. TAN[1], X. WANG[2], F. TUTOR-NEW[1], Z. QUICKSALL[2], M. ATIK[1], J. S. REDDY[2], Y. MIN[1], J. GAO[1], T. KANEKIYO[3], D. W. DICKSON[1], M. ALLEN[1], N. ERTEKIN-TANER[1];
[1]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [2]Dept. of Quantitative Hlth. Sci., Mayo Clin., Jacksonville, FL; [3]Dept. of Neurosci., Mayo Clin. Jacksonville, Jacksonville, FL

Abstract: Background: Alzheimer's disease (AD) affects all brain cells and has complex genomic and immunological alterations. Previous research linked AD to the missense variants in microglial genes *ABI3*-rs616338-T and *PLCG2*-rs72824905-G. Altered expression of these genes has been shown to disrupt microglial function. This study aims to understand protective, and risk microglial molecular signatures associated with these variants to determine their role in microglial subtypes and states in AD. Methods: We previously identified DOCK8 protein as a target for fluorescent activated nuclei sorting (FANS) to enrich microglia nuclei from frozen

human brains. Using this enrichment strategy, we generated microglia enriched snRNAseq data from superior temporal gyrus of 30 donors harboring *ABI3* or *PLCG2* missense mutations, or neither mutation. To check the impact of variant load and Aβ treatment, using CRISPR/Cas9 we generated isogenic *PLCG2*-rs72824905-G homozygote, heterozygote or wild-type carriers from an AD patient iPSC, differentiated these into microglia and generated scRNAseq after Aß treatment vs. control conditions. We annotated microglia clusters using established markers of microglia states and subtypes, identified differentially expressed genes and pathways using pseudo bulk and MAST approaches. To determine the relevance of our findings to AD, we assessed the identified signatures in additional human snRNAseq and scRNAseq and *in vivo* model system datasets. Results: From 30 donors with and without *PLCG2* or *ABI3* variants, we obtained single nuclei transcriptome profiles of 54,000 frozen human brain nuclei, of which 35,000 are microglia. We identified microglial clusters including: Homeostatic, Disease-Associated (DA), Alzheimer's Disease Associated (ADA), Proliferative, and Adaptive. We generated single cell transcriptomic profile of 63,000 iPSC-derived microglia (iMGLs). Our differential gene expression analysis identified novel protective and risk signatures genes in microglia subtypes and states. These were validated using orthogonal experimental methods and further explored for their conservation using external datasets. Conclusion: Our study uncovers microglia subtype specific protective and risk signatures that are associated with AD using snRNAseq from frozen human brain and scRNAseq from IPSC derived microglia cells. These findings nominate novel immune targets and pathways with therapeutic potential.

Disclosures: Ö. Is: None. **J. Bergman:** None. **J. Tan:** None. **X. Wang:** None. **F. Tutor-New:** None. **Z. Quicksall:** None. **M. Atik:** None. **J.S. Reddy:** None. **Y. Min:** None. **J. gao:** None. **T. Kanekiyo:** None. **D.W. Dickson:** None. **M. allen:** None. **N. Ertekin-Taner:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.16/B78

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Doctoral Dissertation Fellowship, Graduate School Fellowship Office at the University of Minnesota
Discretionary Funds, PL

Title: Multifaceted role of specialized neuropeptide-intensive neurons on the selective vulnerability of Alzheimer's disease in the human brain

Authors: *M. LI[1], N. FLACK[2], P. LARSEN[3];
[1]Univ. of Minnesota - Col. of Sci. and Engin., Minneapolis, MN; [2]Univ. of Minnesota, Saint Paul, MN; [3]Univ. of Minnesota, St. Paul, MN

Abstract: Alzheimer's disease (AD) is characterized by selective vulnerability of specific neuronal populations and brain regions. Investigating the basis of this selective neuronal and regional vulnerability is crucial for elucidating the molecular mechanisms underlying AD pathology. However, progress in this area is currently limited by our incomplete understanding of the intricate functional and spatial diversity of neuronal subtypes in the human brain. We have previously shown that neuronal subpopulations with high neuropeptide (NP) co-expression are lost in the entorhinal cortex of AD brains at the single-cell level. Additionally, there was a significant decline in hippocampal NP expression in naturally aging human brains. Here, we hypothesize that neurons expressing higher levels of NPs (HNP neurons) have unique functional characteristics that predispose them to cellular abnormalities, and that this susceptibility can manifest as degeneration in AD with aging. To test this hypothesis, we analyzed multiscale and spatiotemporal transcriptome data from over 1800 human brain samples, including healthy control, mild cognitive impairment (MCI), and AD populations, using publicly available datasets. We report that HNP neurons not only experienced greater metabolic burden but also were more prone to protein misfolding. The specific decrease of NPs during aging and MCI provided temporal evidence corroborating the role of NPs in the progression of AD. In addition, the localization of HNP neurons in AD-associated brain regions supplied neuroanatomical support for cellular/neuronal composition as a key factor in regional AD vulnerability. This study provides novel insights into the molecular and cellular basis of selective neuronal vulnerability in AD, highlighting the role of HNP neurons in the pathogenesis and regional vulnerability of AD in human brains.

Disclosures: M. Li: None. **N. Flack:** None. **P. Larsen:** None.

Poster

PSTR207: Alzheimer’s Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.17/B79

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institute on Aging R01AG068293
Cure Alzheimer's Fund
Alzheimer's Drug Discovery Foundation
Hevolution/American Federation for Aging Research

Title: Highly multiplexed spatial single-cell multi-omic imaging of tau neuropathology in human brain tissue sections

Authors: T. ORR[1], A. ROSENBLOOM[2], A. HECK[3], L. WU[4], C. S. LATIMER[5], C. D. KEENE[6], J. BEECHEM[7], ***M. ORR**[8];
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Univ. of Washington, Seattle, WA; [7]Nanostring Technologies, Piedmont, CA; [8]Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: The microtubule-associated protein tau accumulates in many neurodegenerative diseases. The neuronal deposition of aberrant tau, neurofibrillary tangles (NFTs), closely correlates with neurodegeneration and cognitive decline. Affected cells undergo phenotypic alterations, including disturbances in proteostasis and genomic instability. We have established connections between these alterations and cellular senescence, a complex stress response leading to a change in cell fate. Encouraging Phase I findings targeting the removal of senescent cells in AD underscore the necessity of further exploring senescent cells and their consequences across the brain.Understanding the molecular pathways guiding the transition from a post-mitotic neuron into senescence, the impact on cellular interactions among neighboring cells and the tissue-wide effects of cellular senescence demands a platform capable of high-resolution spatial analyses. Given the association between NFTs and senescence, an ideal platform should simultaneously capture changes in protein, such as tau phosphorylation, and gene expression. A spatial molecular imager (SMI) enables ultra-high plex detection of RNA and protein, offering a unique opportunity to profile molecular alterations in a spatial context. Here we showcase the multi-omic capabilities of the SMI in detecting the entire transcriptome and >68 proteins from the exact same healthy and diseased FFPE human brain sections. This multi-omic assay involves first detecting proteins with oligonucleotide barcode-conjugated antibodies before exposing sections to protease digestion and detecting transcripts with barcoded RNA probes. The targeted proteins encompass neural cell typing and neurodegenerative disease pathology, including tau and phosphorylated tau variants, while the RNA portion of the assay provides an unbiased view of 18,000+ protein-coding genes.Leveraging both protein and RNA data, we refined the segmentation of neural cells and categorized them into neuronal, glial and vascular subtypes. Moreover, we unveiled spatially correlated gene modules and categorized cells into distinct niches, facilitating the exploration of differential expression in gene sets or proteins across these niches for specific cell types. Particularly relevant for dissecting AD neuropathology, we examined changes in protein and RNA expression at defined distances from phospho-tau and amyloid beta labeling. Thus, by combining high-plex neuro protein and whole transcriptome RNA datasets, we gleaned unprecedented insights into NFT-associated cellular senescence, molecular landscapes, and neurotoxicity.

Disclosures: T. Orr: None. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; Employment/Salary (full or part-time):; Nanostring Technologies Inc, Bruker.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies Inc, Bruker. **A. Heck:** A. Employment/Salary (full or part-time):; Employment/Salary (full or part-time):; Nanostring Technologies Inc, Bruker. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies Inc, Bruker. **L. Wu:** A. Employment/Salary (full or part-time):; Nanostring Technologies Inc, Bruker. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder,

excluding diversified mutual funds); Nanostring Technologies Inc, Bruker. **C.S. Latimer:** None. **C.D. Keene:** None. **J. Beechem:** A. Employment/Salary (full or part-time):; Employment/Salary (full or part-time):; Nanostring Technologies Inc, Bruker.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanostring Technologies Inc, Bruker.. **M. Orr:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.18/B80

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NIA Grant 1R03AG073906

Title: Causal relationship between sleep duration and Alzheimer's disease: insights from Mendelian Randomization and Latent Causal Variable analysis

Authors: *S. SONG, M. J. HUENTELMAN, I. S. PIRAS;
TGen, Phoenix, AZ

Abstract: The identification of modifiable risk factors, including lifestyle-related habits, offers opportunities for disease risk reduction or prevention. Sleep disturbances, such as insomnia, sleep apnea, and restless leg syndrome, encompass a wide category of conditions that affect sleep. Several studies have linked sleep disturbances with an increased risk of Alzheimer's Disease (AD).
To explore the causal relationship between sleep disturbances and AD risk, we conducted mendelian randomization (MR) and latent causal variable (LCV) analysis using sleep duration (SD) as the exposure and AD as the outcome. We leveraged publicly available Genome-wide Association Studies (GWAS) summary statistics from self-reported and accelerometer-derived SD from the UK Biobank (PMIDs: 30846698, 30952852) and six AD GWAS. We ran LCV analysis as a discovery analysis using three AD GWAS as exposure (GCST90012877, GCST007320, and GCST90027158), and then focused on the significant phenotypes for the MR analysis, using the same sleep GWAS and also three additional AD GWAS (PMIDs: 2416273, 24162737, 30820047). Inverse Weighted Variance (IWV) was employed as our primary analysis, and subsequently we used MR-Egger and Weighted Median methods. We tested for heterogeneity using the Cochran Q test. LCV analysis revealed a significant association of self-reported long sleep (gcp = -0.644; p = 2.04E-13) and accelerometer-derived sleep duration standard deviation (gcp = -0.507; p = 0.031). We focused on these phenotypes in the MR study, but did not validate the results using the same three AD GWAS. However, we replicated the results on one of the three other GWAS (β = -22.4; p = 4.9E-09). The results were not significant when we used MR-egger (β = -18.5; p = 0.260) and Weighted Median methods (β = -21.0; p =

0.518). Three SNPs were responsible for the signals (rs4577128, rs4727449, and rs79456170; p < 0.001), located in *PRKCA*, *STAG3*, and *COMETT* genes.
Our results do not suggest clear evidence of a causal association between SD and AD. Although the results were highly significant with two different methods in two independent GWAS, we failed to find consistency across the different datasets. Other studies failed to detect evidence of causal association between SD and AD by MR (PMIDs: 35918656 and 33150399). However, a recent study (PMID: 38301285) using the English Longitudinal Study of Ageing, was able to identify increased risk of dementia and AD associated with long sleep. The risk of AD associated with SD might be potentially independent from genetic factors but mediated by other processes such as astrocyte and microglia dysregulations (PMIDs: 35755779, 29563238).

Disclosures: S. Song: None. **M.J. Huentelman:** None. **I.S. Piras:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.19/B81

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Single Cell Profiling of Cerebrospinal Fluid Immune Populations Reveals Unique Macrophage Population with Relevance to Neurodegenerative Disease

Authors: *S. MARSH[1,2], H. ZETTERBERG[3], A. KANE[4], E. MACOSKO[5], B. A. STEVENS[6,2,7];
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Abstract: Immune dysregulation is a common factor across the neurodegenerative disease spectrum implicated in both the development and progression of disease. Indeed, multiple single-nucleus RNA-seq (snRNA-seq) studies of neurodegeneration have shown dramatic alterations in the brain's tissue-resident immune cells, microglia, in animal models and more importantly from post-mortem human tissue. Interestingly, these alterations are not limited to microglia; data from animal models has shown that alterations in peripheral immune function dramatically impacts multiple brain cell populations and can alter disease pathogenesis. Studies of these peripheral immune populations in human patients, however, have been limited by lack of concurrent brain neuropathological and transcriptomic data.
We have access to paired brain, CSF, and peripheral blood from a rare cohort of living individuals with varying degrees of Alzheimer's disease (AD) pathology that has allowed us to directly examine both brain and peripheral immunity in the same patients. We have performed

multi-modal scRNA-seq and snRNA-seq from the brain, CSF immune cells, and peripheral blood from these patients.
Our analysis has found a unique population of macrophages present in the CSF that are distinct from CNS microglia/macrophages and peripheral monocytes. These cells are abundant in the CSF of controls and patients with neurological diseases. Strikingly, we find that these cells exhibit enrichment of polygenic Alzheimer's disease heritability, including very high expression of the two most significant AD genetic variants, APOE and TREM2. Furthermore, we find that these macrophages display altered gene expression profiles in patients with AD pathology or clinical AD diagnosis, underscoring the importance of understanding these enigmatic cells. To further our understanding, we have also performed large-scale integrative analysis with previously published CSF scRNA-seq datasets (>200 patients and 400,000 cells) which has led to identification of disease-/condition-specific differentially expressed genes/pathways in AD, PD, MS, and viral infection. Further analysis will better help clarify the role of CSF immunity in AD and provide a comprehensive reference for future studies.

Disclosures: S. Marsh: None. **H. Zetterberg:** None. **A. Kane:** None. **E. Macosko:** None. **B.A. Stevens:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.20/B82

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R0AG078797-02
NIH Grant S10OD032242-01A1

Title: Spatial lipidomics of Aβ plaques in a mouse model of Alzheimer's disease via multiscale mass spectrometry imaging

Authors: *T. J. TRINKLEIN[1], S. S. RUBAKHIN[1], M. ASADIAN[1], K. SABITHA[2], O. LAZAROV[2], F. LAM[1], J. V. SWEEDLER[1];
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Abstract: The pathogenesis of Alzheimer's disease (AD), including Aβ plaque formation, is associated with changes in the brain's spatiotemporal lipid content. For instance, the greatest genetic risk of late onset AD, apolipoprotein E4 (APOE4), plays a major role in lipid transport. Despite extensive research, the complete suite of lipids present in the Aβ plaque microenvironment has not been fully characterized. Mass spectrometry imaging (MSI) is a tool of choice for spatiochemical localization and quantification of lipids. We applied MSI to profile lipids in the brains of 5xFAD and wild type mice with unprecedented 5-µm-spatial resolution. We then analyzed thousands of single cells with MSI and mapped their lipid profiles to tissue (Xie 2024). This multiscale dataset of lipid alterations in Aβ plaques could guide the discovery

of potential therapeutic and diagnostic targets. We analyzed female 5xFAD mice at 5 months of age (*n*=3) and age- and sex-matched wild type (*n*=3) controls. Immediately after sacrifice, brains were perfused with ice-cold physiological solution and removed. For MSI, tissue was frozen and cryosectioned into 12-µm-thick sections. For single cell profiling, fresh tissue was dissected from the same brains, dissociated into single cells, and dispersed onto glass slides. Using matrix assisted laser desorption ionization (MALDI) MSI, we imaged tissue slices (*n*=12, two per animal) and over 10,000 individual cells from all six animals. After MSI, sections were stained with Thioflavin S and imaged by fluorescence microscopy to locate Aβ plaques. Statistically significant differences in lipid signals between 5xFAD and wild type mice were discovered by the Wilcoxen rank-sum test with Benjamini-Hochberg correction. Coregistration of images of histological staining for Aβ plaques and MSI data enabled identification of plaque-colocalized and depleted lipids. Classes of lipids that accumulated in Aβ plaques included monogangliosides, hexosylceramides, phosphatidic acids, phosphatidylethanolamines, phosphatidylinositols, and lysophosphatidylecholines. In contrast, only sulfatides were depleted in plaques. Sulfatides are abundant myelin lipids and are essential for maintenance of myelin sheath and axonal structure (Marcus 2005), and have been suggested as biomarkers for neurodegeneration (Blomqvist 2021). The results of our work indicate that myelin is excluded from the plaques. Plaque-associated lipids in tissue were also detected in single cells, recapitulating the trends shown in tissue-level data. Work is ongoing to extend our approach to through disease progression and to other models, such as APOE3/4 knock-in animals.

Disclosures: T.J. Trinklein: None. **S.S. Rubakhin:** None. **M. Asadian:** None. **K. Sabitha:** None. **O. Lazarov:** None. **F. Lam:** None. **J.V. Sweedler:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.21/B83

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A systems biology approach for biomarker discovery in Alzheimer's Disease using multi-omics and Bayesian Artificial Intelligence.

Authors: *M. A. KIEBISH[1], G. M. MILLER[1], S. DAS[2], R. SARANGARAJAN[1], P. SHAH[1], V. BUSSBERG[1], B. GREENWOOD[1], P. P. NARAIN[1], S. E. ARNOLD[2], B. HYMAN[2], N. R. NARAIN[1];
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Abstract: Alzheimer's disease (AD) is a progressive dementia disorder which represents a growing, unsustainable healthcare and economic burden globally, with no reliable effective treatments or biomarkers available to provide adequate guidance for intervention. Recent experience in AD drug development demonstrates the urgent need for novel therapeutic targets as well as reliable diagnostic, prognostic, and companion biomarkers. Herein, BPGbio deployed

BPGbio's NAi® platform to address discovery of causal biomarkers through the integration of proteomics, lipidomics, and metabolomics. Molecular omic and clinical phenotype data was integrated using Bayesian artificial intelligence. A causal network map was generated, and regression analysis was performed using multi-omic analysis of plasma and buffy coat samples from Cognitively Unimpaired Controls (ND, N=50), Mild Cognitive Impaired (MCI, N=75), and AD (N=75) samples from the Massachusetts Alzheimer Disease Research Center's longitudinal study. A total of 3,465 data features including proteomics (buffy coat and plasma), lipidomics (plasma), metabolomics (plasma), and clinical features were utilized for generation of the model network. The longitudinal time point assessment enabled dynamic modeling and identification of possible predictive signatures of disease progression. Several AD specific, statistically significant markers with relationship to clinical endpoints, such as MMMSE/MOCA, TRAILA, and days until cognitive decline were identified. Very Important Nodes (VIN) representing drivers of model networks for all patients at all time points include N-Acetylglucosamine-1-Phosphate Transferase (GNPTG; plasma) and Immunoglobulin kappa (IGKV1-5; buffy coat); Hepatocyte growth factor activator (HGFAC; plasma), and Beta-actin like protein 2 (ACTBL2; plasma) were markers of causal relevance associated with clinical features to infer potential biomarker utility. These biomarkers will be further examined with additional cohorts for bioanalytical and clinical validation to demonstrate the greatest diagnostic utility providing a valuable tool for the diagnosis and progression of Alzheimer's disease (AD).

Disclosures: M.A. Kiebish: A. Employment/Salary (full or part-time):; BPGbio Inc. **G.M. Miller:** A. Employment/Salary (full or part-time):; BPGbio Inc.. **S. Das:** None. **R. Sarangarajan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BPGbio, Inc. **P. Shah:** None. **V. Bussberg:** None. **B. Greenwood:** None. **P.P. Narain:** A. Employment/Salary (full or part-time):; BPGbio Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BPGbio, Inc. **S.E. Arnold:** None. **B. Hyman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dewpoint, Novartis, Lattus. **N.R. Narain:** A. Employment/Salary (full or part-time):; BPGbio Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BPGbio, Inc.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.22/B84

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG056287
R01 AG057915
R01 AG068279
U19 AG065156

UH3 CA246633
P30 AG066515
T32 AI007290

Title: Proteo-spatial signatures of human resilience to Alzheimer’s Disease captured through multiplexed ion beam imaging.

Authors: *B. CANNON;
Stanford Univ., Stanford, CA

Abstract: Neurodegenerative diseases such as Alzheimer's Disease are characterized by aggregation of misfolded proteins and the progressive loss of neurons and synapses in the brain. However, some individuals show cellular and symptomatic resilience to these diseases despite accumulating a buildup of protein aggregates. In this study, we aimed to identify the local proteomic signatures associated with human neurodegenerative resilience through multiplexed ion beam imaging (MIBI). We analyzed FFPE human brain tissue samples from individuals with Alzheimer's Disease, those who were cognitively healthy and without protein aggregation, and those who were resilient to the development of Alzheimer’s Disease symptoms in the face of existing protein aggregation. By using MIBI we simultaneously measured the expression of 40 proteins in individual cells and tissues across multiple brain regions in 31 patients. Using cell segmentation, pixel clustering, and spatial enrichment analysis, we aim to find proteomic signatures associated with resilience that are distinct from those of the disease states. Our integrative approach aims to unveil the microglial proteomic configurations that could possibly confer neuroprotection against AD pathology. The resultant datasets promise to shed light on the proteomic immune and microenvironmental phenotypes associated with neurodegenerative resilience. Ultimately, the insights gained from this study have the potential to propel the development of targeted therapeutic strategies, seeking to emulate or enhance these naturally occurring protective mechanisms in Alzheimer's Disease.

Disclosures: B. Cannon: None.

Poster

PSTR207: Alzheimer’s Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.23/B85

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The National Key R&D Program of China (2021YFE0203000)
The Research Grants Council of Hong Kong (the Collaborative Research Fund [C6027-19GF], the Theme-Based Research Scheme [T13-605/18W] and the General Research Fund [HKUST16103122]
The Areas of Excellence Scheme of the University Grants Committee (AoE/M-604/16)

The Innovation and Technology Commission (InnoHK, and ITCPD/17-9)
The Guangdong Provincial Key S&T Program Grant (2018B030336001)
The Guangdong Provincial Fund for Basic and Applied Basic Research (2019B1515130004)
The Fundamental Research Program of Shenzhen Virtual University Park (2021Szvup137)

Title: Soluble TREM1 is a putative causal factor of Alzheimer's disease identified by unbiased Mendelian randomization analysis

Authors: *H. UHM[1,2], Y. JIANG[1,2], X. YANG[1,2], D. Y. WONG[1,2], W. WU[1,2], Y. QIAO[1,2], W.-Y. FU[1,2], F. C. IP[1,2,3], T. KWOK[4], A. WONG[1,2], Y. DUAN[1,2], A.-Y. FU[1,2,3], N. Y. IP[1,2,3];
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Abstract: Several blood proteins can modulate neuronal function and immune response in the brain either by crossing the blood-brain barrier or acting on infiltrating peripheral immune cells. Given that the blood proteome is altered in patients with Alzheimer's disease (AD), certain dysregulated blood proteins may play a functional role in AD pathogenesis. Here, we identified blood proteins that are causally associated with AD risk by performing a large-scale unbiased Mendelian randomization analysis in the European population. We then performed functional analysis on the top candidate disease-causing factor to delineate the molecular mechanism by which it contributes to AD pathogenesis. Accordingly, we identified 32 blood proteins, including 11 immune-related proteins, which are causally associated with AD. We further assessed the causal associations between the 32 candidate proteins and AD-associated endophenotypes, such as tau pathology and neurodegeneration. As soluble triggering receptor expressed on myeloid cells 1 (sTREM1) was causally associated with the most endophenotypes tested (i.e., 6 out of 8), we selected it for further functional study. Consequently, we showed that sTREM1 level in the blood is elevated in patients with AD from both European and Chinese cohorts. Furthermore, immunohistochemical, transcriptomic, and proteomic analysis of patients with AD and mouse models of amyloidosis revealed that higher levels of sTREM1 in the blood promote inflammation, impair microglial clearance of amyloid-beta, and exacerbate neurodegeneration. Taken together, we demonstrated the causative role of sTREM1 in AD.

Disclosures: H. Uhm: None. **Y. Jiang:** None. **X. Yang:** None. **D.Y. Wong:** None. **W. Wu:** None. **Y. Qiao:** None. **W. Fu:** None. **F.C. Ip:** None. **T. Kwok:** None. **A. Wong:** None. **Y. Duan:** None. **A. Fu:** None. **N.Y. Ip:** None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.24/B86

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: The National Key R&D Program of China (2021YFE0203000)
The Research Grants Council of Hong Kong (the Collaborative Research Fund (C6027-19GF), the Theme-Based Research Scheme (T13-605/18W) and the General Research Fund (HKUST16103122)
The Areas of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
The Innovation and Technology Commission (InnoHK, and ITCPD/17-9)
The Guangdong Provincial Key S&T Program Grant (2018B030336001)
The Guangdong Provincial Fund for Basic and Applied Basic Research (2019B1515130004)
The Fundamental Research Program of Shenzhen Virtual University Park (2021Szvup137)

Title: Rare TREM2 variant is associated with compromised microglial function and dysregulated immune response in the Chinese AD population

Authors: *S. TSUI[1,2], D. AU[1,2], H. UHM[1,2], W. HO[1,2], M. LO[1,2], F. C. IP[1,2,3], T. KWOK[4], K. MOK[1,2,5], H. W. TSANG[1,2], A. WONG[1,2], A.-Y. FU[1,2,3], N. Y. IP[1,2,3];
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Abstract: Genome-wide association studies (GWAS) have revealed many AD risk genes, most of which (i.e., TREM2, CD33, etc.) are related to microglial functions. Studies on the role of disease-associated genetic variants in cellular functions and disease progression are critical to our understanding of disease mechanisms. While most genetic studies are carried out in the European population, emerging evidence suggests that studies in other ethnic groups may provide novel insights on protein functions in disease progression. In this study, we examined the roles of the TREM2 variant p.H157Y, a rare AD risk variant found mainly in the Chinese population with a minor allele frequency ~5-fold higher than in European-descent populations. TREM2 is predominantly expressed in microglia in the brain and is an important mediator of microglial functions. Other AD-associated TREM2 loss-of-function genetic variants have been found to decrease microglial metabolic fitness and chemotactic ability. TREM2 is also known to play an important role in the peripheral immune system, suggesting a potential role of TREM2 in mediating the crosstalk between the peripheral system and the brain. To investigate the role of p.H157Y in AD pathology, we recruited patients with AD carrying a single copy of p.H157Y variant and their families, and found its association with AD-related plasma biomarkers and brain atrophy. Moreover, blood multi-omics studies revealed an early dysregulation of blood transcriptomic and proteomic profiles in younger variant carriers with normal cognition, suggesting a role of TREM2 in the peripheral immune system during early development of AD-

related pathology. Using induced pluripotent stem cell-derived microglia-like cells generated from variant carriers, we found that the p.H157Y variant leads to phenotypic changes resembling partial TREM2 loss-of-function, with compromised capacity to respond to external stimuli. In summary, we show that the p.H157Y variant of TREM2 leads to microglial dysfunction and dysregulation of the peripheral immune system, providing a deeper understanding on the role of TREM2 in AD pathology.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

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The Research Grants Council of Hong Kong (the Collaborative Research Fund [C6027-19GF]
the Theme-Based Research Scheme [T13-605/18W]
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The Areas of Excellence Scheme of the University Grants Committee (AoE/M-604/16)
The Innovation and Technology Commission (InnoHK, and ITCPD/17-9)
The Guangdong Provincial Key S&T Program Grant (2018B030336001)
The Guangdong Provincial Fund for Basic and Applied Basic Research (2019B1515130004)
The Fundamental Research Program of Shenzhen Virtual University Park (2021Szvup137)

Title: Il-33/st2 signaling regulates microglial functions and their interaction with aβ in alzheimer's disease

Authors: *A. WONG[1,2], W. WU[1,2], X. WANG[1,2], Y. JIANG[1,2], S. GILL[1,2], A. YUEN[1,2], W.-Y. FU[1,2], A.-Y. FU[1,2,3], N. Y. IP[1,2,3];
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Abstract: Alzheimer's disease (AD), the commonest cause of dementia worldwide, is characterized by the formation of amyloid plaques from insoluble beta-amyloid peptides (Aβ)

deposition and neurofibrillary tangles (NFT) from pathological tau, resulting in axonal toxicity and neurodegeneration. Genetic and functional studies reveal that clearance of Aβ is mainly carried out by microglia, the brain's resident macrophages. The cytokine interleukin-33 (IL-33) interacts with its full-length receptor, ST2L, on microglia to induce a functional state transition that enhances microglial chemotaxis towards Aβ and subsequent Aβ clearance capacity. Our group reported a common genetic variant rs1921622 located in the *IL1RL1* gene region, which is associated with the levels of soluble ST2 (sST2), the decoy receptor of IL-33/ST2 signaling. Carriers of rs1921622 A allele is associated with decreased sST2 levels, as well as amyloid plaque load and increased Aβ-microglia interaction in female AD patients who carry ε4 allele of apolipoprotein E (*APOE*). Thus, it is important to understand the effect of genetic variant rs1921622 on the regulation of microglial phenotypes and their interaction with Aβ in detail. We examined the molecular and cellular phenotypes of microglia in post-mortem brain samples of AD patients who carry the rs1921622 A. The snRNA-sequencing and immunohistochemical analysis of post-mortem human brain samples showed a change in the subpopulation of microglia (i.e. increase of activated microglia) in carriers of rs1921622 A allele. Furthermore, spatial analysis of Aβ and microglia revealed higher Aβ-microglial interaction and phagocytosis in carriers of rs1921622 A allele, suggesting that the decrease in sST2 plasma level is associated with upregulated microglial activation and enhanced Aβ clearance in AD. Further studies on the roles of sST2 in microglial clearance of Aβ may provide insights into the development of sST2 modulators for treatment of AD.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.26/B88

Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant R21 AG056901
NIH Grant R21 AG059195
NIH Office of Dietary Supplements

Title: Integrative DNA methylomic and transcriptomic profiling of APP^{NL-G-F} Alzheimer's disease model mice across age and brain region: modulation by perinatal choline diet

Authors: *A. KRUNIC[1], T. BELLIO[2], T. J. MELLOTT[2], J. K. BLUSZTAJN[2];
[1]Pharmacology, Physiology, and Biophysics, [2]Pathology & Lab. Med., Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA

Abstract: Alzheimer's disease (AD) is the most common form of dementia and has limited options for treatment and prevention. Aberrant brain DNA methylation (DNAm) is associated with many of the pathological hallmarks of AD, such as β-amyloid accumulation and gliosis. Previous studies have shown that dietary supplementation during fetal- and early postnatal life with the essential nutrient choline - a metabolic precursor for phosphatidylcholine, acetylcholine, and S-adenosylmethionine - can alter brain DNAm patterns in developing rat brain and ameliorate neuropathology and inflammation, while improving cognition in mouse models of AD. This study aimed to characterize the brain DNA methylome and transcriptome of the App^{NL-G-F} mouse model of AD and its modulation by perinatal choline supplementation (PCS) across disease progression. Male and female C57BL/6J and App^{NL-G-F} AD model mice were reared on a perinatally choline supplemented- or control diet and cerebral cortical and hippocampal DNA methylation was measured at 3, 6, 9, or 12 months of age by reduced representation bisulfite sequencing (n = 96 total, n = 3 per group). App^{NL-G-F} mice were characterized by extensive age and brain region-dependent DNA methylation abnormalities. PCS induced genotype-specific DNAm changes and reversed DNAm abnormalities at a subset of CpG sites in App^{NL-G-F} mice. By associating DNAm with matched transcriptomics, we found that DNAm in App^{NL-G-F} mice was correlated with the RNA expression of multiple genes involved in microglial activation and inflammation. Moreover, DNAm changes in PCS mice were associated with expression of synaptic genes, in line with the cognition-enhancing effects of PCS. The methylation levels of a set of CpGs correlated with amyloid burden, thus associating DNAm with neuropathology. DNAm levels of multiple of these plaque-associated CpGs (PACs) correlated with the expression of previously-identified plaque-induced genes (e.g. *Apoe, C1qc, Trem2, Tyrobp*), and some were modulated by PCS. Overall, the results show that brain DNAm is highly influenced by the AD-like disease progression in the App^{NL-G-F} mice and suggests that targeting DNAm with an early life nutritional strategy may be a promising avenue for preventing AD.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

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Program #/Poster #: PSTR207.27/B89

Topic: C.02. Alzheimer"s Disease and Other Dementias

Support: RF1AG057768
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HHSN2682015000011
I01BX005933

Title: App$^{nl\text{-}g\text{-}f}$ alzheimer's disease (ad) model mice recapitulate the transcriptomic abnormalities seen in postmortem human ad brain: attenuation by perinatal choline supplementation

Authors: *T. A. BELLIO[1], A. KRUNIC[2], T. D. STEIN[3], T. J. MELLOTT[4], J. K. BLUSZTAJN[5];
[1]Pathology, Boston Univ. Sch. of Med., Boston, MA; [2]Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA; [3]Boston VA Med. Ctr., Boston, MA; [4]Pathology & Lab. Med., Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA; [5]Pathology, Boston Univ., Boston, MA

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia worldwide. Although AD has been extensively studied, a cure remains elusive and treatment options are inadequate to effectively benefit the patient's life. We have shown that perinatal choline supplementation in *App*$^{NL\text{-}G\text{-}F}$ AD model mice prevents cognitive deficits, reduces Aβ42 deposition across the brain [PMID: 38315685], and abolishes or dampens transcriptomic brain alterations [SfN annual meeting 2023]. In this study, we investigated the effects of the *App*$^{NL\text{-}G\text{-}F}$ genotype and perinatal choline supplementation on the brain transcriptome across the lifespan and correlated our findings with an analogous postmortem human data set. We used cortical and hippocampal RNA-sequencing data from wildtype (WT) and *App*$^{NL\text{-}G\text{-}F}$ mice that received a perinatal diet containing either 1.1 g/kg (control) or 5 g/kg (supplemented) of choline chloride during gestation through weaning. Mice were aged until 3, 6, 9, or 12 months of age (n=96 total, 6 per group). Analyses of differentially expressed genes due to both genotype and choline supplementation within *App*$^{NL\text{-}G\text{-}F}$ mice revealed enrichment in pathways involved in immune, synaptic/neuronal function, oxidative phosphorylation, and metabolism. We identified abnormally-expressed genes in *App*$^{NL\text{-}G\text{-}F}$ mice as compared to WT mice reared on the control diet. The abnormal expression of a subset of these genes was ameliorated by perinatal choline supplementation and we termed these genes choline-responsive AD pathology-associated genes (CRADPAGs). We identified over 200 CRADPAGs in the hippocampus and over 100 CRADPAGs in the cortex in immune, synaptic, energetic, and metabolic pathways. The human homologs of these murine CRADPAGs were then examined in the previously published RNA-sequencing data set from the postmortem dorsolateral prefrontal cortex of the brain donor participants of the Framingham Heart Study [PMID 34480088] and correlated with AD diagnosis; CERAD stage; Braak and Braak stage; cortical Aβ40, pTau396, and alpha-synuclein protein levels; cortical Iba1 staining density; and clinical dementia rating. The expression of the majority of the human homologs of the CRADPAGs correlated with at least one of the AD traits. These, and prior data suggest that perinatal choline supplementation reduces AD-like neuropathology in the *App*$^{NL\text{-}G\text{-}F}$ AD model mice by normalizing the expression of genes characteristically dysregulated in AD brain and support the notion that adequate maternal dietary choline consumption may be a valuable method to prevent or reduce characteristic AD cognitive impairments and neuropathology.

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Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.28/B90

Topic: H.12. Aging and Development

Support: AFAR BIG21042
RF1AG068292
R01AG070934

Title: Serum metabolome profiling in patients with mild cognitive impairment reveals sex differences in lipid metabolism

Authors: *R. DIAZ ESCARCEGA;
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Abstract: Serum metabolome profiling in patients with mild cognitive impairment reveals sex differences in lipid metabolism

Rocio Diaz Escarcega[1], M. J. Vijay Kumar[1], Guadalupe J. Ortiz[1], Aaron M. Gusdon[2], Huihui Fan[1], Pedram Peesh[1], Maria P. Blasco Conesa[1], Gabriela Delevati Colpo[1], Hilda W. Ahnstedt[1], Lucy Couture[1], Stella H. Kim[1,3], Miriam Hinojosa[1], Christine M. Farrell[1], Sean P. Marrelli[1], Akihiko Urayama[1], Bhanu P. Ganesh[1], Paul E. Schulz[1], Louise D. McCullough[1,3] and Andrey S. Tsvetkov[1,3,4]
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Key words: sex differences, cognition, metabolomics, lipids

AbstractMore women than men are affected by Alzheimer's disease (AD). Although women live longer than men, it is not longevity alone, but other factors, including metabolic changes, that contribute to the higher risk of developing AD in women. Metabolic pathways have been implicated in AD progression, but studies to date have examined targeted pathways, leaving many metabolites unmeasured. Sex is often a neglected biological variable and most metabolomic studies have not been specifically designed to investigate sex differences in metabolomic profiles. Here, we performed untargeted metabolomic profiling using serum from male and female patients with mild cognitive impairment (MCI), a common precursor to the development of AD, as well as age-matched controls. We discovered significant metabolic changes in individuals with MCI, with several pathways being strongly associated with sex. Peptide energy metabolism demonstrated sexual dimorphism. Lipid pathways exhibited the strongest differences between female and male MCI patients. Specific dicarboxylic fatty acids, phosphatidylcholine lipids, lysophospholipids, long chain fatty acids, and monoacylglycerols. 1-palmitoleoyl glycerol and 1-arachidonoyl glycerol (1-AG) were higher in female MCI subjects than in male MCI subjects with no differences seen between control males and females. In cultured male and female astrocytes, 1-AG promoted epigenetic changes and chromatin remodeling in a sex specific fashion. Overall, we identified novel sex-specific metabolites in

MCI patients that could serve as biomarkers of MCI in both sexes, help further define AD etiology, and reveal new potential prevention strategies for AD.

Disclosures: R. Diaz Escarcega: None.

Poster

PSTR207: Alzheimer's Disease: Genomics and Other Omics Approaches II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR207.29/B91

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Swiss National Science Foundation (SNSF) 211053

Title: Spatial and single cell transcriptomics reveal neuron-astrocyte interplay in long-term memory

Authors: *W. SUN;
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Abstract: Memory encodes past experiences, thereby enabling future plans. The basolateral amygdala (BLA) is a center of salience networks that underlie emotional experiences and thus plays a key role in long-term fear memory formation. Here we used spatial and single-cell transcriptomics to illuminate the cellular and molecular architecture of the role of the basolateral amygdala in long-term memory. We identified transcriptional signatures in subpopulations of neurons and astrocytes that were memory-specific and persisted for weeks. These transcriptional signatures implicate neuropeptide and brain-derived neurotrophic factor (BDNF) signaling, mitogen-activated protein kinase (MAPK) and cAMP response element-binding protein (CREB) activation, ubiquitination pathways, and synaptic connectivity as key components of long-term memory. Strikingly, upon long-term memory formation a neuronal sub-population defined by increased Penk and decreased Tac expression constituted the most prominent component of the BLA's memory engram. These transcriptional changes were observed both with single-cell RNAseq and with single-molecule spatial transcriptomics in intact slices, thereby providing a rich spatial map of a memory engram. The spatial data enabled us to further discover that this neuronal subpopulation interacts with adjacent astrocytes, and functional experiments then showed that neurons require interactions with astrocytes to encode long-term memory.

Disclosures: W. Sun: None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.01/B92

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG067419 (WDH, MKE, MAR, CCS)
NIH NS092988 (CCS, WDH)

Title: Cortical calbindin-immunoreactive neurons are protected from the effects of aging and Alzheimer's disease-like pathology in the chimpanzee brain

Authors: *M. K. EDLER[1,2], C. C. SHERWOOD[3], P. R. HOF[4], W. D. HOPKINS[2], M. RAGHANTI[1];
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Abstract: Calbindin D28k (CB) is a calcium-binding protein that regulates intracellular calcium (Ca^{2+}) levels in the brain and is involved in neurotransmitter release and neuronal membrane excitability and plasticity. Dysregulation of Ca^{2+} homeostasis has been implicated in Alzheimer's disease (AD), characterized neuropathologically by amyloid-beta protein (Aβ) plaques and tau-associated neurofibrillary tangles (NFT). CB can modulate Aβ generation and NFT formation, while Aβ triggers increased intraneuronal Ca^{2+} leading to excitotoxicity and reduced CB protein expression levels. Recently, we demonstrated the sporadic occurrence of Aβ plaques, NFT, and cerebral amyloid angiopathy (CAA) in aged chimpanzees. Here, we used immunohistochemistry and stereology to examine whether aging or AD-like neuropathology affected CB-immunoreactive (ir) neuron density (Nv) in the dorsolateral prefrontal cortex (DLPFC, layer III), middle temporal gyrus (MTG, layer III), entorhinal cortex (EC, layer II), and hippocampus (pyramidal layer, CA1) of 82 chimpanzees (16-61 y; male = 30, female = 52), including individuals previously identified with severe CAA (Aβ40 and Aβ42) or NFT lesions (AT8). Repeated-measures ANOVA revealed CB-ir Nv was significantly higher in the DLPFC (20,197/mm^3), while the EC had the lowest CB-ir Nv (9,081/mm^3; p's $\leq$ 0.001). Age was not associated with CB-ir Nv in any region (p's $\geq$ 0.06), nor was the presence of severe CAA or NFT (p's $\geq$ 0.10). No differences were observed between males or females (p's $\geq$ 0.28). Like humans, CB-ir Nv in chimpanzees was preserved in the DLPFC, EC, and CA1 during aging. Conversely, chimpanzees lacked age-related changes in CB-ir Nv seen in the human MTG as well as the extensive loss of CB-expressing neurons observed in association with NFT during late stages of AD. These data indicate that CB-ir neurons in the chimpanzee brain are protected from the effects of aging, severe CAA, and NFT, although whether these findings are due to species' variation in levels of AD-like pathology or CB remains to be investigated.

Disclosures: M.K. Edler: None. **C.C. Sherwood:** None. **P.R. Hof:** None. **W.D. Hopkins:** None. **M. Raghanti:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.02/B93

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UTMB NVRCL startup package
UTMB Zahra Kolahchi scholarship

Title: Amyloid β and Tau oligomers disrupt blood-brain barrier in a human based in-vitro microfluidic neurovascular unit model

Authors: *Z. KOLAHCHI[1], P. P. MARTINEZ CUEVAS[2], E. CUEVAS[3];
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Abstract: Amyloid β and Tau Oligomers Disrupt Blood-Brain Barrier in a Human Based in-vitro MicrofluidicNeurovascular Unit ModelBackground: Alzheimer's disease (AD) is the most common neurodegenerative disorder seen in age-dependent dementia and accounts for 60-70% of worldwide cases. A growing body of evidence supports the idea that neurovascular unit (NVU) dysfunction and blood-brain barrier (BBB) breakdown are major contributors for AD development. However, the mechanism underlying NVU dysfunction contributing to AD is still not fully understood.**Methods:** In this study, a three-dimensional in vitro NVU model was used as a compartmentalized microfluidic device. It contains five cell types in a dynamic and tunable microenvironment, resulting in an in vivo-like response. Each chip contains two fluidic compartments separated by a porous membrane. The vascular compartment is lined with endothelial cells (EC), while the brain compartment contains cortical neurons, astrocytes, pericytes, and microglia. Cultured cells on both sides of the porous membrane, provided cell-cell interactions resembling in-vivo microenvironment. Imitating AD pathophysiology, we evaluated the toxic effects of Amyloid β oligomers (Aβo) and Tau oligomers (TauO) by adding both to the brain compartment and adding only Aβo to the vascular compartment. We confirmed the aggregation of Aβ using Simple Western blot assay. Twenty-four hours after the treatment, lactate dehydrogenase (LDH) test was tested to asses *cytotoxicity*, and indicators of *permeability* were measured using Dextran Cascade Blue 3 kDa, and occluding levels. **Results:** After 24 hours of treatment, our preliminary data showed that Aβo and TauO produce cytotoxicity as indicated by a significantly increased level of LDH, compared to the control chips. This toxic effect was further supported by a significant increase in the crossing of Cascade Blue 3KDa between the compartments, as an indicator of increasing permeability, as well as a decrease in occludin levels. **Conclusions:** This is a human-based in vitro model that offers dynamic cell-cell interactions to investigate BBB function continuously with and without Aβo and TauO as well as discover therapeutic target capabilities. Our preliminary results suggest that Aβo and TauO provoke vascular cytotoxicity, BBB dysfunction and increased permeability. These data together suggest that NVU-on-a-chip is a good model for testing NVU dysfunction in AD

Disclosures: Z. Kolahchi: None. **P.P. Martinez Cuevas:** None. **E. Cuevas:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.03/B94

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1AG077826
VA Merit Award 101 BX004161-01
NIH R01 ES028104
NIH RO1 R01AG076142
NIH R01 ES029835GW

Title: Ozone Dysregulates Vasculature-Associated Proteins in the Amyloid Plaque Microenvironment

Authors: *C. AHMED[1], H. J. GREVE[2], C. GARZA-LOMBO[3], J. A. JOHNSON, Jr.[4], A. OBLAK[5], M. BLOCK[6];
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Abstract: Urban air pollution exposure, including ozone (O_3), has been associated with increased Alzheimer Disease (AD) risk with recent suggesting that urban air pollution exposure may increase amyloid plaque pathology. However, the underlying cellular mechanisms driving this effect are poorly understood. The CNS vasculature is known to be dysregulated early in AD and the accumulation of amyloid pathology in the vasculature is known to be a key component of the disease. While O_3 has long been linked to cerebral vascular effects such as stroke, how O_3 may affect the vasculature characteristics in AD pathology is largely unknown. To begin to explore this, 10-11 week old male 5xFAD mice were exposed to filtered air (FA) or 1 ppm O_3 for 13 weeks (4 hours/day and 3 days/week). Our analysis showed both increased plaque load in the parenchyma and increased vessel associated Amyloid-beta (Aβ) deposition with O_3 exposure. O_3 also reduced vascular density in plaque microenvironment, potentially affecting neuronal health. The NanoString GeoMx Digital Spatial Profiling (DSP) platform was used to assess multiplex protein expression co-localized with laminin positive vessels, that were either plaque-associated or plaque distant, in the cortex. DSP analysis comparing plaque-associated vessels to plaque distant ones revealed a baseline change in expression of 14 proteins in FA exposed group. While 8 of these proteins were also differentially expressed in the O_3 exposed group, 6 proteins were changed in only FA exposed mice, and not in O_3 exposed mice, implying O_3 alters the baseline vascular response to plaque deposition. Notably, IDE upregulation, important for Aβ degradation, on periplaque vessels in FA group was abolished from O_3 exposure, potentially suggesting obstructed Aβ degradation and clearance. Additionally, multiple proteins, such as C9, CD11b, and olig2, were dysregulated in plaque associated laminin-positive vessels in O_3 exposed mice only, implicating an altered cell to cell interaction and communication between vascular

cells and others, specifically microglia, with ozone exposure. Taken together, these findings support that O_3 modifies the amyloid-associated changes in the vasculature, potentially dysregulating Aβ degradation and clearance, and vasculature associated cellular communication. These findings may provide much needed insight into how urban air pollution affects amyloid cerebral vascular pathology.

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Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.04/B95

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC Grant: MR/X003418/1
MRC Grant: MR/W006944/1
Battelle—Dr Jeff Wadsworth studentship
Wellcome Trust/Royal Society, Sir Henry Dale Fellowship: 105586/Z/14/Z

Title: Investigating the therapeutic potential of increasing CBF in mouse models of dementia

Authors: B. EYRE[1], L. M. LEE[1], R. SIDHU[2], D. DREW[2], J. SIMPSON[2], C. J. MARTIN[3], J. BERWICK[2], ***C. HOWARTH**[2];
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Abstract: Approximately 54 million people are living with dementia worldwide, a number expected to treble by 2050. Alzheimer's disease (AD), the most prevalent cause of dementia, is a devastating condition for which we have very few disease-modifying treatments. The link between impaired cerebral blood flow (CBF) and AD remains understudied, not only in terms of understanding the disease but also as a potential AD therapeutic strategy. Increasing CBF could lead to increased brain oxygenation and reduced accumulation of proteins, such as amyloid beta. We hypothesise that experimental modulations of CBF will affect disease progression in mouse models of AD. Unlike sensory stimulation, CO_2 is known to increase CBF without increasing neural activity, avoiding additional metabolic demand in a disease in which a mismatch between energy supply and demand may exist. Therefore, the effect of a daily 1 hour treatment with 5% CO_2 for 3 months will be assessed in two mouse models of AD: APP/PS1 (6-9 months old) and P301S(PS19) tauopathy model (3-6 months old), each replicating a different hallmark pathology of AD. Male and female mice will be used. Cognition will be assessed using the Novel Object Recognition test and Barnes Maze, and pathology (amyloid-beta, tau) will be assessed using post-mortem immunohistochemistry. CO_2-treated AD mice will be compared with sham-treated

AD and wild-type (WT) littermates. Experimenters are blinded to genotype and treatment, wherever possible. In order to characterise neurovascular function in these mouse models of AD, we have applied 2D-OIS to assess cortical haemodynamic responses to whisker stimulation in awake APP-PS1 mice (9-12 months old, n=12), P301S(PS19) mice (9-11 months old, n=2) and wild type mice (9-12 months old, n=11). Preliminary results indicate that haemodynamic responses are preserved in AD, suggesting that the neurovascular machinery is functional and thus can be targeted to increase CBF as a therapeutic strategy. This research will further our understanding of neurovascular function in AD and will demonstrate whether increasing CBF in the absence of increasing neural activity has a potential disease-modifying effect in AD.

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Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.05/B96

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: K01 AG078407
P51 OD011092
P30 AG066518
U24 AA025473
grant from the M.J. Murdock Charitable Trust

Title: Quantifying perivascular spaces in aged rhesus macaques with Magnetic Resonance Imaging (MRI)

Authors: *A. R. WEISS[1,2], W. LIGUORE[1], D. SCHWARTZ[2], A. RAJENDRAN[4], G. JENSEN[4], J. PIANTINO[2], J. KARPF[3], M. M. CHERNOV[1], S. G. KOHAMA[1], L. C. SILBERT[2]; [1]Neurosci., Oregon Natl. Primate Res. Ctr., Beaverton, OR; [2]Neurol., [3]Behavioral and Systems Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [4]Reed Col., Portland, OR

Abstract: Perivascular spaces (PVS) are fluid channels hypothesized to be involved in regulating the interchange between cerebrospinal fluid (CSF) and interstitial fluid (ISF) to clear solutes and waste products from the parenchyma, or "glymphatic" function. A feature common to many types of dementia are PVS that can be visualized on Magnetic Resonance Imaging (MRI) scans. MR-visible PVS are typically elongated and cylindrical in shape, observed in white matter and basal ganglia structures, and isointense to CSF. In humans, retrospective studies have indicated that the number of MR-visible PVS increases starting in mid-life, and are seen prior to cognitive decline, suggesting that they may be an early marker of ADRD (Alzheimer's Disease and Related Dementias). The aging process of nonhuman primates (NHP) shares many features in common with humans including mild cognitive impairment, as well as increased white matter

diffusivity and gray matter atrophy, but the presence of MR-visible PVS in aged NHPs is unexplored. To address this gap, we quantified MR-visible PVS in a population of 69 rhesus macaques, ranging in age from 5 to 28 years old (41F/28M). MR-visible PVS were segmented on high-resolution (0.5mm isotropic) T2-weighted SPACE images using an automated method for brain-wide quantification. The resulting segmentations were used to calculate the number (count) and total volume (mm^3) of MR-visible PVS for each animal. We identified significant relationships between age and MR-visible PVS number ($r(67)=0.46$, $p<0.0001$), as well as volume ($r(67)=0.47$, $p<0.0001$). In order to assess the accuracy of the automatic detection method, a subset of the automated segmentations were correlated with manual counts from trained observers ($r(56)=0.850$, $p<0.0001$). Additional work is underway to co-localize the *in vivo* radiological findings with post-mortem MRI and histological markers. The establishment of a NHP model of MR-visible PVS will allow for preliminary evaluation of some of the fundamental tenets of the glymphatic hypothesis: age-related alterations in CSF kinetics leads to decreased glymphatic function, and MR-visible PVS are a marker of such glymphatic dysfunction.

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Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.06/B97

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant from Arabian Gulf University Bahrain #: (E006-PI-04/17).

Title: Effects of caffeine on the blood brain barrier of type 2 diabetic rats, a histological study

Authors: *M. A. OTHMAN[1,2];
[1]Anat., Arabian Gulf Univ. Col. of Med. and Med. Sci. Bahrain, Manama, Bahrain; [2]Histology & Cell Biology, Assiut University Faculty of Medicine, Assiut, Egypt

Abstract: Abstract
Background: Type 2 diabetes (T2D) is one of the primary pathological factors that contribute to cognitive impairments and central nervous system (CNS) degeneration. Dysfunction of pericytes, cells located around microvascular endothelial cells of the CNS, can affect the blood-brain barrier (BBB), which may undergo hyperpermeability with diabetes progression. T2D can result in degenerative changes of the brain. The present study focuses on the effects of an animal model of T2D on the role of vascular pericyte dysfunction, which may play a role in BBB integrity. The ameliorative role of caffeine in these changes is also addressed. **Materials & Methods:** T2D was induced in rats by feeding them with high caloric diet and giving them a single

intraperitoneal injection of streptozotocin at a lower dose. Caffeine was administered to diabetic rats orally for 5 weeks. After sacrificing the animals, brains were dissected out and processed for general histological evaluation. Immunohistochemistry was also performed using the following primary antibodies: alpha-smooth muscle actin (α-SMA, a marker for pericytes), occludin 1 (tight junction marker) and transforming growth factor-beta (TGF beta). **Results:** There was disruption of the general morphological structure of the hippocampus and downregulation of α-SMA and occludin 1 and upregulation of TGF beta. These results demonstrate that the impaired hippocampus structure is associated with BBB impairment. The administration of caffeine to diabetic rats resulted in reversal of immunomarkers' expression. **Conclusion:** In this model of T2D, there was disruption of pericytes and BBB integrity and improvement of these structures and related biomarkers with caffeine administration.

Disclosures: M.A. Othman: None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.07/B98

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research Council VR (2022-02727)
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Title: Loss of glymphatic homeostasis in heart failure

Authors: *M. KRITSILIS[1,2], L. VANHERLE[1,2], M. ROSENHOLM[3], R. IN T ZANDT[4], Y. YAO[5], K. M. SWANBERG[1,2], P. WEIKOP[3], M. GOTTSCHALK[4], N. C. SHANBHAG[1,2], J. LUO[5], K. BOSTER[6], M. NEDERGAARD[3,7], A. MEISSNER[1,2,8], I. LUNDGAARD[1,2];
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Abstract: Heart failure (HF) is a progressive and potentially fatal condition affecting more than 10% of people over 65 years old, a prevalence expected to increase in the following decades. HF is characterized by progressive reduction in cerebral blood flow (CBF) and neurodegenerative

changes resulting in cognitive decline. The glymphatic system plays a key role in waste disposal from the brain and its impairment is implicated in neurodegenerative processes. The aim of this project was to investigate how HF affects the glymphatic system. We used a mouse model of HF, induced by myocardial infarction (MI), to study the effects of reduced ejection fraction (EF) in the brain. Using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and high-resolution fluorescence microscopy, we found an increase in glymphatic influx at 12 weeks post-MI compared with sham controls. Interestingly, the EF showed an inverse correlation with glymphatic influx. Glymphatic clearance, however, did not increase proportionately with influx, consistent with dysregulation of brain fluid dynamics. 2-photon microscopy demonstrated that cerebral arterial pulsatility, a well-established driver of the glymphatic system, was increased at 12 weeks post-MI, providing a plausible mechanism for our results. Our findings also revealed a strong correlation between glymphatic clearance and CBF, suggesting an involvement of the glymphatic system in the progression of HF-induced decline in brain health in HF patients affected by lowered CBF.

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Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.08/B99

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Brain endothelial GSDMD activation mediates inflammatory BBB breakdown

Authors: *C. WEI;
Chinese Inst. for Brain Research, Beijing, Beijing City, China

Abstract: Abstract: The blood-brain barrier (BBB) protects the central nervous system (CNS) from pathogen infections or harmful substances, and its impairment renders various CNS diseases. However, mechanisms for BBB disruption in infection and inflammatory conditions are poorly defined. Here, we find that GSDMD activation, causing plasma membrane pore-formation/pyroptosis in brain endothelial cells, but not TLR4-induced cytokine production, determines circulating lipopolysaccharide (LPS)-caused BBB breakdown. Mice deficient in the *Casp11-Gsdmd* noncanonical inflammasome or the *Lbp-Cd14* LPS transfer/internalization resisted LPS-induced BBB disruption. Brain endothelial cells highly expressed GSDMD and responded robustly to LPS that primed *Casp11* and *Cd14* expression. Circulating LPS acted on brain endothelial cells, inducing GSDMD-mediated plasma-membrane permeabilization/pyroptosis *in vitro* and in BBB-disrupted mice. Electron microscopy revealed ultrastructural damages in LPS-disrupted BBB, featuring pyroptotic endothelia with abnormal

appearance of tight junctions, vasculature detachment from basement membrane, enlargement of perivascular space, and collapse of basement membrane. *Gsdmd*$^{-/-}$ specifically in brain endothelia blocked LPS-caused BBB disruption, which was restored by re-expression of *Gsdmd* selectively in these cells. Targeted expression of active GSDMD in brain endothelial cells opened the BBB in the absence of LPS. Infection of Gram-negative *Klebsiella pneumoniae*, notorious for causing severe nosocomial infections, disrupted the BBB in *CASP4*-humanized mice, which was blocked by a GSDMD-neutralizing nanobody expressed selectively in brain endothelial cells. Our findings shift the paradigm of understanding inflammatory BBB breakdown and offer insights into potential therapeutics of BBB impairment-associated CNS diseases. **Keywords:** lipopolysaccharide (LPS), *CASP4-GSDMD/Casp11-Gsdmd* pathway, brain endothelial cells, BBB breakdown, bacterial infection, *Klebsiella pneumoniae*

Disclosures: C. Wei: None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.09/B100

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Lundbeck Foundation (Grant number R400-2022-1213)

Title: New evidence linking a rat model of hypertension to human sporadic Alzheimer's disease

Authors: *J. A. BASTRUP[1], J. FOLKE[2], S. AZNAR[2], T. JEPPS[1];
[1]Dept. of Biomed. Sci., Univ. of Copenhagen, Copenhagen N, Denmark; [2]Ctr. for Neurosci. and Stereology, Bispebjerg-Frederiksberg Hosp., Copenhagen Univ. Hosp., Copenhagen NV, Denmark

Abstract: Cerebrovascular dysfunction and subsequent development of Alzheimer's disease (AD) hallmarks are observed in a rat model of hypertension (spontaneously hypertensive rat, SHR). This may suggest that cerebrovascular dysfunction has a causal role in triggering an AD-like pathology. Do the molecular changes observed in the SHR resemble the underlying pathological features in AD? We used a combination of immunostaining and in-depth proteomics to establish a translatable reference map of protein changes in the prefrontal cortex (PFC) of aged hypertensive rats (SHR, 30 to 40-week-old, n = 12), age-matched normotensive WKY controls (n=8), human AD (Braak stages 4-6, n = 30), cerebral amyloid angiopathy (n=5) and human non-demented controls (Braak stages 0-3, n = 37). Demographic analysis confirmed similar age, gender, post-mortem interval and APOE alleles (ε3, ε4) in human samples. Our proteomic analysis identified >4000 unique proteins across all rat and human samples. The statistical analysis identified 199 differentially expressed proteins (DEPs) in the PFC when comparing SHR rats to WKY control rats. Pathway analysis revealed significant association to "regulation of amyloid precursor protein catabolic process". The statistical analysis of human

samples identified >1300 DEPs when comparing AD to non-demented controls (Braak stage 0). Pathway analysis revealed associations to biological processes and cellular components, such as “oxidative phosphorylation” and “extracellular exosome”. We quantified the intensity ratio between an Aβ peptide over the upstream full-length APP. This approach confirmed significant increase in the Aβ:APP ratio in AD and hypertensive rat samples compared to WKY rats and non-demented human controls (Braak stages 0-3). Cross-species comparison identified 24 candidate proteins in the hypertensive rat model, which trajectory pattern over the development of hypertension, is mirrored in AD Braak stages 0-6. Among the identified candidate proteins, pathway analysis revealed associations to “Extracellular exosomes” and “MHC class II protein complex binding”. Together, these findings demonstrate cross-species translatability between the SHR and human AD. Our findings provide novel insights into the association between hypertension and AD.

Disclosures: J.A. Bastrup: None. **J. Folke:** None. **S. Aznar:** None. **T. Jepps:** None.

Poster

PSTR208: Alzheimer’s Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.10/B101

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01AG065836

Title: Utilizing voluntary wheel running to attenuate cerebral blood flow in the J20 mouse model

Authors: *J. L. BROWNING[1], A. KALOSS[2], J. LI[3], M. THEUS[2], M. L. OLSEN[4];
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Abstract: Decades of research indicate that a global reduction in cerebral blood flow (CBF) represents one of the earliest observable pathologies in individuals with Alzheimer disease (AD), a phenotype that is recapitulated in animal models of AD with Aβ accumulation. Reduced CBF occurs prior to significant parenchymal plaque accumulation or neurological decline and is now considered predictive and a potential diagnostic indicator for early-stage AD, and may be modified through exercise. Chronic reduction in CBF and the subsequent reduction in parenchymal tissue oxygenation and glucose may be sufficient to drive neurodegeneration, yet, the underlying cause of global reduced CBF is not understood. Using methoxy-X04 to label vascular Aβ, and vascular labeling approaches, we identified significant Aβ burden on the pial artery/arteriole network in J20 (PDGF-APPSwInd) AD mice. Imaris image analysis of Aβ accumulation on reconstructed vessels indicated a progressive increase in Aβ burden on both the MCA and ACA, reaching an average of 23% coverage in mice one year of age, with males

expressing a higher plaque burden than females. Additionally, the leptomeningeal anastomoses or pial arteriole collateral vessels, i.e. the by-pass system responsible for re-routing blood flow in the event of an occlusion, displayed active outgrowth and remodeling, including an increase in MCA-ACA collateral diameter and collateral tortuosity in J20 male and female mice by one year of age. Despite increased collateral diameter, overall surface hemisphere perfusion measured by laser speckle imaging indicate CBF was decreased by 15% in 12-month J20 mice compared to age matched WT littermates. We further assess the impact of voluntary wheel running in J20 and WT mice to assess the impacts exercise has on AD progression. Our findings indicate significant Aβ burden on the meningeal arteries, the inflow for parenchymal perfusion, may serve to restrict global CBF observed in animal models of AD. Further we have identified retrograde reperfusion initiated through collateral vessel remodeling as a potential compensatory mechanism to restore CBF to affected brain tissue.

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Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.11/B102

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Boston University Marion and Henri Gendron Faculty Fund

Title: Increased blood-brain barrier (BBB) permeability and white matter structural damage detected in vivo using non-contrast MRI in the hippocampus of young APOE4 mice

Authors: *C. CHENG[1], Y. GUAN[2], B.-B. KOO[1], J. A. WELLS[3];
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Abstract: The Apolipoprotein E (APOE) is important for lipid metabolism and is primarily expressed by astrocytes in the brain. Specifically, the e4 (APOE4) genotype is associated with the greatest risk for late onset Alzheimer's Disease (LOAD), accounting for more than 95% of AD cases, through inflammatory glial activation and other mechanisms during aging (Frisoni et al., 2022; Steele et al., 2022). Recent studies also revealed that the APOE4 gene leads to blood brain barrier (BBB) breakdown (Montagne et al., 2020). Using a novel and non-invasive multiple echo time arterial spin labeling (multi-TE ASL) magnetic resonance imaging (MRI) (Ohene et al., 2020), we successfully detected faster water exchange rates ($p<0.01$, i.e., more leaky BBB) in the hippocampi of young APOE4 mice (3M/2F, mean age: 5.6 months old) compared to APOE3 mice (2M/2F, mean age: 7.5 m.o.), associated with increased levels of the BBB degradation and

inflammation marker Matrix Metalloproteinase-9 (MMP9) protein localized at the blood vasculature in immunostaining (p=0.02). In nearby white matter (WM) structures, we observed lower fractional anisotropy (p=0.01) in the E4 group using both diffusion tensor imaging and diffusion kurtosis reconstruction on multi-b diffusion MRI (b=1000, 2000s/mm^2). The near hippocampal WM diffusion measures were also correlated with water exchange rate in the hippocampus (p=0.03, rho=0.79). Whereas peripheral cortical (i.e., perirhinal cortex) and other subcortical brain regions (thalamus) did not show clear patterns in either BBB permeability or WM integrity changes. Additionally, we developed a semi-automated image processing pipeline that incorporates image registration, precise template labeling, and noisy voxel elimination for processing multi-TE ASL data, which provides robust and replicable BBB permeability measures compared to the manual drawing approach. Here we demonstrated for the first time that APOE4-related early BBB disruption could be detected by non-invasive MRI with increased water exchange rates as early as 6-8 months of age in the hippocampus, associated with local WM disruptions. Results suggest potential interaction between early BBB functional alterations and region-specific WM changes that require further investigation to monitor the structural and functional properties of the BBB, which may be considered as important biomarkers for studying LOAD in the APOE4 carrying population.

Disclosures: C. Cheng: None. **Y. Guan:** None. **B. Koo:** None. **J.A. Wells:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.12/B103

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BBSRC LIDo PhD Studentship
ERC Advanced Investigator Award (740427)
Wellcome Trust Senior Investigator Award (219366)
Howard Hughes Medical Institution Grant

Title: Mural cell calcium channels reduce cerebral blood flow, increase hypoxia and promote immune cell stalling in capillaries in Alzheimer's disease

Authors: *N. KORTE[1], A. BARKAWAY[2], J. A. WELLS[4], F. FREITAS[3], H. SETHI[5], S. ANDREWS[6], J. SKIDMORE[7], B. A. STEVENS[1], D. ATTWELL[2];
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Abstract: Early in Alzheimer's disease (AD) pericytes constrict capillaries, increasing their hydraulic resistance and trapping of immune cells, and thus decreasing cerebral blood flow (CBF). Therapeutic approaches to attenuate pericyte-mediated constriction in AD are lacking. Using in vivo two-photon imaging with laser Doppler and speckle flowmetry and magnetic resonance imaging, we show that Ca2+ entry via L-type voltage-gated calcium channels (CaVs) controls the contractile tone of pericytes. In AD model mice we identify pericytes throughout the capillary bed as key drivers of an immune reactive oxygen species (ROS)-evoked and pericyte [Ca2+]i-mediated decrease in microvascular flow. Blocking CaVs from early in AD improved CBF, reduced leukocyte stalling at pericyte somata and attenuated brain hypoxia. Aβ-evoked pericyte contraction in human cortical tissue was also greatly reduced by CaV block. Lowering mural cell [Ca2+]i early in AD may thus offer a therapeutic strategy to enhance brain energy supply and cognitive function in AD.

Disclosures: N. Korte: None. **A. Barkaway:** None. **J.A. Wells:** None. **F. Freitas:** None. **H. sethi:** None. **S. Andrews:** None. **J. Skidmore:** None. **B.A. Stevens:** None. **D. Attwell:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.13/B104

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R21 AG080216 (ME)
NIH NS092988 (CSS, WDH)

Title: Serotoninergic neuron density in the dorsal raphe nucleus is preserved during aging and in the presence of Alzheimer's disease-like pathology in chimpanzees

Authors: *C. HEARN[1], W. HOPKINS[2], C. SHERWOOD[3], P. R. HOF[4], M. RAGHANTI[1], M. K. EDLER[1];
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Abstract: The dorsal raphe nucleus (dRN) is comprised mainly of projection neurons producing serotonin (5-hydroxytryptamine; 5-HT), which play a role in learning, memory, and mood regulation. The dRN also is a site of early tau pathology in humans, and loss of 5-HT in this region likely contributes to behavioral changes (e.g., disrupted stress coping and exaggerated anxiety response) seen in Alzheimer's disease (AD). While AD is unique to humans, the pathologic hallmarks of amyloid-beta (Aβ) protein plaques, cerebral amyloid angiopathy (CAA), and tau-associated neurofibrillary tangles (NFT) occur in elderly chimpanzee brains. Here, we

investigated the effect of physiological aging, CAA, and NFT on 5-HT-immunoreactive (ir) neurons in the chimpanzee dRN. Using immunohistochemistry and stereology, we quantified 5-HT-ir neuron density (5-HT Nv), Nissl Nv (total Nv), 5-HT/total Nv ratio, Nissl glia density (total Gv), and Nissl glia:neuron (G:N) ratio in the postmortem brainstems of 21 captive chimpanzees including young controls (n = 7, 19-34 y), aged controls (n = 7, 36-54 y), and aged with moderate to severe CAA individuals based upon Aβ40 and Aβ42 immunoreactivity within cerebral blood vessels (n = 7, 36-58 y). Occasional Aβ-ir plaques and vessels were observed in the dRN of aged chimpanzees with CAA. Surprisingly, one-way ANCOVA analyses found that dRN total Nv was significantly higher in chimpanzees with CAA compared to young and aged controls ($p = 0.001$), while 5-HT Nv, ratio of 5-HT/total Nv, total Gv, and G:N ratio did not differ (p's ≥ 0.26). NFT characterized by AT8 immunoreactivity were identified in 12 chimpanzees (36-58 y) and qualitatively more prevalent than Aβ lesions in the dRN, although the presence of tau lesions did not correlate with cell densities or ratios (p's ≥ 0.65). Age and sex were not associated significantly with any measure ($p \geq 0.55$). Similar to this report, 5-HT-ir neuron numbers are preserved in dRN of elderly humans. However, human AD brains exhibit NFT and extensive 5-HT-ir cell loss, which contrasts with our observations in chimpanzees with CAA and NFT. Our findings suggest either varying levels of pathology between species or the presence of a potential protective mechanism within the chimpanzee brain that prevents or reduces the neurotoxic effects of NFT and CAA, which have been well documented in humans. Alternatively, a higher density of 5HT-ir neurons within the dRN may render this region more susceptible to CAA in chimpanzees.

Disclosures: C. Hearn: None. **W. Hopkins:** None. **C. Sherwood:** None. **P.R. Hof:** None. **M. Raghanti:** None. **M.K. Edler:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.14/B105

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MR/X003418/1

Title: Understanding cerebral vasomotion and its implications for the interpretation of neurovascular data in health and disease.

Authors: R. WANG[1], S. O'CONNOR[2], R. SIDHU[2], O. SHABIR[3], C. J. MARTIN[1], C. HOWARTH[4], M. OKUN[6], ***J. BERWICK**[5];
[1]Psychology, [2]The Univ. of Sheffield, Sheffield, United Kingdom; [3]Univ. of Sheffield Fac. of Med., [4]Psychology, [5]Univ. of Sheffield, Sheffield, United Kingdom; [6]Sch. of Life Sci., Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Vasomotion is a pervasive low frequency oscillation that can occur in any tissue in the body at a frequency of 0.1 Hz (once every 10 seconds). Its existence has been known for decades; in fact its first appearance can be traced back to the Roy and Sherrington paper (1890), which is often credited with proposing the existence of neurovascular coupling. With the emergence of wide-field whole cortex awake mouse neurovascular imaging technologies the term vasomotion is now routinely used as a general term referring to spontaneous neurovascular low frequency dynamics, while the original concept has to a large extent become extinct. In fact, vasomotion in its original meaning of 0.1 Hz oscillation remains poorly understood: we do not understand why vasomotion occurs, its structure within the brain and whether it is completely independent of neuronal activation. To address these questions, we manipulated baseline blood pressure and the fraction of inspired oxygen to generate and inhibit distinct vasomotion oscillations, and measured concurrent neuronal and hemodynamic activity and tissue oxygenation changes within the cortex of anaesthetised rats and mice. We find that vasomotion oscillations are driven by the arterial tree causing pronounced saturation changes in downstream draining veins. Vasomotion is associated with low tissue oxygen and is largely independent from neuronal activity. Vasomotion is separate and distinct from baseline low frequency neurovascular activity, measured in anaesthetised mice undergoing a gas challenge, which had a broad spectrum peak around 0.1 Hz that represented spontaneous neurovascular coupling. Vasomotion has the potential to become an important biomarker and target for therapeutic treatment of neurodegenerative disease but understanding when and why it arises needs further research.

Disclosures: R. Wang: None. **S. O'Connor:** None. **R. Sidhu:** None. **O. Shabir:** None. **C.J. Martin:** None. **C. Howarth:** None. **M. Okun:** None. **J. Berwick:** None.

Poster

PSTR208: Alzheimer’s Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.15/B106

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG062006

Title: Brain connectivity of the olfactory cortex in metabolic syndrome and cerebral small vessel disease

Authors: *H. REYES[1], C. FRANK[3], J. MONDRAGON URIBE[2], C. MURPHY[4];
[1]Psychology, [2]San Diego State Univ., San Diego, CA; [3]SDSU/UC San Diego, SAN DIEGO, CA; [4]San Diego State University/UC San Diego, San Diego, CA

Abstract: Understanding the neurological underpinnings of metabolic syndrome (MetS) and small vessel disease (SVD), both important risk factors for Alzheimer’s Disease (AD), can provide insight into the development of dementia (Cai et al., 2015; Han & Lean, 2015; Liu et al.,

2018). Olfactory dysfunction is one of the early indicators of mild cognitive impairment (MCI) and AD, and neuropathology associated with AD shows early manifestations in olfactory processing areas, including the olfactory cortex (OC; Albers et al., 2015; Frank et al., 2024; Jung et al., 2019; Murphy, 2019). Therefore, we sought to explore brain connectivity patterns in the olfactory cortex for individuals with MetS and SVD. Thirty-seven cognitively unimpaired participants (female = 22) were divided into two groups of interest, MetS (n =11) and SVD (n =19) and seven without either condition. Functional MRI data were acquired while participants engaged in an odor identification task. Seed-to-voxel functional connectivity of the OC, with the seed located primarily in the orbitofrontal cortex and part of the piriform cortex, during correct odor identification was analyzed using CONN toolbox. Initial analyses revealed opposing patterns of brain connectivity among MetS and SVD groups between the OC and other regions. Significantly higher (k = 2767, p-FDR = .0204) OC connectivity was detected in the MetS group with the following areas: frontal pole right, frontal pole left, frontal medial cortex, precentral gyrus left, postcentral gyrus right, middle frontal gyrus left, superior parietal lobule left, and superior frontal gyrus left, while significantly lower (k = 12895, p-FDR <.001) brain connectivity was detected in the SVD group with these same areas. When controlling for MetS status, SVD was associated with less OC connectivity with the medial frontal cortex (k = 10805, p-FDR < .001) and increased OC connectivity with the middle cingulate (k = 3671, p-FDR = .0026). Previous literature has found a relationship between increased connectivity in olfactory areas and higher BMI and its connection with memory, taste, and reward regions, consistent with these MetS analysis findings (Jacobson et al., 2019, 2017). The SVD results raise important questions about the relationship between MetS and SVD**.** We discuss the potential implications for this contrasting effect, and the important insight that the olfactory cortex can provide for understanding the effect of MetS and SVD on the brain and the progression of Alzheimer's Disease.
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Disclosures: H. Reyes: None. **C. Frank:** None. **J. Mondragon Uribe:** None. **C. Murphy:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.16/B107

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: In Vitro Modeling of the Blood-Brain Barrier in Sporadic Alzheimer's Disease Using Patient iPSCs

Authors: *K. LIM;
The Salk Inst., San Diego, CA

Abstract: In Vitro Modeling of the Blood-Brain Barrier in Sporadic Alzheimer's Disease Using Patient iPSCs **AUTHOR BLOCK: *K. LIM**[1] M. WANG[1], F. GAGE[1]; [1]Salk Inst. for Biol. Studies, San Diego, CA
Alzheimer's disease is the most common form of dementia and as much as 95% of all AD cases manifest as a late onset sporadic form (sAD). While most AD research has focused primarily on neuronal and glial dysfunction, compelling data are emerging identifying a significant vascular component in the pathology of AD. Evidence shown by cerebrospinal fluid biomarker[1] and post mortem neuroimaging studies of AD-affected brains show blood-brain barrier (BBB) disruption in the form of cerebral microbleeds, amyloid beta (Aβ) peptides in and along the walls of cerebral blood vessels, and deficits in Aβ clearance. Furthermore, one risk factor for developing sAD is the apolipoprotein E (APOE), specifically the APOE4 allele. While the accumulation of Aβ in the vasculature of Alzheimer's disease (AD) has been proposed as one mechanism explaining the impact of APOE4 on cerebrovascular integrity, **the specific role of different APOE genotypes (APOE ε2/ε2, APOE ε3/ε4, APOE ε4/ε4) in the systemic breakdown of the blood-brain barrier (BBB) in sporadic AD remains uncertain.** Using various endothelial, pericyte, and astrocyte differentiation protocols, I established an induced pluripotent stem cell-based in vitro model of the BBB, composed fully of all cell types derived from 16 individuals (8 AD/ 8 CTRL) from various APOE backgrounds (2/3, 3/3, 4/4). By employing the use of transwells, we were able to analyze the baselines permeability in the BBB model of AD patients when compared to age-matched control. Additionally, transport assays that utilize BBB-specific transporters, such as PGP, MRP1, and BCRP1 were used to assess if the pathological breakdown of the BBB results in impaired efflux of toxic substances from the brain to the blood. We hypothesize that the APOE genotype of AD patients exacerbates the pathological breakdown of the BBB when compared to unaffected age-matched controls. The findings from this study will elucidate the role of each cell type in BBB dysfunction, contributing to the development of strategies to mitigate these disruptions in sporadic Alzheimer's disease.

Disclosures: K. Lim: None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.17/B108

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH and NINDS Grant 7RF1NS132291-02.

Title: Protein arginine methyltransferase 4 can modulate neurovascular coupling in sickle cell disease

Authors: *J. ZACCARELLI MAGALHÃES[1], M. UDO[2], C. T. CITADIN[3], L. H. MATUGUMA[4], J. LANGMAN[1], D. SMITH[1], H. LIN[4];

[1]Univ. of Texas, Houston, TX; [2]Neurol., UT Hlth. at Houston, Houston, TX; [3]Neurol., UTHealth Houston, Houston, TX; [4]Neurol., Univ. of Texas, Houston, TX

Abstract: Background: Sickle cell disease (SCD) is one of the most common recessive inherited blood disorders in African Americans in the United States. SCD is caused by mutations in the β-globin gene that makes hemoglobin to have an atypical shape of a rigid sickle, which compromise their oxygen-carrying capacity. Thus, SCD patients chronically suffer from oxygen deprivation, and have hemoglobin aggregations along the vessel walls causing inflammation and vaso-occlusive crisis. Consequently, a lot of patients, particularly aged females, develop SCD-related vascular dementia, characterized by loss of cerebral blood flow autoregulation and impaired cognitive function related to chronic brain hypoxia. Thus, the aim of this study was to determine the mechanisms of vascular dementia in aged female Townes mice (model of sickle cell disease), particularly the role of protein arginine methyltransferase 4 (PRMT4), an important enzyme for inflammation and neurovascular function that has potential to be a target for new treatments for this disease. **Hypothesis**: Sickle cell mice have deranged PRMT4 signaling pathways that can lead to impaired neurovascular coupling. **Results**: Our preliminary results suggest that 1: brains of Townes mice have enhanced levels of PRMT4, dimethylarginine dimethylaminohydrolase 2 (DDAH2), and asymmetric dimethylarginine (ADMA), but reversed with PRMT4 inhibition. nNOS, eNOS, and iNOS were elevated in Townes mice. 2: Two-photon laser scanning microscopy combined with whisker stimulation showed that Townes mice, presented with neurovascular coupling deficiency, evidenced by a decrease in microvessel diameter and leukocyte rolling velocities (more adhesion and inflammation). 3: This is further supported by the fact that glial fibrillary acidic protein (GFAP) was enhanced in Townes mice. **Conclusions**: SCD-related vascular dementia may be prevalent in SCD and can decrease memory and cognitive function including shortened attention spans, spatial function, and reading. Vascular deficiency also include loss of autoregulation of cerebral blood flow and impaired cognitive function related to chronic hypoxia of the brain. To study PRMT4 signaling pathways may be important for the regulation of neurovascular coupling and neuroinflammation in sickle cell disease. **Acknowledgements**: NIH and NINDS Grand 7RF1NS132291-02.

Disclosures: J. Zaccarelli Magalhães: None. **M. Udo:** None. **C.T. Citadin:** None. **L.H. Matuguma:** None. **J. Langman:** None. **D. Smith:** None. **H. Lin:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.18/B109

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG074552

Title: The role of plasminogen activator inhibitor, PAI-1 in microvascular remodeling in the 5xFAD Alzheimer's disease mouse model

Authors: *A. F. CITALAN-MADRID[1], D. A. LAWRENCE[2], G. G. MURPHY[3], K. STANGIS[4], E. J. SU[5];
[1]Michigan Neurosci. Dept., Univ. of Michigan, Ann Arbor, MI; [2]Intrnl. Med., Univ. of Michigan, Ann Arbor, MI; [3]MNI/MIP, Univ. of Michigan, Ann Arbor, MI; [4]Molec & Behav Neurosci Inst. (MBNI), Univ. of Michigan, Ann Arbor, MI; [5]Intrnl. Med. - Cardiovasc. Div., Univ. of Michigan, Ann Arbor, MI

Abstract: Alzheimer's disease (AD) is the leading cause of dementia in the elderly. The pathophysiology of AD is complex and the precise mechanism behind the onset is unknown. In correspondence with the impaired cognition and the accumulation of beta amyloid (Aβ) plaques and neurofibrillary tangle (NFT) formation, histological observations have demonstrated decreased synaptic function and neuronal loss. Furthermore, there is a relationship between vascular remodeling and impaired cognition, as demonstrated for the reduction in the cerebral blood flow in AD patients. In addition to its role in fibrinolysis, plasminogen activator inhibitor-1 (PAI-1) has been shown to regulate angiogenesis and vascular remodeling. In preliminary studies in the 5xFAD mouse model, we find that inhibition of PAI-1 with a small molecule PAI-1 inactivator reduces aberrant vascular remodeling and increases microvascular density in 5XFAD mice. These data suggest that PAI-1 may play an important role in the development of AD by promoting abnormal vascular remodeling, and that inhibiting PAI-1 may protect against microvascular loss in AD. To test this hypothesis, we quantified the expression level of PAI-1 and analyzed the brain vascular remodeling and Aβ plaques deposition in 5xFAD AD mouse model on the C57BL6Ntac background and corresponding controls at 10 months of age. We found that the 5xFAD mice showed a significant increase in PAI-1 expression in the brain but not in plasma in comparison with mice controls. Next, we analyzed the effects of PAI-1 on the remodeling of the cerebral microvasculature by a high-resolution imaging approach in combination with the SeeDB method for clearing of thick brain sections. Visualization by confocal microscopy of blood vessels labeled with Rhodamine B/lectin showed an apparent reduction of vascular density and vessel diameter and increase in vessel length in the hippocampus of 5xFAD in comparison with mice treated for 6 months with the PAI-1 inhibitor. Interestingly, this was not associated with changes in the accumulation of Aβ plaques in the brain parenchyma. In conclusion, our data suggest that PAI-1 may have an important role in the neurovascular remodeling and its inhibition could contribute to the preservation of brain microvasculature in the context of AD.

Disclosures: A.F. Citalan-Madrid: None. **D.A. Lawrence:** None. **G.G. Murphy:** None. **K. Stangis:** None. **E.J. Su:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.19/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Open Philanthropy grant

Title: Alterations in the gut microbiome are associated with impaired blood-brain barrier integrity in ApoE4 transgenic mice

Authors: *M. ZHANG[1], A. P. SAGARE[1], K. KISLER[1], B. V. ZLOKOVIC[2];
[1]Physiol. and Neurosci., USC, Los Angeles, CA; [2]Physiol. and Neurosci., Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Alterations in the gut microbiome are associated with impaired blood-brain barrier integrity in *ApoE4* transgenic mice
Zhang M, Sagare AP, Kisler K, Zlokovic BV
Department of Physiology and Neuroscience and the Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, USA
Recent studies suggest that brain vascular dysfunction contributes to cognitive impairment and Alzheimer's disease (AD). *APOE4*, a major genetic risk factor for AD, exacerbates blood-brain barrier (BBB) breakdown and degeneration of brain vascular pericytes, dysregulates cerebral blood flow and increases amyloid deposition, thereby affecting, neuronal function. In addition to genetic risk factors, emerging evidence suggests that the gut microbiome is closely related to aging-associated neurodegenerative diseases including AD. However, it remains unclear whether the gut microbiome impacts BBB integrity in an APOE-dependent manner. To addres this question, we investigated the difference in gut bacterial taxa in *ApoE3* and *ApoE4* transgenic mice and its effects on BBB permeability by treating animals with a cocktail of amoxicillin-clavulanic acid antibiotics (ABX) through drinking water for two weeks. We found that the alteration induced by ABX in the gut microbiome restored BBB as shown by reduced accumulation of pericapillary fibrinogen deposits. Further analysis using shotgun metagenomics of fecal pellets from before and after ABX treatment and analyses of inflammatory cytokines in serum, identified disruptions in the balance of three major bacterial families from the dominant phyla Bacteroidetes and Firmicutes. These disruptions may contribute to the compromised integrity of the BBB in *ApoE4* transgenic mice. These preliminary findings suggest that differences in gut microbiome between *ApoE3* and *ApoE4* transgenic mice may influence BBB permeability, and that ABX treatment suppressed the proinflammatory Cyclophilin A-matrix-metalloprotienase-9 BBB-degrading pathway in pericytes which may help restore *APOE4*-mediated BBB disruption in mice after ABX treatment.
This work is supported by the Open Philanthropy grant to B.V.Z. **Theme**: Theme C: Neurodegenerative Disorders and InjuryCategory: C.02. Alzheimer’s disease and other dementiasSection: C.02.f. Vasculature, BBB, and AD

Disclosures: M. Zhang: None. **A.P. Sagare:** None. **K. Kisler:** None. **B.V. Zlokovic:** None.

Poster

PSTR208: Alzheimer’s Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.20/B110

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RO1 AG050049
NIH RF1 AG057842
TRIBA/ Physiology Faculty Startup Fund from Augusta University

Title: Unveiling the vascular role of sodium-glucose cotransporter 2 in cognitive impairments

Authors: A. GREGORY[1], C. TANG[1], S. WANG[2], C. CANTWELL[1], D. BUNN[1], S. SHIN[3], R. J. ROMAN[2], H. YU[3], P. J. O'HERRON[1], ***F. FAN**[1];
[1]Physiol., Med. Col. of Georgia at Augusta Univ., Augusta, GA; [2]Pharmacol., Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Anesthesiol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Recent research has highlighted a robust connection between cerebral vascular dysfunction and Alzheimer's Disease and Alzheimer's Disease-Related Dementias (AD/ADRD). Diabetes mellitus (DM) exacerbates cerebral vascular dysfunction and stands as a primary risk factor for ADRD. In prior studies, we demonstrated that inhibiting sodium-glucose cotransporter 2 (SGLT2) significantly enhanced learning and memory in DM-ADRD rats (T2DN) while also improving myogenic response, cerebral blood flow autoregulation, brain perfusion, and functional hyperemia. These protective effects on both vascular and cognitive functions were linked with increased neuronal cell counts and reduced plasma glucose levels in the DM-ADRD rats without impacting blood pressure. Interestingly, SGLT2 inhibition also bolstered cerebral vascular and cognitive functions in an AD rat model (Tg-F344AD), independently of changes in plasma glucose and blood pressure levels. The current study delves into the potential vascular role of SGLT2 inhibition in AD and DM-associated ADRD. We found that SGLT2 is highly expressed in cerebral vascular cells (VSMCs). It is also expressed in brain microvascular pericytes and endothelial cells but not detected in astrocytes. We also found that SGLT2 inhibition enhances the contractility of primary cerebral VSMCs exposed to high glucose levels, as assessed through a collagen gel-based assay. Moreover, bulk RNA-seq analysis of primary cerebral VSMCs isolated from AD rats elucidates that SGLT2 inhibition reinstates disrupted molecular pathways crucial for VSMC contractility and vascular function. These pathways encompass the regulation of the actin cytoskeleton, oxidative phosphorylation, tight junction, focal adhesion, AGE-RAGE signaling, and ECM-receptor interaction, suggesting that SGLT2 inhibition holds promise as a therapeutic target for interventions targeting vascular dysfunction in AD. Our results provide a compelling rationale for further clinical exploration into the vascular and neuroprotective potential of SGLT2 inhibitors in AD/ADRD.

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Poster

PSTR208: Alzheimer’s Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.21/B111

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Vascular cognitive impairment (VCI) decreases cognitive flexibility in a murine model of Alzheimer's disease

Authors: J. LOPEZ[1], *G. P. BAENA CALDAS[1], J. LI[2], F. C. BARONE[2], H. W. MORENO[2], J. M. LIBIEN[1], A. I. HERNANDEZ[1];
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Abstract: Vascular cognitive impairment (VCI) and Alzheimer's disease (AD) are neurological disorders characterized by progressive neuronal death and cognitive decline. Previously it has been hypothesized that VCI and AD may have a relationship where VCI can lead to AD; thus, the co-occurrence of vascular disease and AD is the rule (not the exception) in the context of cognitive impairment and dementia, conceptualizing VCI as an important component of AD. Therefore, we prepare a murine model using the partial occlusion of the left common carotid artery (LCCA) as a VCI model on transgenic-AD (APP/PS1) mice compared with WT animals. These mice were subjected to cognition tasks: novel object recognition (NOR) on days 33-36 and active place avoidance (APA) on days 37 and 38, and its conflict (APAc) on day 39 after the surgery. We found a trend but no statistical difference in the NOR task associated with phenotype or surgery. Whereas APA shows significant inter-trial and intra-trial differences associated with phenotype, the APAc task shows a statistically significant synergistic effect of phenotype and surgery, suggesting that hypoperfusion exacerbates cognitive problems in APP/PS1 mice. Since a decrease in performance in the APAc task has been associated with decreased cognitive flexibility and neurogenesis impairment in the dentate gyrus, we will now be comparing the biogenesis of ribosomes and neurogenesis across the groups. For that, we will perform immunohistochemistry and confocal microscopy to determine whether we find differences amongst groups.

Disclosures: J. Lopez: None. **G.P. Baena Caldas:** None. **J. Li:** None. **F.C. Barone:** None. **H.W. Moreno:** None. **J.M. Libien:** None. **A.I. Hernandez:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.22/B112

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SBIR Grant R44MH119989

Title: Brain-wide parenchymal quantification of antibody therapeutics crossing the BBB

Authors: C. REDD[1], N. GUANZON[1], Y. GALLEGOS[2], C. S. JENSEN[3], S. B. VERGO[4], J. G. ZEITOUN[2], A. REKSOATMODJO[2], P. CHEUNG[5], ***S. GANDHI**[6], D. G. WHEELER[7]; [1]Translucence Biosystems, Irvine, CA; [2]Translucence Biosystems, Inc., Irvine, CA; [3]Merck, Copenhagen, Denmark; [4]Biotherapeutic Discovery, H. Lundbeck A/S, Valby, Denmark; [5]Translucence BioSystems, SAN DIEGO, CA; [6]Univ. of California, Irvine, CA; [7]Translucence Biosystems Inc, Irvine, CA

Abstract: To look deep inside tissues, traditional histological methods cut specimens into thin slices. Providing access to the intricate anatomy of intact organs, tissue clearing offers neuroscientists unbiased and complete views of brain anatomy and function. One area where these methods have particular utility is in the development of CNS therapeutics where they can be used to examine the regional distribution of the therapeutics in the brain as well as brain-wide target engagement and phenotypic efficacy. We have developed a pipeline that provides unbiased and complete cellular resolution measurements of brain-wide therapeutic biodistribution in pre-clinical rodent brains. With our optimized iDISCO-based clearing method and our Mesoscale Imaging System for ZEISS Lightsheet microscopes, we can image cellular-resolution immunoreactivity across entire mouse brains in <20 min. Our AI-powered whole-brain object segmentation, and quantification software, the Translucence Teravoxel Toolkit (3TK) and new statistical methods for anatomics, produce regionalized read-outs of micron-scale immunoreactivity across 100's of brain areas. Here, we examined whether our technology can detect antibody therapeutics crossing the blood-brain barrier (BBB). To do this, we took advantage of a bispecific antibody engineered to bind to the Alzheimer's Disease target, BACE1, as well as the transferrin receptor (TfR1), which helps shuttle the antibody across the brain endothelium and into the brain parenchyma. We demonstrated that our methods can detect IV-dosed BACE1/TfR1 bispecific antibody throughout the brain. In mice dosed with a monospecific control antibody that does not bind TfR1, immunoreactivity was at background levels, similar to that seen in mice not dosed with any antibody. The bispecific therapeutic antibody was detected in the parenchyma and enriched in brain regions with high BACE1 expression, indicating that the antibody crosses the BBB and engages the target. However, there are high levels of bispecific antibody bound to TfR1 in the vasculature where is does not have access to drug targets. To quantify the effective parenchymal levels of the bispecific antibody, we have extended out AI-powered quantification pipeline to segment the staining in the vasculature and measure only the therapeutically relevant bispecific antibody signal in the brain parenchyma. These data provide a clear demonstration of the utility of tissue clearing methods for quantitative brain-wide monitoring of Alzheimer's Disease therapeutic antibody biodistribution.

Disclosures: C. Redd: A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **N. Guanzon:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **Y. Gallegos:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc.. **C.S. Jensen:** None. **S.B. Vergo:** A. Employment/Salary (full or part-time):; H. Lundbeck A/S. **J.G. Zeitoun:** None. **A. Reksoatmodjo:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **P. Cheung:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **S. Gandhi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc. **D.G. Wheeler:** A. Employment/Salary (full or part-time):; Translucence Biosystems, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems, Inc..

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.23/B113

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Tulane Brain Institute Pilot Award (Engler-Chiurazzi and Zwezdaryk)
IDSA Pilot Grant (Zwezdaryk)
NIA R01 AP1639G1 (Zwezdaryk)

Title: Increased blood bran barrier permeability in intermittent MCMV infected mice.

Authors: *S. R. WROBLEWSKI[1], S. L. MORRIS[2], H. WANG[4], E. B. ENGLER-CHIURAZZI[5], K. J. ZWEZDARYK[3];
[1]Dept. of Microbiology & Immunol., Tulane Univ. Neurosci. PhD Program, New Orleans, LA; [3]Dept. of Microbiology & Immunol., [2]Tulane Univ. Sch. of Med., New Orleans, LA; [5]Neurosurg., [4]Tulane Univ., New Orleans, LA

Abstract: Viruses are a potential driver of Alzheimer's Disease (AD) etiology, as some viruses are found at higher levels in AD patients versus age matched non-AD patients. However, data supporting the association between AD and viruses is correlative. We explored the role of cytomegalovirus (CMV) in AD, because epidemiological data shows that CMV increases the risk of dementias, including AD. Our previous work demonstrated that intermittent murine (MCMV) infection over 1-year significantly reduced cognitive function using a Y-maze assay. This correlated with increased host metabolic stress. In the blood brain barrier, we measured altered mitochondrial function, including increased reactive oxygen species using Seahorse and flow cytometry assays. Alterations in blood brain barrier permeability and cellular activation were observed using immunofluorescence. Interestingly, changes were not uniform throughout the brain. The hippocampus displayed an increased neuroinflammatory state compared to the striatum. Significant increases in GFAP and Iba-1 were observed in the hippocampus of MCMV infected mice at 12-months post initial infection. The permeability markers claudin-5, fibrinogen and IgG were also significantly altered in MCMV infected mice, but only in the hippocampus

and not in the striatum. Using spatial transcriptomics we are mapping the location of differential effects and defining altered regulatory mechanisms. Together, this data suggests that intermittent MCMV infection contributes to differential regulation of blood brain barrier permeability and associated cell activation.

Disclosures: S.R. Wroblewski: None. **S.L. Morris:** None. **H. Wang:** None. **E.B. Engler-Chiurazzi:** None. **K.J. Zwezdaryk:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.24/B114

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R33NS124970
R61NS114353
NIH/NCATS UL1TR002003

Title: Identifying compounds that modulate inflammation-induced permeability disruption in APOE4-brain endothelial cells

Authors: *A. VALENCIA-OLVERA[1], K. RATIA[2], D. BALU[1], F. M. MAROTTOLI[1], G. THATCHER[3], L. M. TAI[1];
[2]Pharmaceut. Sci., [1]Univ. of Illinois, Chicago, Chicago, IL; [3]Univ. of Arizona, Tucson, AZ

Abstract: Compared to *APOE3*, *APOE4* is associated with greater cognitive dysfunction in older adults, increases Alzheimer's disease risk, and exacerbates the progression of vascular dementia, stroke, and traumatic brain injury. Evidence supports that blood-brain barrier (BBB) dysfunction, including leakiness, is greater with *APOE4* and could contribute to neuronal dysfunction in all these conditions. Specialized brain endothelial cells (BEC) are central to all the complex functions of the BBB. Our previous *in vitro* and *in vivo* data demonstrate that the *APOE* genotype of BECs is important for BBB function. We found that *APOE4*-BECs have a unique basal phenotype that renders them more vulnerable to barrier dysfunction with inflammatory stimuli compared to *APOE3*-BECs. Peripheral inflammation has been linked to neurodegeneration and *APOE4*, and therefore, due to their unique location, *APOE4*-BECs may be particularly susceptible to dysfunction in neurodegenerative disorders. Thus, we explored whether our *in vitro* assay could be adapted as a high-content screen to identify novel compounds that mitigate inflammation-induced permeability disruption to *APOE4*-BECs. Primary BEC cultures were treated with lipopolysaccharide (LPS) to increase permeability as assessed by trans-endothelial electrical resistance (TEER). First, we identified a dose of LPS and frequency for permeability assessment that gave an optimal assay range. LPS caused a dose-dependent lowering of TEER with no impact on capacitance (an indication of cell viability) and 1000Hz gave the greatest assay range. LPS (0.8 µg/ml) reduced TEER values by ~ 40% with no

edge or drift across the plates. After validating the phenotypic screen, we performed a pilot screening campaign with a 900+ compound library that included primarily kinase inhibitors as well as compounds that we predicted would target the *APOE4*-BEC phenotype. We identified two main groups of active compounds: those that decrease TEER without affecting BEC viability and those that protect against LPS-induced TEER reduction. Most of the hits on the first group inhibited mTOR, and we found that mTOR activators could protect against LPS-induced TEER reduction. Additional active compounds could be grouped into those that inhibit growth factor receptor signaling (e.g. VEGFR, EGFR), cell cycle re-entry (e.g. aurora B, CDK4/6), cytoskeleton remodeling (e.g. focal adhesion kinase) as well as specific signaling cascades (e.g. PDE, JAK/STAT, PKD, CaMKII). Overall, our data support the potential of our *in vitro* screen to identify compounds to prevent *APOE4*-associated BEC dysfunction in response to inflammation and other stressors.

Disclosures: A. Valencia-Olvera: None. **K. Ratia:** None. **D. Balu:** None. **F.M. Marottoli:** None. **G. Thatcher:** None. **L.M. Tai:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.25/B115

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG061114
R33NS124970
R01MH086507

Title: Endothelial APOE4 Protects Neural Function in vivo

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Abstract: *APOE4* has been empirically linked to poorer neurovascular health in aging and in neurodegenerative disorders when directly compared to *APOE3* and *APOE4* is overwhelmingly designated a toxic gain of function. Therefore, current therapeutic strategies aim to reduce the levels or physiological activity of apoE4. However, an alternative explanation is that *APOE4* is beneficial for neural function, but less so than *APOE3*, i.e. a loss of positive function. Furthermore, *APOE4* could represent a toxic gain of function or a loss of positive function depending on the cell type and specific functions or pathways within each cell type. The neurovasculature comprises multiple cell types, but it is the specialized brain endothelial cells that are indispensable for maintaining the homeostatic barrier between the central and peripheral

environments. Previous studies have identified that *APOE*-modulated pathways in the brain and periphery can impact brain endothelial cell function. Meanwhile, data on the contribution of endothelial *APOE* to neurovascular function is scarce and remains controversial. We recently found that loss of endothelial cell *APOE3* disrupts neurovascular and synaptic function. Yet, whether endothelial *APOE4* is detrimental or protective for neural function under physiological conditions is unknown. Therefore, the goal of this study was to determine the role of endothelial cell *APOE4* in regulating brain function *in vivo*. To this end, we developed *APOE4*$^{fl/fl}$/Cdh5(PAC)-CreERT2$^{+/-}$ and *APOE4*$^{fl/fl}$/Cdh5(PAC)-CreERT2$^{-/-}$ (control) mice and induced knockdown of endothelial cell *APOE4* at ~4-5 weeks of age. Experiments were conducted at 9 months of age to evaluate neurovascular and neuronal function via biochemistry, immunohistochemistry, behavior tests, and electrophysiology. Endothelial cell *APOE4* knockdown resulted in higher neurovascular permeability, lower claudin-5 vessel coverage, impaired trace fear memory extinction, and dysregulation of cortical excitatory-inhibitory balance of synaptic activity. Our data support the novel concept that endothelial cell *APOE4* is protective for neurovascular and neuronal function.

Disclosures: F.M. Marottoli: None. **D. Balu:** None. **E. Flores-Barrera:** None. **E. Artur De La Villarmois:** None. **K. Tseng:** None. **L.M. Tai:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.26/B116

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Understanding the Blood-Brain Barrier Breakdown and Vascular Dysfunction in New AD Mouse Models

Authors: *Y. CHIU;
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Abstract: Background: Alzheimer's disease (AD), the leading cause of dementia accounting for 70% of cases, involves complex pathogenesis with amyloid, tau, and cerebrovascular dysfunction contributing significantly. Vascular changes and impairment are strongly associated with AD pathogenesis in new knock-in models from the MODEL-AD consortium - early-onset AD (EOAD) and late-onset AD (LOAD) mice - which can be influenced by spatial transcriptomic genetic factors.Method: We will thoroughly characterize vascular dysfunction in these models over time: 3, 6, 9 months for EOAD mice; 4, 8, 12 months for LOAD mice. Age-matched C57BL/6J mice will serve as controls. Additionally, we will perform behavioral tests e.g., novel object recognition (learning and memory function) and other social behavioral tests. Lastly, we will apply cutting-edge spatial transcriptomics with subcellular resolution (Stereo-seq) to one EOAD and one LOAD model to uncover molecular changes underlying neurovascular dysfunction. We will integrate the obtained data with existing resources.Result:

The pathological phenotypes of the new AD mouse models are expected to express microglial responses that are positively correlated with amyloid beta aggregation, leading to tau pathology. Vasculature is also expected to be impaired. The distribution of the phenotypes tends to be in cortical and hippocampal regions. Conclusion: Our outcomes will provide an enhanced understanding of blood-brain barrier (BBB) breakdown and vascular contributions in physiological AD models, guiding future mechanistic and therapeutic studies of vascular-directed strategies to mitigate neurodegeneration.

Disclosures: Y. Chiu: None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.27/B117

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS108763
NIH grant NS127392
NIH grant NS106592
Taiwan grant MOST 111-2320-B-110-003-MY2

Title: Unraveling the Pathophysiological Mechanisms of Hypertension-Induced Cognitive Impairment: Insights from a Mouse Model of Chronic Hypoperfusion

Authors: *Y.-Y. SUN[1], Y.-M. KUO[2], H.-R. CHEN[3], C.-Y. KUAN[4];
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Abstract: Hypertension is a significant contributor to cerebral small vessel disease (cSVD), a primary cause of vascular dementia, yet the precise pathophysiological mechanisms behind hypertensive-induced cognitive impairment remain elusive. Here, we employed a mouse model of chronic hypoperfusion, inducing unilateral common carotid artery occlusion (UCCAO) for 14 days in transgenic renin overexpression mice (RenTg). Our investigation revealed a hypertensive phenotype in RenTg mice as early as one month of age, marked by elevated plasma Angiotensin II levels. While cerebral vascular density reduction was evident only in 8-month-old RenTg mice, diminished cerebrovascular function was observed across age groups, particularly in response to cerebral blood flow (CBF) regulation challenges. Upon UCCAO insult, RenTg mice displayed reduced CBF within one week, leading to motor and cognitive deficits indicative of hypoperfusion-induced cSVD. Histological analysis unveiled pathological alterations in RenTg brains, including diminished expression of extracellular matrix proteins, microvessel abnormalities, thrombosis, hemorrhaging, and compromised blood-brain barrier integrity. Collectively, our study underscores the deleterious impact of chronic hypertension and

hypoperfusion in RenTg mice, manifesting as cerebrovascular dysfunction, neurological impairments, and extracellular matrix abnormalities. These findings illuminate potential mechanisms underpinning hypertension-related cerebrovascular pathology.

Disclosures: Y. Sun: None. **Y. Kuo:** None. **H. Chen:** None. **C. Kuan:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.28/B118

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Functional characterization of a blood-brain barrier model using human ipsc-derived cells

Authors: R. VAIDYANATHAN[1], M. CASCO[2], R. FIENE[1], S. HILCOVE[1], *E. JONES[1], C. B. CARLSON[1];
[1]FUJIFILM Cell. Dynamics, Madison, WI; [2]Visikol, Inc., Hampton, NJ

Abstract: Objective: The blood-brain barrier (BBB) is a specialized network of cells that function to maintain a tightly controlled microenvironment around the brain. Modeling the BBB in vitro is needed to evaluate barrier function, test drug permeability, and study the diseases that affect it. Induced pluripotent stem cell (iPSC) technology is a powerful tool to generate the cells that compose the BBB and establish such a model. Methods: FUJIFILM Cellular Dynamics, Inc. developed three different iPSC-derived cell types from the same human donor needed to create an in vitro BBB model, including astrocytes, brain microvascular endothelial cells (BMEC), and pericytes. In collaboration Visikol, the BBB model was functionally tested for barrier formation using trans-endothelial electrical resistance (TEER), drug permeability analyzed by LC-MS/MS (with control compounds caffeine (positive) and Atenolol (negative) in triplicate, receptor mediated transcytosis (using fluorescently-tagged transferrin protein), and P-glycoprotein (Pgp) assay to measure the effect of P-gp inhibitors (e.g., Cyclosporine and Elacridar) on the efflux ratio of substrates such as Digoxin and Loperamide. Results: The iPSC-derived iCell BMEC have distinctive cellular structures (tightly packed, cobblestone morphology, proper organization of tight junctions), appropriate marker expression (transporters: GLUT1, CD98hc and efflux/influx proteins: BCRP, P-gp, MRP1, transferrin receptor), and functional assay performance (effective barrier formation, low permeability). iPSC-derived pericytes show a characteristic stellate morphology, appropriate marker expression, and demonstrate phagocytosis function. When all three cell types are co-cultured in the optimized media from FCDI, high TEER signal (>1500 ohms) is observed in assays using transwell inserts. Permeability assays showed that Caffeine readily crossed the BBB but Atenolol did not. Fluorescently-tagged transferrin could cross the BBB and it was inhibited by Ferrostatin II, a compound that prevents the transferrin protein from binding to its receptor. Finally, compelling evidence was generated to illustrate that the efflux ratio of Digoxin and Loperamide was significantly affected by inhibiting the function of P-gp. Conclusion: FCDI succeeded in

establishing an isogenic, fully human iPSC-derived BBB model, manufacturing a consistent supply of cells at-scale, and cryopreserving the material for subsequent on-demand use. The functional characterization together with Visikol also provides confidence in the reliability and usefulness of this BBB model as an in vitro system for preclinical compound testing.

Disclosures: R. Vaidyanathan: A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics. **M. Casco:** A. Employment/Salary (full or part-time):; Visikol, Inc. **R. Fiene:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics. **S. Hilcove:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics. **E. Jones:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics. **C.B. Carlson:** A. Employment/Salary (full or part-time):; Fujifilm Cellular Dynamics.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.29/B119

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AMED JP19dm0207073

Title: The role of cerebrovasculature as the reservoir of seed-competent amyloid beta species

Authors: *T. HASHIMOTO[1], H. UCHIGAMI[2], M. KASHIWAGI-HAKOZAKI[3], S. MURAYAMA[4], Y. SAITO[5], T. TODA[6], T. IWATSUBO[7];
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Abstract: Massive deposition of amyloid beta peptide (Abeta) as senile plaques (SPs) in the brain parenchyma or as cerebral amyloid angiopathy (CAA) around blood vessels or within the leptomeninges is a pathological hallmark of Alzheimer's disease (AD). Despite extensive research, the precise role of SPs and CAA in the pathogenesis of AD remains unclear. Our recent findings have shed light on a specific form of TBS-soluble Abeta oligomers with more than 100 kDa (hereafter referred to as peak 1 Abeta), which we have identified in the brains of both AD patients and Abeta precursor protein transgenic (APP tg) mice. The injection of peak 1 Abeta into the hippocampus of young APP tg mice induced Abeta plaques and CAA in the brain, indicating a seeding role for peak 1 Abeta in beta-amyloidosis. To elucidate the pathological significance of peak 1 Abeta in AD brains, we first examined whether CAA affects the level of peak 1 Abeta. We compared the levels of peak 1 Abeta or insoluble Abeta between the brains of AD patients with abundant CAA (CAA-rich, n=10) and those with minimal CAA (CAA-

minimal, n=8). We found significantly higher levels of peak 1 Abeta in the CAA-rich brains compared to those in the CAA-minimal brains. Conversely, the levels of insoluble Abeta remained consistent across both groups. Furthermore, the comparison of peak 1 Abeta levels in meningeal tissues between CAA-rich and CAA-minimal brains also revealed significantly higher levels in CAA-rich meningeal tissues. Injection of meninges-derived peak 1 Abeta from CAA-rich brains into the hippocampus of young APP tg mice induced Abeta deposits after 4 months, suggesting that meninges-derived peak 1 Abeta acts as a "seed" for beta-amyloidosis. Secondly, we investigated the impact of the APOE ipsilon4 allele, which is known to correlate with increased CAA prevalence in AD patients, on the level of peak 1 Abeta. We found a notably higher level of peak 1 Abeta in the brains of AD patients with APOE ipsilon4/ipsilon4 genotype (n=12) compared to those with APOE ipsilon3/ipsilon3 genotype (n=10), suggesting that apoE may exert isoform-dependent effects on the level of peak 1 Abeta. These results highlight that seed-competent peak 1 Abeta was abundantly present in the cerebrovasculature of AD patients compared to the brain parenchyma, potentially serving as a reservoir for peak 1 Abeta. Further biochemical investigations focusing on meninges-derived peak 1 Abeta may provide crucial insights into the mechanisms underlying the formation of CAA.

Disclosures: T. Hashimoto: None. **H. Uchigami:** None. **M. Kashiwagi-Hakozaki:** None. **S. Murayama:** None. **Y. Saito:** None. **T. Toda:** None. **T. Iwatsubo:** None.

Poster

PSTR208: Alzheimer's Disease: Vasculature

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR208.30/B120

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 2021YFA0804900
32100796

Title: Elevated vascular BACE1 causes vascular dysfunction and white matter lesions in a mouse model

Authors: *D. BI;
Univ. of Sci. and Technol. of China, Anhui, China

Abstract: Cerebral amyloid angiopathy (CAA) is an age-associated condition characterized by the deposition of β-amyloid (Aβ) in the vascular wall of small- and medium-sized cerebral arteries. The protein levels of β-site APP-cleaving enzyme 1 (BACE1), the key enzyme for Aβ production, are significantly elevated in the cerebral vessels of CAA patients. In addition to vascular amyloid deposition, CAA patients also exhibit typical clinical symptoms including white matter hyperintensities and cerebral hypoperfusion, both of which are related to the impairment of vascular function. Growing evidence shows that brain vascular alteration occurs before Aβ deposition and may contribute to the initial stages of disease. Therefore, we proposed

that the elevated vascular BACE1 may directly contribute to vascular dysfunction and dementia independent of Aβ. To examine this hypothesis, we generated a mouse model overexpressing human BACE1 in vascular smooth muscle cells (smN-BACE1 mice) and found that, smN-BACE1 mice showed (1) over-excited pyramidal neuron activity in the prefrontal cortex (PFC) and impaired working memory; (2) gradual white matter myelin loss; and (3) cerebral hypoperfusion and arterial dysfunction. Our data suggest that vascular BACE1 elevation can directly lead to vascular dysfunction and white matter lesions similar to the symptoms observed in CAA patients, which eventually causes memory deficits in the smN-BACE1 mice. This research emphasizes the pathological role of BACE1 on cerebral vasculature, in addition to an enzyme in Aβ generation.

Disclosures: D. Bi: None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.01/B121

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant PJT- 173540
A.P.S is supported by the Krembil-Rossy Chair program

Title: Exploring APOE4's role on cognitive impairment in Parkinson's disease: Insights from structural MRI and sex differences in the PPMI cohort

Authors: *A. E. ROSAL[1,2], S. L. MARTIN[1], A. GRAFF-GUERRERO[3,2], A. P. STRAFELLA[1,2,4];
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Abstract: Cognitive impairment is a prevalent non-motor symptom of Parkinson's Disease (PD), yet its development in PD individuals remains poorly understood. Previous studies have linked *Apolipoprotein E4 (APOE4)*, a genetic risk factor of Alzheimer's Disease, with PD-related cognitive impairment. However, *APOE4's* role in this aspect of PD is still unclear due to conflicting findings. Reduced gray matter volume (GMV) is observed in both PD individuals with cognitive impairment and *APOE4* carriers with cognitive deficits. However, no study has explored the relationship between *APOE4* status, GMV changes, and cognitive function all together in a PD cohort. This study aims to examine *APOE4's* role on cognitive impairment in PD by analyzing its impact on GMV using data from the Parkinson's Progression Markers Initiative (PPMI). T1-weighted MRI images of 52 PD *APOE4* carriers (mean age = 58.9 ± 9.2

years ; 16 females, 36 males) and 123 PD *APOE4* non-carriers (mean age = 63.1 ± 9.1 years ; 48 females, 75 males) underwent Voxel-Based Morphometry (VBM) using the Computational Anatomy Toolbox in SPM12 to quantify GMVs in Region of Interests (ROIs) associated with cognition and/or are affected in PD *APOE4* carriers. This included the anterior/posterior cingulate, middle temporal/frontal gyrus, hippocampus, precuneus, caudate nucleus, and insula. VBM analyses in SPM12 revealed no GMV differences in ROIs between carriers and non-carriers. Linear regression models in RStudio then showed no significant associations between GMV in ROIs and cognition (measured by Montreal Cognitive Assessment scores) based on *APOE4* status. Both analyses used age, sex, and total intercranial volume (TIV) as co-variates. Given previous reports of worser cognitive decline in female *APOE4* carriers than males, sex differences were also considered. ANCOVA models in RStudio, adjusted for age and TIV, revealed no GMV differences between sexes and not influenced by *APOE4* status, as no significant *APOE4*-sex interactions were found. These preliminary results suggest that in PD, *APOE4* may not play a role in GMV changes of specific ROIs nor cognitive function, and that other factors may influence GMV alterations. However, further structural MRI analyses is needed to clarify these controversial findings. Future work of this study will explore *APOE4*'s role on other structural brain changes using MRI data, including cortical thickness, that may be linked to PD-related cognitive impairment.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.02/B122

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Society UK PhD studentship 521 (AS-PhD-19A-007)

Title: Increased APOE abundance in Down syndrome-Alzheimer's disease compared with early onset-Alzheimer's disease is associated with non-neuronal cells and elevated APP and C-terminal fragment abundance

Authors: *C. FARRELL[1], Y. BUHIDMA[2], W. HEYWOOD[3], J. HÄLLQVIST[3], S. TASO[1], C. SALA FRIGERIO[1], T. LASHLEY[2], K. MILLS[3], C. TOOMEY[2], F. K. WISEMAN[4];
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Abstract: Trisomy of chromosome 21 (Hsa21), the cause of Down syndrome (DS), is the most commonly occurring genetic cause of Alzheimer's disease (AD), caused by triplication of the amyloid precursor protein (APP). Although three-copies of *APP* are sufficient and necessary to cause Down syndrome-Alzheimer's disease (DSAD), we have shown that other triplicated genes on Hsa21 modify DSAD compared to AD in the general population. How the proteome of the brain in DSAD differs to AD in the general population is unknown but is relevant for considering AD therapeutic strategies for people with DS. Here, we used label-free mass spectrometry to compare the proteome of frontal cortex tissue from people who had DSAD with demographically matched (age at death, sex, Braak stage, *APOE* genotype) cases of early-onset AD and healthy ageing controls from the general population. The abundance of several Hsa21-encoded proteins, including APP, CSTB and PDXK, are increased 1.5-fold in DSAD compared to both comparative groups. Moreover, wider dysregulation of the proteome occurs beyond proteins encoded by Hsa21, with over 300 proteins having differential abundances between case types. The altered non-Hsa21-encoded proteins includes an increase in the abundance of the key AD-associated protein apolipoprotein E (APOE), in people with DSAD compared to matched cases of early-onset AD, which occurs independently of the individuals *APOE* genotype. To understand the cell-types which may contribute to these changes in protein abundance we undertook single-nuclei RNA-sequencing on the same cases. This demonstrated that trisomy of Hsa21 altered transcription of Hsa21-encoded proteins in a wide range of cell-types. Moreover, *APOE* expression was raised in a subtype of astrocytes, endothelial cells and pericytes. We further found that the abundance of APOE in our case series correlates with the abundance of full-length APP and APP-C-terminal-fragment-α levels, but not with amyloid-β, the key constituent of AD plaque pathology. These data highlight that trisomy 21 alters both the transcriptome and proteome of people who have DSAD and that these differences compared to AD in the general population should be considered when selecting therapeutic strategies for this important group of individuals who are at high risk of early-onset dementia.

Disclosures: C. Farrell: None. **Y. Buhidma:** None. **W. Heywood:** None. **J. Hällqvist:** None. **S. Taso:** None. **C. Sala Frigerio:** None. **T. Lashley:** None. **K. Mills:** None. **C. Toomey:** None. **F.K. Wiseman:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.03/B123

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG063175
NIH Grant R01AG081374

Title: Apoe4 promotes lipid droplet accumulation and impairs dendritic arborization in human neurons

Authors: *K. A. STEPHENSON[1], H. ZHANG[1], W. G. WOOD[1], S. ZHANG[1], X. ZHAO[1], A. KOZLOVA[1], Z.-P. PANG[2], A. SANDERS[1], J. DUAN[1];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive decline and memory loss, being the leading cause of dementia globally. A significant genetic risk factor for late-onset AD (LOAD) is the apolipoprotein E (APOE) 4 allele. Despite extensive research on APOE, particularly its impact on amyloid-beta (Aβ) peptides and Tau pathology, the mechanism through which APOE4 influences AD progression remains elusive, hindering effective treatment. There's a pressing need to shift paradigms in understanding the disease mechanisms related to APOE4 and other major LOAD risk factors. The role of lipid droplets (LD) accumulation in glial cells is increasingly recognized in AD progression, yet the implications for neuronal LD remain underexplored. This study examines how APOE allelic variations affect LD accumulation in excitatory neurons (iNs) derived from human induced pluripotent stem cells (hiPSCs), with or without astrocyte co-culture, and in hiPSC-derived cortical organoids. Initially, LD formation in iNs was compared across different APOE alleles (APOE2, 3, or 4, each with two donor iPSC lines). Robust LD accumulation was observed in day-15 iNs, with APOE2 showing the highest LDs, followed by APOE3 and APOE4 lines. Notably, co-culture with astrocytes led to disappearance of LDs in APOE2 and APOE3 lines but persistence in APOE4 lines, suggesting a detrimental effect of neuronal APOE4 on LD accumulation. Further analysis examined LD accumulation and neuron branching in day-27 mature iNs with the APOE4 allele, revealing increased LD volume and decreased neurite length and branches compared to APOE3 iNs. CRISPR-Cas9-CBE editing induced a premature stop codon in the APOE4 gene, resulting in APOE4-iSTOP, which mirrored APOE3 genotype traits, indicating a potential therapeutic avenue. Comparative analysis of iNs of all three genotypes (APOE3, APOE4, and APOE4-iSTOP) with and without LDs revealed that LD presence correlated with reduced dendritic branching, implying a potential causal link between LD and abnormal neuron dendritic arborization. Further, transcriptomic analyses of iNs with APOE3, APOE4, or APOE4-iSTOP unveiled differentially expressed genes supporting observed LD accumulation and reduced dendritic branching in APOE4 iNs. These findings highlight the influence of APOE alleles on neuronal LD dynamics and advocate for further investigation into LD and cellular lipid metabolism in 3D cortical organoid models, potentially shedding light on AD pathogenesis.

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Poster

PSTR209: Alzheimer’s Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.04/B124

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG058081
NIH R01 AG081426
University of Minnesota Office of Academic Clinical Affairs (FRD #19.29)

Title: Targeting APOE4-associated dysfunction with a clinically tested HDL mimetic peptide in human induced pluripotent stem cell (iPSC)-derived models

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Abstract: APOE is the greatest genetic risk factor for developing late onset Alzheimer's disease (AD). In humans, APOE has three isoforms- APOE2 (E2), APOE3 (E3) and APOE4 (E4); E4 increases AD risk, while E3 is neutral and E2 decreases risk. In the brain, APOE is mainly produced and secreted by astrocytes, where it binds lipids to form HDL-like particles. In addition to maintaining lipid homeostasis, APOE plays a central role in other AD-related processes including Aβ aggregation and clearance and neuroimmune modulation. The lipidation state of APOE is crucial for its proper function. E4 is poorly lipidated compared to E2 and E3. Mounting evidence suggests that aberrant E4-associated lipid metabolism underlies its pathogenic impact on AD. Therefore, emerging therapeutic strategies for AD include enhancing E4 lipidation and ameliorating E4-related dysfunction. Previous studies in our lab have shown that the small clinically tested HDL-mimetic peptide 4F increases APOE secretion and lipidation in primary mouse and human astrocytes. 4F also rescues the inhibitory effect of Aβ42 on APOE secretion and lipidation. In the current studies, we sought to determine the ability of 4F to rescue E4-associated dysfunction using human induced pluripotent stem cells (iPSCs). Isogenic E2, E3 and E4 iPSCs were differentiated into astrocytes and subjected to 4F treatment with or without aggregated Aβ42. The results show that 4F enhances APOE secretion and lipidation in iPSC-derived astrocytes in the presence and absence of aggregated Aβ42. Furthermore, E4 astrocytes are more susceptible to the inhibitory effect of Aβ42 on APOE secretion and lipidation, and 4F ameliorates this effect. To assess the functional outcomes of 4F treatment on lipid homeostasis, cholesterol efflux was measured using a BODIPY-cholesterol assay. While E4 astrocytes efflux cholesterol less efficiently than E3 astrocytes, 4F promotes cholesterol efflux and rescues the deficit in E4 astrocytes. In addition, E4 astrocytes contain abundant lipid droplets compared to E2 and E3 astrocytes, particularly upon exposure to fatty acids. Importantly, 4F reduces lipid droplet accumulation in E4 astrocytes to similar levels as in E2 and E3 astrocytes. Transcriptomic and lipidomic analyses are underway to further elucidate the molecular mechanisms contributing to the beneficial effects of 4F in iPSC derived astrocytes. These results highlight the therapeutic potential of HDL-mimetic peptides for E4-associated dysfunction and sporadic Alzheimer's disease.

Disclosures: K. Fredriksen: None. **S. Joshi:** None. **A. Chang:** None. **L. Li:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.05/B125

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH National Institute on Aging
EPAAD ApoE Pathobiology in Aging and Alzheimer's disease
BrightFocus Foundation

Title: Dysregulation of APOE4-enriched matrisome signatures in human iPSC-derived mixed cortical culture proteomes

Authors: *L. QIAN[1], A. PELLETIER[2], T. TCW[2], J. TCW[2];
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Abstract: Apolipoprotein E (APOE) is primarily expressed by astrocytes in the brain to facilitate cholesterol and phospholipid transport to neural cells expressing receptors such as LDLR and LR8/LRP2. Polymorphism of APOE allele ε4 (APOE4) is the most significant genetic risk factor in Alzheimer's disease (AD), but the specific mechanism underlying APOE4-induced AD phenotypes remains unclear. To interrogate APOE4 impact on human brain cells, we collected human induced pluripotent stem cell (hiPSC) lines from Caucasian subjects (N=6 for APOE 44, N=6 for APOE 33) at age > 65 years, balanced for gender, disease status (CDR), and genetic risk scores. The reprogrammed iPSCs were differentiated mixed cortical cultures (MCC) comprising primarily excitatory neurons and ~20% glia. The derived MCC were subjected to LC-MS/MS proteomics/phospho-proteomics followed by DESeq2, WGCNA and fGSEA pathway analyses (FDR < 0.1). Network co-expression and pathway analyses uncovered significant upregulation of matrisome signatures (extracellular matrix organization) in APOE 44 vs. 33 MCC, confirming matrisome as the primarily altered module driven by APOE4 in AD human brains in both transcriptomes and proteomes. Other proteomic changes in APOE 44 MCC included upregulation of actin binding and downregulation of chromatin organization and RNA splicesome, implicating aberrant cytoskeletal rearrangements, exacerbated DNA damage, and defected alternative splicing that might contribute to neurodegeneration in AD. Co-staining of neuronal MAP2/TUJ1 confirmed cytoskeletal abnormality, revealing significant neuronal loss, disorganized MAP2 structures, compromised synaptic networks, and shortened neurite projections in APOE4. Granule formation by G3BP1 aggregation also increased, suggesting APOE4-induced cellular stresses and mRNA cytosolic sequestration. Together, these results evidenced matrisome as a significant signature for AD therapeutics and unraveled APOE4-associated compromised synaptic development and aberrant RNA splicing for neurodegeneration.

Disclosures: L. Qian: None. **A. Pelletier:** None. **T. Tcw:** None. **J. Tcw:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.06/B126

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: In vitro model of Alzheimer's disease based on neurons and astrocytes generated from iPSCs derived from Alzheimer's disease patients with APOE4 genetic polymorphisms

Authors: *R. TANAKA[1], R. YAMOTO[2], T. SAMESHIMA[3], Y. CHEHREGHANIANZABI[4], T. HOSOYA[5];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia. Since no animal models fully recapitulate the AD phenotype and drug responses, and it is hoped that useful models will be developed that contribute to drug discovery research. The major pathological phenotypes of AD are extracellular accumulation of amyloid plaques and intracellular neurofibrillary tangles of phosphorylated Tau. *In vitro* modeling based on iPSC-derived cells are a highly valuable in the investigation of the AD pathophysiology, since accumulation of Aβ and/or phosphorylated Tau has been observed in neurons generated from iPSCs derived from AD patients. The APOE4 gene polymorphism is well-known a risk factor associated with the development of AD. Neurons and astrocytes from AD patients with APOE4 for understanding provide unique opportunity for the understanding of the pathogenesis of AD that occurs in association with APOE dysfunction and for the drug discovery. In this study, we asked whether the pathological phenotypes can be observed in iPSC-derived neurons and astrocytes from AD patients with APOE4 generated by the transcription factor-based technology for rapid differentiation (Quick-Neuron™ and Quick-Glia™ Astrocytes). iPSC-derived neurons generated from a sporadic AD patient with a genetic polymorphism in the APOE4 gene (AD neurons) were cultured for one to eight weeks, with neuros from a healthy donor as control. After six weeks of culture, the AD neurons exhibited significantly higher accumulation of Aβ40 and Aβ42 in the culture media compared to the control neurons. Tau accumulation and Tau phosphorylation were also significantly higher in the AD neurons after four weeks of culture. β-secretase inhibitors that inhibit Aβ production significantly reduced the accumulation of Aβ40 and Aβ42 in the AD neurons. These results imply that the patient-derived Quick-Neuron™ exhibit the pathological phenotypes of Alzheimer's disease within four to six weeks of culture. Because astrocytes play a central role in APOE4 mediated cholesterol transport to neurons, we also investigated the phenotypes of astrocytes generated from the patient iPSCs using the rapid differentiation technology (Quick-Glia™). Lipid accumulation was observed in AD astrocytes, suggesting that this may be involved in the dysfunction. Based on these results, contribution of the neurons and astrocytes generated from AD patients using the rapid differentiation technology will be discussed in the context of the disease mechanism investigation and phenotypic drug screening.

Disclosures: R. Tanaka: None. **R. Yamoto:** None. **T. Sameshima:** None. **Y. Chehreghanianzabi:** None. **T. Hosoya:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.07/B127

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1RF1AG079517-01

Title: Investigating the bioactivity of microglia-derived extracellular vesicles on neurons in the context of apolipoprotein-E genotype and inflammation

Authors: *A. VERDUZCO[1], N. NA[1], P. NGO[1], K. K. BALDWIN[2], H. T. CLINE[1];
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Abstract: Intercellular communication mediates glial regulation of neuronal homeostasis and synaptic dynamics. The fact that in early asymptomatic Alzheimer's disease (AD) dysregulation of astrocyte and microglia metabolism accompanies a dysregulation of synaptic function suggests an important role for intercellular communication in the onset and progression of neurodegenerative diseases like AD (Johnson et al., 2020). Additionally, apolipoprotein E ε4 (APOE4), a genetic risk factor for AD, is known to alter glia metabolism and inflammatory responses yet the mechanism for how these glial phenotypes result in neuronal dysregulation remains unclear (Raman et al., 2018). We are interested in glia-derived extracellular vesicles (EVs) and their potential to regulate neuronal metabolism and synaptic function. Using human induced pluripotent stem cells (iPSCs) from donors with homozygous APOE3 genotypes and isogenic CRISPR-edited APOE4 lines, we generated induced neurons (iNs) and microglia (iMGLs) to study EV-mediated signaling in the context of inflammation. We treated APOE3 iMGLs with a chronic inflammation paradigm where they received either PBS as a control or LPS (50ug/mL) every 2 days for one week. We then used sequential ultracentrifugation to purify the extracellular vesicles that accumulated in iMGL conditioned media in response to the treatments. APOE3 iNs were then treated with either PBS, PBS-EVs, or LPS-EVs. After 48hrs, we collected iNs and evaluated lipid droplet (LD) load via immunocytochemistry and confocal microscopy. We found that while PBS-EVs did not alter LD load compared to PBS, LPS-EVs significantly decreased neuronal LDs. These results suggest that the inflammatory state of microglia affects EV bioactivity and that microglia EVs have the potential to modulate neuronal metabolism. Ongoing experiments are aimed at evaluating whether the APOE4 genotype influences the LPS effect on EV signaling, whether neuronal APOE4 genotype modulates the impact of microglia EVs on LD load, and whether these neuronal metabolic changes affect synaptic activity. This work will provide valuable insight into the role of extracellular vesicles in conveying glia APOE genotype- and inflammation-dependent phenotypes to neurons.

Disclosures: A. Verduzco: None. **N. Na:** None. **P. Ngo:** None. **K.K. Baldwin:** None. **H.T. Cline:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.08/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The effects of chronic zinc supplementation on behavior and tauopathy in an Alzheimer's Disease mouse model containing both APOE4 and Tau

Authors: *A. BOOTH, S. NEFF, *A. BOOTH, V. DE LILLO, A. MAREDIYA, J. CAMPITELL, S. GRAY, J. M. FLINN;
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Abstract: Alzheimer's Disease (AD) is a neurological disease characterized by two major biological components: amyloid beta (Aβ) and phosphorylated tau (p-tau). Research suggests that the p-tau aggregation seen in late-onset AD is characterized by two independent pathways, one caused by Aβ buildup, and the other potentially caused by Apolipoprotein 4 (APOE4). However, research examining the relationship between p-tau and APOE4 in the absence of Aβ has been both inconsistent and lacks behavioral results. Likewise, research suggests that zinc exacerbates the effects of both tau and Aβ in AD mouse models, however, there is limited research in how zinc interacts with APOE4. We analyzed 8 groups of mice: 4 genotypes (WT, APOE4, Tau, APOE4xTau), 2 treatment groups (lixit water, zinc water (10 ppm)), none of which contained Aβ. Behavioral tests were run at 3 and 6 months of age to examine activities of daily living (Nesting & Burrowing), anxiety & motor ability (Open Field), risk taking (Elevated Zero Maze), spatial memory (Morris Water Maze), and circadian activity. Initial results at 3 months indicate that Tau and APOE4xTau mice demonstrated impaired activities of daily living (Nesting ($p<.001$), Burrowing ($p<.001$)), higher risk taking ($p<.001$), and impaired spatial memory ($p<.001$) compared to WT or APOE4 mice respectively. APOE4 mice demonstrated improved spatial memory compared to WT ($p<.001$), and APOE4xTau mice demonstrated improved spatial memory compared to Tau mice ($p<.001$). Likewise, both Tau and APOE4xTau mice demonstrated a phase delay in circadian activity compared to WT and APOE4 mice, although this phase delay was less apparent in the APOE4xTau mice ($p<.05$). However, APOE4 and APOE4xTau mice did not show differences in activities of daily living, nor anxiety compared to WT and Tau mice respectively. Additionally, Zinc did not significantly influence behavior at 3 months. Results will be re-analyzed at 6 months of age, and brains will be analyzed for proteins of interest. Our initial findings provide evidence that APOE4 enhances certain behaviors at 3 months of age. This is consistent with previous research that APOE4 may show beneficial effects at a young age in both humans and mouse models. Subsequent analysis at 6 months will determine whether these beneficial effects continue at an older age.

Disclosures: A. Booth: None. **S. Neff:** None. **A. Booth:** None. **V. de Lillo:** None. **A. Marediya:** None. **J. Campitell:** None. **S. Gray:** None. **J.M. Flinn:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.09/B128

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GM105561
NIH GM008074
CSULB Richard D. Green Fellowship
CSULB Monahan Chemistry Research Endowment
CSULB Student Summer Research Award

Title: Development of Nanodiscs with embedded acid sphingomyelinase for the treatment of Niemann-Pick Disease

Authors: V. METKAR[1], C. NGUYEN[2], J. ZHENG[3], N. PLASCENCIA[4], T. GREDIG[4], A. M. ZIVKOVIC[3], *V. NARAYANASWAMI[1];
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Abstract: Lysosomal storage disorders (LSDs) are genetic metabolic diseases with lysosomal enzyme deficiencies. Deficiency of or defective acid sphingomyelinase (ASM) causes Niemann-Pick disease types A and B (NPDA and NPDB, respectively), neurological disorders characterized by accumulation of sphingomyelin. We have developed a nanodisc (ND)-based platform wherein recombinant ASM has been embedded in a bilayer of phospholipids circumscribed by apolipoprotein E3 (apoE3) for targeted delivery to the lysosomes. The rationale is that apoE3 would serve as a high-affinity ligand via its N-terminal (NT) domain for the low-density lipoprotein receptors (LDLr) facilitating receptor-mediated endocytosis of the nanodisc; the nanodisc and its components would eventually disintegrate in the low-pH environment of the lysosomes thereby releasing the embedded ASM. We designed a fusion protein comprising the essential catalytic domain (residues 319-579) of ASM flanked by the transmembrane domain (TMD) of a pulmonary surfactant protein linked via a flexible linker at the N-terminal end to facilitate embedding in a lipid bilayer and a His-tag at the C-terminal end to facilitate purification. The fusion construct termed TMD-ASM was expressed and purified from a bacterial expression system, and the identity confirmed by Western blot using anti-ASM and anti-His tag antibodies. TMD-ASM was reconstituted with phospholipids and apoE3 NT by the cholate dialysis method to promote formation of lipoprotein complexes, followed by density gradient ultracentrifugation to isolate nanodiscs with ASM (TMD-ASM-ND). Empty ND without the embedded ASM were generated in parallel. Physicochemical characterization using SDS-PAGE shows the presence of both ASM (35kDa) and apoE3NT (24 kDa) in the nanodisc preparations, while native PAGE revealed the formation of large complexes (~600 kDa) of ~ 15 nm diameter. Negative-stained electron microscopy imaging of TMD-ASM-ND revealed

nanodisc formation, while atomic force microscopy imaging showed nanodisc thickness of ~15 nm. Co-immunoprecipitation confirmed the presence of ASM in nanodiscs, while sphingomyelinase activity assays revealed robust ASM activity of TMD-ASM-ND. Lastly, glioblastoma cells (GBM A172), which are known to overexpress LDLr were treated with TMD-ASM-ND and the cellular uptake followed by immunofluorescence. Fluorescence microscopy revealed punctate perinuclear vesicles with co-localization of ASM with apoE3, indicative of successful targeting of ASM to the lysosomes. These findings show potential for targeted delivery of ASM using nanodiscs to treat NPDA and NPDB by enzyme replacement therapy.

Disclosures: V. Metkar: None. **C. Nguyen:** None. **J. Zheng:** None. **N. Plascencia:** None. **T. Gredig:** None. **A.M. Zivkovic:** None. **V. Narayanaswami:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.10/Web Only

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GM105561
NIH GMT34149378
NSF LSAMP HRD-1826490
Richard D Green fellowship

Title: Conformational analysis of reconstituted discoidal and spherical high density lipoprotein bearing apolipoprotein E

Authors: *L. ODELL[1], Y. HAN[1], K. M. TAIWO[2], Z. HAGUAR[1], V. NARAYANASWAMI[1]; [1]Chem. and Biochem., California State Univ. Long Beach, Long Beach, CA; [2]Yale Univ., Meriden, CT

Abstract: Apolipoprotein E (apoE) (~34 kDa, 299 residues) is a highly helical, exchangeable protein that can exist in lipid-free and bound states. In the brain, it is found on high density lipoproteins (HDL)-like particles and plays a major role in cholesterol transport. In humans, it is polymorphic expressing two major isoforms, apoE3 and apoE4, the latter strongly linked with Alzheimer's disease. They differ in amino acid at position 112 (Cys and Arg, respectively). Our goal is to understand the conformational organization of apoE3 and apoE4 in reconstituted discoidal and spherical HDL, rHDL(d) and rHDL(s), bearing POPC/cholesterol and POPC/cholesterol/cholesterylester, respectively. We designed a series of single Cys variants of apoE3 (C112S) and apoE4 with Cys located on various helices to monitor the N-terminal and the C-terminal domains. The proteins were labeled with N-(1-pyrene) maleimide, a spatially sensitive -SH specific fluorophore that emits excimer emission peaks if proximal (~10Å) to another pyrene. Fluorescence emission spectra of lipid-free apoE3 and apoE4 variants bearing pyrene label in the NT and CT domains revealed large excimer peak indicative of spatial

proximity. The labeled variants were then allowed to form rHDL(d), which was verified by native PAGE analysis. Fluorescence analysis of rHDL(d) revealed significant loss of excimer emission indicative of distance separation between two specified sites on neighboring apoE molecules. In a complementary approach unlabeled apoE3 and apoE4 single Cys variants were allowed to form rHDL(d) and treated with bismaleimidohexane (BMH), which covalently links two -SH moieties that manifest as dimeric protein bands on SDS-PAGE. Regardless of the location, all variants appeared as monomeric bands following BMH treatment indicating that two neighboring apoE molecules were not aligned parallel to each other in rHDL(d), consistent with the spectroscopic data. Preliminary spectroscopic and biochemical crosslinking analyses of rHDL(s) reveal similar results as those seen with rHDL(d), suggesting that despite the change of lipoprotein shape from discoidal to spherical geometries, two specified sites on neighboring apoE molecules are not proximal to each other. Taken together, our data indicate that 2 apoE molecules are aligned anti-parallel to each other on rHDL(d) and that the corresponding sites on 2 neighboring apoE are not juxtaposed to each other on rHDL(s). Our results offer valuable insights into the conformation of apoE3 and apoE4 and bear the potential to identify the mechanistic role behind the isoform-specific differences noted in brain HDL metabolism in neurodegenerative diseases.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.11/B129

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG068395
NIH R01AG081228
Alzheimer's Drug Discovery Foundation

Title: Md simulations of rare alzheimer's disease-associated apoe isoforms, such as apoe3-christchurch, highlight unique structural, conformational, and dynamical features

Authors: *R. A. TUCKEY[1], R. GREER[2], H. DEAN[3], E. D. ROBERSON[4], Y. SONG[2]; [1]UAB Neurosci. Grad. Programs, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Univ. of Alabama, Birmingham, Birmingham, AL; [4]Neurol., Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: The lipid carrier protein a*polipoprotein E* (*APOE*) is the strongest and most well-known risk factor for late onset Alzheimer's disease (AD). The three most common alleles are *APOE3*, *APOE2*, and *APOE4* which provide neutral, decreased, and increased risk of developing AD, respectively. Several rare variants in APOE have been identified, through a case study and

genome wide association study, to be protective against AD. These rare variants include *APOE3-Christchurch* (*R136S*), *APOE3-Jacksonville* (*V236E*), and *APOE4-R251G*. Previously, we examined common and rare APOE isoforms in a lipid-free state. This study was limited by the starting structure, as APOE begins in a closed state with the C-domain against the N-domain, allowing for few observable changes on a nanosecond timescale. Additionally, APOE is largely in a lipid-bound state *in vivo* and undergoes distinct structural changes to become lipid-bound. Therefore, examining APOE in a more extended lipid-bound conformation may elucidate changes caused by these substitutions that would have otherwise gone undetected. We have obtained a starting lipidated APOE structure, provided by Prakashchand et al., which was generated from coarse-grained molecular dynamics (MD) simulations of a mutant APOE structure (PDBID: 2L7B) in the presence of DPPC lipid molecules. Using this structure, we subsequently converted the mutant APOE to three separate APOE3 molecules using PyMOL after energy minimization. After 300 ns of equilibrated simulation of each lipid-protein complex, we generated a representative structure which was used to convert APOE3 to the remaining APOE isoforms, except for APOE4-R251G which was generated from a representative APOE4 structure. Three replicate simulations for each variant, each with 300 ns of equilibrated simulation, were used for subsequent analysis. Types of analysis included examining changes in APOE structure, conformation, dynamics, and lipids binding interactions. Results indicate that APOE3-Ch has similar conformation stability and dynamics to APOE2. Another variant of which we know very little, APOE4-R251G, appears largely distinct in conformational stability and has different dynamics compared to its most closely related isoform APOE4. Analysis of rare APOE isoforms, in the context of common APOE isoforms, provides insight into unique and shared characteristics of each that could be predictive of downstream functional changes resulting in altered AD risk.

Disclosures: R.A. Tuckey: A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **R. Greer:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **H. Dean:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. **E.D. Roberson:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham. 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.; Grants from NIH, Bluefield Project to Cure FTD, Alzheimer's Drug Discovery Foundation, Site PI for Clinical Trials with Eisai and Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of IP related to tau, Royalties on mouse model licensed to Genentech. F. Consulting Fees (e.g., advisory boards); Member of SAB for AGTC, Member of DMC for Lilly, Member of Editorial Board for SfN. **Y. Song:** A. Employment/Salary (full or part-time):; University of Alabama at Birmingham.

Poster

PSTR209: Alzheimer’s Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.12/B130

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG065819
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Advanced Magnetic Resonance Imaging and Spectroscopy Facility, UF

Title: Age and genotype differences in contextual fear memory between the human APOE4 and APOE3 homozygous targeted replacement mouse models

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[1]Neurosci., Univ. of Florida, Gainesville, FL; [2]Dept. of Psychiatry, Univ. of Florida, Gainesville, FL

Abstract: Apolipoprotein-ε4 (APOE4) homozygosity in humans is the strongest genetic risk factor for Alzheimer's Disease (AD). There is also evidence of greater AD risk in females than males, but the extent to which APOE plays a role is unknown. The combined effects of APOE4 with age on neural communication and behavior, and possible sex-differences, remain to be investigated. In the present work, we tested the hypothesis that age alters contextual fear memory, functional network topology, and diffusion imaging measures, differentially between APOE4 and APOE3 homozygous mice. Male and female mice aged 1.5-5 months and 9-13.5 months, homozygous for the human APOE4 or APOE3 gene, were scanned on an 11.1 Tesla MRI scanner under sedation. Images were processed and analyzed using 148 a priori nodes on the common coordinate framework mouse atlas (version 3) and graph theory algorithms in brain connectivity toolbox. Following fMRI and DTI scans, mice completed a contextual fear conditioning (CFC) protocol over three days. Day 1, mice received CFC training for 17 minutes using a series of 4 trials in the presence of a foot shock (0.90mA, 1 second) applied at equal intervals. Day 2, mice repeated day 1 without the presence of a foot shock in a recall session. Day 3 the recall session was repeated with visual and tactile contextual cues modified.During CFC day 1 all mice developed a robust increasing freezing response, with significant differences between groups. There were significant main effects of trial, age, and genotype. APOE4 and older mice showed greater freezing over the trials compared to young and APOE3 mice. During modified context, we found significance in the main effect of age and interaction effect of genotype and age, with our adult APOE4 mice showing the greatest freezing behavior and our young APOE4 mice showing the least. Both adult APOE4 and APOE3 showed freezing behavior that continued increase throughout the modified context testing period, while young mice exhibited more typical exploratory behavior. We may run an open field test in the future to determine if freezing behavior reflects fear in a modified context, or a decrease in overall activity over time. There were no significant differences in the recall session. The results suggest that in APOE4 mice adults (which have decreased functional network integration) there may be a link of network connectivity changes with increases in fear behavior, and a decreased ability to recognize contextual changes. This decrease in contextual change recognition may also be largely age-based, with genotype interaction as a cofactor. The sex-differences seen in connectivity with our previous pilot cohort are not seen in CFC behavior.

Disclosures: Z. Simon: None. **M. Febo:** None. **P. Chakrabarty:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

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Program #/Poster #: PSTR209.13/B131

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG069196

Title: Impact of Multiple Biological Risk Factors for Alzheimer's Disease on Functional Operation of the Anterior Olfactory Nucleus

Authors: *M. HU, C. UZUN, Y. LI, S. BRUNSON, C. P. VICKNAIR, D. ZHAO, S. LIU; Univ. of Georgia, Athens, GA

Abstract: Olfactory deficits have been recognized to precede other symptoms in Alzheimer's disease (AD) patients for many years to decades thus can potentially serve as a prodromal biomarker for facilitating early AD diagnosis. Although pathological or functional alterations in any or multiple olfactory structures could potentially contribute to this sensory disorder in AD, the anterior olfactory nucleus (AON) stands out from other structural candidates given its pivotal position in the whole olfactory system and early demonstration of AD pathogenesis. Surprisingly, investigation of AD pathophysiology in the AON, which likely appears before AD pathologies, is lacking. In the present study, we explored the impacts of the human apolipoprotein E4 (APOE4) allele, the strongest genetic risk factor for the late onset AD, on neuronal and synaptic functions within the AON of both sexes of mice at different ages. Our behavioral results showed that expression of the human APOE4 gene altered social behaviors in mice, as reflected by declined sociability and social novelty preferences compared to the control animals carrying the human APOE3 gene, which is considered being neutral as for AD development. Given the functional role of AON in social behaviors, this finding suggests dysfunctional operation in the AON of this AD mouse model. Using in vivo electrophysiological techniques to record population level of neuronal activities and network operation in awake animals, we found stronger oscillatory activities in the AON of aged APOE4 mice compared to the APOE3 counterparts, indicating APOE4 genotype-caused network disorder. At the cellular level, the AON excitatory pyramidal neurons and inhibitory interneurons in APOE4 mice displayed differences in intrinsic properties, including a depolarized resting membrane potential, reduced afterhyperpolarization, and prolonged action potentials. At the synaptic level, elevated excitatory synaptic activities were detected in AON pyramidal neurons. These findings are consistent with the speculation of altered network operations inferred from the in vivo data. Intriguingly, our research further revealed that some detrimental actions of APOE4 are sex-dependent, indicating the contribution of sex as a risk factor. Future work will extend to morphological analysis and neurophysiological characterization of more AON neuronal populations at different ages with an aim at fully elucidating the pathophysiological influences of APOE4 on the structural organization and functional operation of AON neurons and circuits and how different risk factors interact to contribute to the initiation of AD pathogenesis in the olfactory system.

Disclosures: M. Hu: None. **C. Uzun:** None. **Y. Li:** None. **S. Brunson:** None. **C.P. Vicknair:** None. **D. Zhao:** None. **S. Liu:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.14/B132

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01AG074216

Title: Mechanistic Analysis of Neuronal and Circuit Vulnerability to APOE4 in the Mouse Olfactory Bulb

Authors: *D. ZHAO[1], S. T. ISLAM[2], C. P. VICKNAIR[1], Y. LI[1], M. HU[1], S. LIU[2];
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Abstract: Alzheimer's disease (AD) represents a formidable public health challenge, profoundly impacting human health and society with its escalating prevalence in the aging populations. Among the human apolipoprotein E (APOE) gene variants, the APOE4 allele as the established genetic risk factor for AD is associated with age-dependent dysfunctions in the olfactory bulb (OB) and piriform cortex. Olfactory disorder, as a prodromal sensory biomarker for AD, may signal AD's onset, presenting opportunities for early intervention. However, the mechanisms at the cellular and synaptic levels remain unclear. In this study, APOE4 mice carrying the human APOE4 gene showed significant deficits in the buried food behavioral test assessing odor detection compared to APOE3 and C57BL/6 control mice at the age of 90 weeks . Specifically, these transgenic APOE4 mice displayed increased latency in locating food, indicating reduced sensitivity to food odors and association with AD. Building on these behavioral outcomes, the in vivo multi-channel extracellular recording approach was utilized to compare the OB network oscillatory activities and spontaneous spiking of mitral cells (MC), the principal OB output neurons, between control and APOE4 mice at multiple ages ranging from 19 to 127 weeks in the awake but head-fixed condition. Additionally, neuronal excitability and synaptic activities in MCs within OB slices were assessed with whole cell patch clamp recordings. Interestingly, our in vivo data indicate sex-dependent differences in both APOE4 and APOE3 mice at the age of 35 weeks, with strong oscillatory activities APOE4 mice, particularly in the low frequency bands. Consistently, our in vitro work at the single cell level revealed sex and age dependent APOE4 effects on neuronal intrinsic properties and excitability in MCs. Taken together, our findings demonstrate age and sex-dependent detrimental actions of APOE4 on the principal OB output neurons and related network operation. Our ongoing research is expanding to disclose how aging, sex, and the APOE4 allele interact in olfactory processing within the OB. Future studies will broaden in vivo analyses across more age groups and enhance in vitro investigations to include interneurons and other output neurons such as the external tufted cells, as well as delve deeper into synaptic transmission dynamics within critical olfactory regions such as the piriform

cortex. These extended studies aim to provide deeper insights into the multifaceted neurobiological mechanisms underpinning olfactory deficits in AD, thereby advancing our understanding of AD early pathogenic processes.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

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Program #/Poster #: PSTR209.15/B133

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH U54AG054349

Title: Phenotypic characterization of a novel hAb-KI^{loxP};hAPOE4 mouse model.

Authors: *A. GOMEZ ARBOLEDAS[1], E. A. KRAMAR[2], S. KAWAUCHI[3], C. DA CUNHA[4], Y.-H. LU[5], G. MILINKEVICIUTE[4], J. NEUMANN[5], M. A. WOOD[6], A. J. TENNER[7], F. M. LAFERLA[8], G. MACGREGOR[5], K. N. GREEN[9];
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Abstract: Background: Genome-Wide Association Studies (GWAS) identified ApoE4 as the strongest genetic risk factor for late-onset Alzheimer's Disease (LOAD). As part of our efforts to develop mouse models that better recapitulate LOAD, at Model Organism Development & Evaluation for Late-Onset Alzheimer's Disease (MODEL-AD) consortium at University of California - Irvine, we have created a double homozygous mouse model that combines our previously developed hAβ-KI^{loxP} mice (Jackson Lab #031050) and a humanized ApoE4, which replaces part of the murine ApoE locus, (Jackson Lab #027894) to evaluate the interactions between aging, hAPOE4, and hAβ.**Methods:** By breeding the hAβ-KI^{loxP} and hApoE4, we obtained double homozygous (HO) mice and we then generated three different groups: WT (C57BL6/J), hAβ-KI^{loxP} HO and hAβ-KI^{loxP} HO;hApoE4 HO. All three groups were then aged to 4, 12, 18 and 24 months of age, when coronal hippocampal slices were prepared, and long-term potentiation recordings were obtained. Synaptic density was also assessed by super-resolution microscopy in three different brain regions. Additionally, we also measured soluble Aβ40 and Aβ42 as well as plasma neurofilament light chain (NfL).**Results:** hAβ-KI^{loxP} mice showed a significant reduction in mean potentiation 50-60 minutes post TBS, when compared to WT mice at 4, 12, 18 and 24 months of age, indicative of an LTP deficit associated to human Aβ.

Interestingly, the presence of hAPOE4 rescued the LTP deficits observed on the hAβ-KI^{loxP} mice from 4 months of age. Additionally, we observed a significant pre-synaptic loss on the hAβ-KI^{loxP} mice (vs WT) that was prevented in hAβ-KI^{loxP} HO;hApoE4 HO mice, which correlates with the LTP measurements. Finally, plasma NfL levels were increased in the hAβ-KI^{loxP} mice at 24 months of age, indicative of axonal damage in this mouse model, but no differences were observed by the addition of hApoE4.**Conclusion:** hAβ induces robust LTP deficits that are prevent by hAPOE4, from 4 months of age. These results are in agreement with the excessive synaptic loss observed in the hAβ-KI^{loxP} mice, which is also rescued by the addition of the hAPOE4 variant. However, the axonal damage induced by the hAβ at 24 months of age was not reduced by the hAPOE4 variant. Further studies are needed in order to fully understand the differences between murine and human APOE4 and how this could be modulating the effects of hAβ on synaptic integrity and function.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: UNMC Alzheimer's Disease Scholarship
NIH T32 Grant
UNMC Fellowship

Title: Apoe4 induces synaptic mitochondrial lipidome, proteome, and funtional alterations

Authors: *N. ROLAND, J. MANGANARO, K. L. STAUCH;
Dept. of Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Alzheimer's disease (AD) is the world's leading cause of dementia characterized by the accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs, composed of hyperphosphorylated tau) with NFTs correlating closely with the resulting cognitive decline. *APOE4* is the strongest genetic risk factor for developing AD; however, the mechanisms involved are not well understood, specifically its contribution to AD-related synaptic impairment. Using 3-month-old, male and female, human *APOE* targeted replacement (APOE3-TR and APOE4-TR) mice with APOE3-TR as a control, we have uncovered synaptic mitochondrial lipid, function, and proteomic changes. We first used a Bioluminescent assay to measure levels of cholesterol at the whole brain, synaptosome, and mitochondria levels. Although unchanged at the whole brain and synaptosome levels, the synaptic mitochondria showed a decrease in cholesterol in APOE4-TR versus APOE3-TR mice. Considering these

cholesterol changes, mass spectrometry was used to assess the synaptic mitochondrial lipidome and uncovered overall decreased levels of lipids across lipid classes. The Seahorse Extracellular Flux assay revealed synaptic bioenergetic dysfunction in APOE4-TR mice, in particular increased maximal mitochondrial respiration. Further, mass spectrometry-based proteomic analysis on isolated synaptosomes from APOE3-TR and APOE4-TR mice uncovered differentially expressed proteins further highlighting changes in the mitochondrial proteome at the synapse due to *APOE4*. Together, these findings further illuminate the molecular mechanisms contributing to *APOE4* genetic risk in AD and its contribution to synapse dysfunction through modulation of mitochondria.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG058081
NIH R01 AG08142
University of Minnesota Office of Academic Clinical Affairs (FRD #19.29)

Title: Investigating the impact of APOE4 on mitochondrial function in AD and the therapeutic potential of HDL-mimetic peptides in ameliorating APOE4-linked AD pathology

Authors: *S. JOSHI[1], L. LI[2], K. FREDRIKSEN[3];
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Abstract: Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases. Genome-wide association studies have identified apolipoprotein E4 (APOE4) as the greatest genetic risk factor for developing sporadic AD; however, the exact underlying mechanism remains undeciphered. APOE, mainly produced by astrocytes in the brain, is encoded by three alleles—ε2, ε3, ε4. Lipidation of APOE is isoform dependent (ε4<ε3<ε2) and is crucial for high-density lipoprotein (HDL) formation and function, including cholesterol homeostasis, synaptic growth, anti-inflammation, and protein clearance. Poor lipidation of APOE4 may be a potential mechanism driving pathological hallmarks of AD including amyloid plaque load, neurofibrillary tangles and inflammation. Moreover, bioenergetic alterations including decreased glucose utilization, decreased mitochondrial respiration and increased oxidative stress have been implicated in AD. However, the impact of APOE4 on mitochondrial function and bioenergetics in AD is poorly understood. We hypothesize that APOE4-associated lipidation deficiency

decreases mitochondrial function and alters bioenergetic pathways in AD, and that correcting APOE4's lipidation and mitochondrial deficits using the clinically tested HDL-mimetic peptide 4F ameliorates APOE4 linked AD pathology. In this study, we observed the secretion and lipidation deficit of APOE4 compared to APOE3 in both primary mouse astrocytes and human iPSC derived astrocytes expressing APOE3 and APOE4 using gel electrophoresis. Furthermore, mitochondrial respiration and mitochondrial membrane potential (MMP) was assessed using Seahorse Flux assays and JC-1 dye, respectively. As expected, APOE4 astrocytes exhibit impaired mitochondrial respiration and MMP in comparison to APOE3 astrocytes. Intriguingly, APOE4 astrocytes mainly rely on glucose as a fuel and are unable to utilize endogenous fatty acids for energy metabolism. Furthermore, APOE4 astrocytes treated with aggregated amyloid-β (Aβ) and APOE4 astrocytes overexpressing APP/PS1 that model Aβ pathology *in vitro* exhibit aggravated mitochondrial dysfunction. Importantly, treating these cells with 4F improved lipidation of APOE4 as well as APOE3, which persists in the presence of Aβ. In conclusion, this study provides experimental evidence of APOE4 linked mitochondrial dysfunction and of HDL-mimetic peptides as potential APOE4 modulating agents. Future studies are warranted to assess whether HDL-mimetic peptides ameliorate APOE4-associated mitochondrial dysfunction and restore the beneficial functions of HDL to mitigate AD pathology.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG081426
University of Minnesota College of Pharmacy

Title: Aav-mediated delivery of apoe4ch reduces amyloid deposition and associated neuronal dystrophy

Authors: *M. KIM[1], K. FREDRIKSEN[2], H. KORTHAS[1], L. LI[1];
[1]Exptl. and Clin. Pharmacol., [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Apolipoprotein E (APOE) is the most influential genetic risk factor for Alzheimer's disease (AD), with the E4 variant of APOE (APOE4) conferring an increased risk of developing AD. A recent case study revealed that the presence of a rare APOE R136S Christchurch mutation (APOECh) was associated with delayed onset of symptoms in an individual harboring a mutation known to cause AD. Imaging analyses for amyloid-beta (Aβ), a peptide that aggregates into plaques and contributes to pathogenesis in AD, showed that this individual had surprisingly high levels of Aβ deposition compared to control AD patients. However, the lack of available age-matched controls in the case study leaves the effect of APOECh on Aβ deposition unclear, with

one recent study done in AD mice suggesting that APOECh can decrease Aβ deposition. Furthermore, the effect of APOECh on Aβ deposition in the context of APOE4 has not yet been studied *in vivo.* To assess the effect of APOECh on AD pathology in the context of APOE4, and to provide a translational aspect to current understandings of APOECh, we created adeno-associated viruses (AAV) expressing either APOE4 or APOE4 with the Christchurch mutation (APOE4Ch) and stereotaxically injected them into 6-8 month-old APP/PS1 mice, a well characterized model of AD. We then used immunofluorescent imaging and biochemical analysis to evaluate the pathology in these mice three months after injection. The results showed that mice injected with APOE4Ch had a reduced Aβ-plaque load compared to mice injected with APOE4, particularly in the fraction of diffuse or soluble Aβ. Microglia were found to be more associated with Aβ plaques in mice injected with APOE4Ch than mice injected with APOE4, possibly contributing to the decrease in Aβ load. Additionally, a decoupling of neuronal dystrophy to plaques was observed in APOE4Ch injected mice, suggesting that APOECh may help rescue Aβ-associated functional deficits in these mice. Overall, these findings show that APOECh rescues amyloid-related pathology in APP/PS1 mice and suggest that AAV-mediated expression of APOECh may be a potential therapy for AD.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant # R01AG055545
NIA grant # R56AG077814

Title: Impact of APOE4 on the Spinal Motor System Under Normal and Stressed Conditions

Authors: *M. C. LOPES, R. W. CASTRO, G. VALDEZ;
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Abstract: Spinal cord motor neurons are central for initiating and modulating all voluntary movements and rely on spinal cord glia to remain viable and properly function. During aging, injuries, and diseases, microglia aggregate around stressed motor neurons to reorganize the motor circuit and phagocytose debris. However, the molecular mechanisms that mediate and potentially exacerbate these unique motor neuron-glia interactions are unknown. Recent work from our lab and others has found Apolipoprotein E (APOE) is elevated in spinal cord motor neurons and microglia under stress. In humans, APOE4 has been shown to increase the risk of Alzheimer's Disease (AD) while APOE2/3 do not alter AD risk. Additional research has shown that APOE4 impairs synaptogenesis partly by increasing neuroinflammatory and glia responses in the brain. Here, we asked whether APOE4 has a similar effect on spinal cord motor neurons

and glial cells. We examined spinal cord motor neurons and associated glial cells in adult humanized APOE4KI and APOE3KI mice. Our initial analysis has revealed that APOE4 causes microglia acquire an age- and disease-associated phenotype. We also found fewer synaptic inputs on motor neurons of APOE4 KI mice. To determine if APOE4 compromises the response of spinal cord motor neurons and glial cells to stress, we axotomized motor neuron axons in humanized APOE3KI and APOE4KI mice. Axotomy has been shown to reduce the number of synaptic inputs on motor neurons and increase microglia targeting of motor neurons. Our initial analysis suggests that axotomy does not cause the additional loss of synaptic inputs on motor neurons nor their targeting by microglia in APOE4KI mice. Together, these initial data are beginning to reveal the role of APOE in motor neurons and glial cells in the spinal cord.

Disclosures: M.C. Lopes: None. **R.W. Castro:** None. **G. Valdez:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

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he Bluefield Project to Cure FTD
Harrington Discovery Institute (HDI2019-SI-4479)
Circle of Friends Pilot Synergy Grant
the Presbyterian Village North Foundation
the Robert J. Kleberg Jr. & Helen C. Kleberg Foundation.

Title: Inhibition of NHE6 corrects the defect in endolysosomal trafficking in Alzheimer's disease

Authors: *M. LAMBERT[1], M. DURAKOGLUGIL[1], L. CALVIER[1,2], H. T. ZHAO[4], J. HERZ[1,2,3];
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Abstract: Apolipoprotein ε4 allele (ApoE4) is the most prevalent genetic cause of late-onset Alzheimer's Disease (AD). Individuals carrying one ε4 allele have a 3-4-fold elevated risk to develop late onset AD and individuals carrying two ε4 have a 9-to-15-fold increased risk. Indeed, amyloid-β plaque deposition, a characteristic feature of AD is increasing with ApoE4 and its clearance is impaired. The molecular mechanisms by which ApoE4 accelerates the onset

and progression of the disease remains unclear. Endolysosomal function and trafficking is needed to clear cellular waste. Endosomal trafficking is regulated by the pH of early endosomes (EE) which is balanced by opposing functions of the v-ATPase proton pump and the proton leak channel, Na+/H+ exchanger, NHE6. However, during aging and AD, v-ATPase activity is reduced. The resulting diminished acidification lead to abnormal endosomal trafficking and impaired autophagy. Previously, our laboratory has demonstrated that ApoE4 impairs early endosomal recycling in neurons. Indeed, the pH of EE and the isoelectric point of ApoE4 are equivalent, leading to the retention of ApoE4 with its receptor in EE and the disruption of endosomal trafficking of ApoE4 containing vesicles. We hypothesize that the inhibition or depletion of NHE6 decreases the pH of EE, restores endolysosomal function and trafficking, and so enhances the removal of protein aggregates in AD and other neurodegenerative diseases. Endosomal pH measurement in living neurons shows that the inhibition of NHE6 significantly increases acidity in endosomal vesicles. Moreover, ApoE4 recycling assays show that the inhibition of NHE6 restores normal trafficking of ApoE4 in neurons. In vivo, NHE6 was depleted using specific antisense oligonucleotides (ASO) as a potential therapeutic strategy. Treatment of 5xFAD mice, an AD mouse model with NHE6 ASO significantly corrects cognitive deficits compared to control mice in Y-maze experiments. Taken together, we propose genetic or pharmacological inhibition of NHE6 as an innovative therapeutic strategy to prevent AD.

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Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.21/B139

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG082315

Title: Apoe deficiency impairs hpa axis stress response and increases vulnerability for cognitive impairment in mice.

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Abstract: The E4 allele of the apolipoprotein E (APOE) gene confers the largest risk for Alzheimer's disease (AD). While this allele is associated with reduced APOE functionality, the mechanisms by which the E4 allele contributes to AD pathogenesis are unclear. Elevated plasma glucocorticoid (GC) levels have been observed in rodents with APOE deficiency and humans carrying the E4 allele, suggesting a possible role for APOE dysfunction in stress sensitivity. Exposure to chronic stress or glucocorticoids impairs cognition, and the effects of exposure are more pronounced in females than males, which may contribute to the disproportionate occurrence of AD in females. Here, we investigated the role of APOE in the susceptibility to stress and resultant cognitive impairment. Using the APOE knockout (KO) mouse, we studied the effects of APOE deficiency on the corticosterone (CORT) response to stress and hippocampal-dependent learning and memory in males and females. In addition, we investigated the role of APOE deficiency on the expression and DNA methylation of hypothalamic pituitary adrenal (HPA) axis-associated genes in mice. Prior to 14-day chronic variable stress (CVS), CORT response to restraint was similar between WT and KO males, but female KO mice took longer to recover to baseline ($P=0.04$). In the Barnes maze, both male and female KOs had delayed acquisition learning ($P=0.03$). After CVS, female KOs had delayed recovery of their CORT response to restraint stress compared to WT ($P=0.03$). Both male and female KO took longer than WT to complete the Barnes maze recall task ($P=0.005$). In reversal learning, females were impaired relative to males ($P=0.02$), and female KOs were impaired compared to all other groups ($P<0.05$). Lastly, APOE KO showed altered epigenetic DNA methylation and gene expression of the FKBP5 gene, which is involved in the regulation of GC signaling. These data suggest a role for APOE deficiency in aberrant stress signaling and subsequent cognitive deficits in AD. Further, the interaction between APOE deficiency and stress may serve as a physiological basis for the sex dimorphism in AD prevalence. Thus, future investigation to further uncover the connection between APOE genotype and HPA axis function is warranted to aid in the understanding of AD and the development of novel treatments.

Disclosures: J.H. Freiman: None. **Z. Cordner:** None. **L. Macias:** None. **C. Munro:** None. **R. Lee:** None. **K.L. Tamashiro:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.22/B140

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 5T32AG06795202

Title: Apoe genotype influences polymorphism and behavior of tau oligomers

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Abstract: Risk of developing late-onset Alzheimer's disease (LOAD), the most prevalent form of AD, is predominantly influenced by the apolipoprotein E (APOE) gene. Humans express three isoforms of APOE (2,3,4), with APOE2 associated with a reduced risk of developing LOAD and APOE4 associated with an increased risk compared to APOE3. In AD, the microtubule-associated protein Tau becomes hyperphosphorylated and assembles into toxic aggregates, and studies have shown that APOE genotype differentially influences tau pathology in AD. The current project builds off this knowledge to demonstrate that APOE genotype influences the structure and functional properties of toxic tau aggregates. Here, we used sucrose density gradient ultracentrifugation to isolate brain-derived tau oligomers (BDTOs) from frontal cortex tissue of 14 individuals with varying APOE genotypes. Biochemical and immunological assays demonstrate genotype-dependent differences in immunoreactivity of the tau oligomers, suggesting structural differences. Electrophysiological recordings further reveal APOE-dependent differences in synaptotoxicity of the tau oligomers within the hippocampus. Ultimately, our results suggest that APOE genotype influences polymorphism and cellular behavior of tau oligomers, and these findings could be used to advance the development of tau-targeting therapeutics for AD based on APOE genotype.

Disclosures: N. Moreno: None. **L. Fung:** None. **A. Limon:** None. **R. Kayed:** None.

Poster

PSTR209: Alzheimer's Disease: ApoE and Associated Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR209.23/B141

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA RF1AG057933
NIA R01AG055798

Title: Apolipoprotein E as a critical factor in regulating Alzheimer's disease pathologies

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Abstract: Alzheimer's disease is neuropathologically defined by two major pathologies – intraneuronal tau deposits and extracellular amyloid deposits. Apolipoprotein (APOE) E4 is the major genetic risk factor for Alzheimer's disease, while the APOE3 is the reference allele widely present in the population. In previously published studies, we have demonstrated that relative to APOE3, APOE4 regulates lifespan in a mouse model of tau, without directly affecting the levels of aggregated tau. In recent work from our lab, we have focused on the relative roles of APOE3 and APOE4 on amyloid β neuropathology in rodent models. Using three distinct rodent model systems that develop intracerebral Aβ, we confirmed that APOE4 was more amyloidogenic than APOE3 – one model with diffuse amyloid deposits and two models with mixed dense-cored

plaques. These findings were consistent with the well-regarded dogma that APOE4 is more amyloidogenic than APOE3. However, we also found that APOE4 and APOE3 show similar amyloidogenicity in a brain homogenate-seeded model of diffuse amyloid, indicating that any differential effects of human APOE alleles on Aβ could be mitigated by accelerating Aβ deposition through a templating process. In these amyloidosis models, the presence of human APOE alleles did not impact the innate morphology of deposits that arise naturally or through seeding. Additionally, in all our experiments, we found that mouse Apoe had significantly higher amyloidogenic potential and gliosis induction relative to human APOE4. We further examined the relative contribution of anti-inflammatory signaling mediators on APP/PS1 transgenic mice with mouse Apoe or human APOE4. Our preliminary results indicate that intracerebral expression of Interleukin-10, a key anti-inflammatory cytokine, modestly reduces amyloid burden in young APP/PS1 mice with APOE4 but increases amyloid burden in APP/PS1 mice with mouse Apoe. Together, our study provides insights into the differential role of APOE isoforms in determining the severity and morphology of Aβ deposits under different conditions.

Disclosures: P. Chakrabarty: None. **G. Xu:** None. **T.L. Williams:** None. **Q. Vo:** None. **D.R. Borchelt:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.01/B142

Topic: C.03. Parkinson's Disease

Support: NWO-ALW (Nederlandse Organisatie voor Wetenschappelijk Onderzoek-Aard en Levenswetenschappen) VICI Grant (865.09.002) awarded to M.P. Smidt

Title: Neuronal Epigenetic Regulation by Dot1l Mediated H3K79 Methylation

Authors: *M. V. R. LINGL, M. P. SMIDT;
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Abstract: Epigenetic regulation of the chromatin state through post-translational modifications of histones in neuronal cells is a fast developing field in molecular neurobiology, but there is still much unknown how epigenetic regulation affects gene expression levels. Disruptor of telomeric silencing 1-like (Dot1l) is the sole histon methyltransferase capable of catalyzing the addition of up to three methyl groups on histon 3 at lysine residue 79 (H3K79). H3K79 methylation is generally associated with active gene transcription and has been linked with aging and Parkinson's disease in human midbrain dopaminergic (DA) neurons. In order to investigate the effect of Dot1l mediated H3K79 methylation on gene expression patterns of (DA) neurons, we modulate H3K79 methylation levels and measure transcriptional responses. Using a conditional

knockout mice model of Dot1l in post-mitotic mesodiencephalic DA neurons, we found a broad upregulation of respiratory chain complex (RCC) genes, suggesting a critical role for Dot1l as a transcriptional repressor of the mitochondrial electron transport chain. To that end, we used a specific Dot1l inhibitor to modulate H3K79 methylation levels in DA MN9D cells and investigated the effect of catalytic inhibition on gene expression levels. In concordance with the Dot1l conditional KO model, we found upregulation of RCC genes following ablation of global H3K79 methylation, indicating a regulatory function of Dot1l mediated H3K79 methylation on RCC gene expression patterns. In addition, our lab developed a Dot1l chimeric construct with distinct catalytic properties in order to modulate H3K79 methylation in-vitro and assessed the effects of global H3K79 hypermethylation on gene expression levels. Moreover, we performed H3K79me2 chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) in order to investigate the relation between H3K79me2 enrichment on a locus specific level and differential gene expression patterns. Our data showed that modulation of global H3K79 hypermethylation via overexpression of the Dot1l chimeric construct resulted in a broad downregulation of RCC genes. Suprisingly, ChIP-seq data revealed decreased H3K79me2 enrichment on RCC gene bodies and increased intergenic H3K79me2 enrichment, suggesting that the decrease in transcriptional activity is following the decrease in H3K79me2 level. This is in discordance with previous data, where decreased H3K79me2 levels correlated with increased transcription of RCC genes, indicating that H3K79me2 enrichment on RCC gene bodies is not critical for transcriptional activity.

Disclosures: M.V.R. Lingl: None. **M.P. Smidt:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.02/C1

Topic: C.03. Parkinson's Disease

Title: Efficacy of novel human plasma-derived products to attenuate parkinsonian-like pathology in human dopaminergic neurons

Authors: *N. A. SMITH, R. DHANUKATE, A. HOSSEINI, M. KERRISK CAMPBELL, A. TENNSTAEDT, M. KILINC;
Alkahest, San Carlos, CA

Abstract: With a steady rise in the world's aging population, there is an immediate need for novel therapeutics targeting age-related diseases such as Parkinson's Disease (PD). An essential goal in developing such therapies for PD is to mitigate the effects of increased reactive oxygen species (ROS) production and dopaminergic neuronal degeneration. It has been shown that neurotoxins induce ROS and cell death in dopaminergic neurons. Therefore, neurotoxin-based PD models are an attractive platform for evaluating pharmacological agents for PD. Human

plasma is a rich source of bioactive proteins used for the manufacturing of many therapeutics. Additionally, plasma is known to change with age and aging-related diseases, including PD. Plasma fractions (PFs) are comprised of a subset of plasma proteins and are geared towards multimodal activity and poly-pharmacology driving a specific outcome/phenotype. We have previously demonstrated that a specific PFs derived from healthy human donors can increase neuronal survival, reduce neuroinflammation, and improve cognition in aged wild-type mice and a mouse model of Parkinson's disease. However, whether this plasma fraction decreases ROS production and improves dopaminergic neuron survival remains unknown. To address this, we selected several candidate fractions to screen for potential protective effects in PD *in vitro* models. In this study, we established a screening platform for PFs on immortalized human dopaminergic neurons stressed with neurotoxins. Through the initial screening paradigm, we observed improvements in neuronal survival and reduction of cellular ROS abundance upon PFs treatment. We validated our results in iPSC-derived dopaminergic neurons to evaluate PF effectiveness in a more clinically relevant model of PD. Our results demonstrate the multimodal activity of PFs and highlight the fact that PFs can alleviate specific contributors to the pathogenesis of aging-related diseases such as PD.

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Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.03/C2

Topic: C.03. Parkinson's Disease

Support: NIH NRSA Grant 5F31CA268947
Melanoma Research Alliance 936151
Michael J. Fox Foundation MJFF-022590

Title: Identifying the association between melanoma and Parkinson's

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Abstract: In order to identify cellular events that drive a shared risk for melanoma and Parkinson's, we developed a microscopy-based analysis pipeline centered on tissue clearing. We investigate GFP-tagged YUMM1.7 melanoma allografts in wildtype (WT) mice and in mice

carrying a knock-in mutation for one of the most common genetic risk factors for Parkinson's: *Lrrk2*-G2019S (GS). This point mutation increases the kinase activity of LRRK2 by 2-to-3-fold, but the downstream effects of such gain of function are poorly understood.
We clear and 3D reconstruct each melanoma allograft using non-toxic Life Canvas clearing reagents, light sheet microscopy, and Imaris software. After reverse-clearing the tumor, we immunolabel cell types of interest. Host melanocytes and sympathetic axons are visualized by Cre-dependent tdTomato expression driven by a tyrosine hydroxylase promoter.
We have found that host melanocytes are widely recruited to melanomas in WT and GS mice. Sympathetic innervation patterns are notably distinct between WT and GS melanomas: In GS melanomas, there is a higher area of sympathetic innervation than in WT melanomas. Furthermore, globally inhibiting LRRK2 kinase activity using Merck LRRK2 inhibitor-2 (MLi-2) in rodent diet at a concentration of 55 mg/kg/day reduces melanoma growth, an effect that is even stronger in GS mice. In cultured YUMM1.7 cells, MLi-2 reduces LRRK2 kinase autophosphorylation and downstream Rab8A phosphorylation, but it does not affect proliferation rates. These data suggest that LRRK2 kinase is active in melanoma allografts, but its impact on tumor growth is not directly mediated by melanoma cells. Instead, the impact of LRRK2 kinase on melanoma growth appears to require the tumor microenvironment and may be mediated by sympathetic innervation. Consistent with this idea, co-cultured sympathetic neurons and melanoma cells interact dynamically *in vitro*.
Investigating the impact of LRRK2-G2019S on the melanoma microenvironment will define key events in melanoma progression and reveal how changes in cellular dynamics drive shared risk between melanoma and Parkinson's.

Disclosures: P. Del Valle: None. **J. Bravo-Cordero:** None. **D.L. Benson:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.04/C3

Topic: C.03. Parkinson's Disease

Support: 1R01NS119690-01
P50 DA044121-01A1
R01 NS097901
NINDS 1F31NS115524-01A1

Title: Molecular and spatial transcriptomic classification of midbrain dopamine neurons and their alterations in a prodromal LRRK2^{G2019S} mouse model of Parkinson's disease

Authors: *C. P. ORAM[1], Z. GAERTNER[2], A. SCHNEEWEIS[3], C. BOLDUC[4], D. A. DOMBECK[5], L. PARISIADOU[6], J.-F. POULIN[1], R. AWATRAMANI[6];
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Chicago, IL; [3]Pharmacol. and Physiol., Georgetown Univ., Washington, DC; [4]The Neuro - McGill Univ., Montréal, QC, Canada; [5]Neurobio., Northwestern Univ., Evanston, IL; [6]Northwestern Univ., CHICAGO, IL

Abstract: Several studies have revealed that midbrain dopamine (DA) neurons, even within a single neuroanatomical area, display heterogeneous properties. In parallel, studies using single cell profiling techniques have begun to cluster DA neurons into subtypes based on their molecular signatures. Recent work has shown that molecularly defined DA subtypes within the substantia nigra (SNc) display distinctive anatomic and functional properties, and differential vulnerability in Parkinson's disease (PD). Based on these provocative results, we reasoned that a more granular understanding of these putative subtypes, and how they might be altered in PD models, is imperative. We developed an optimized pipeline for snRNASeq, and generated a high-resolution hierarchically organized map, now revealing the presence of three main families, and 20 molecularly distinct DA neuron subtypes. We integrated this data with spatial MERFISH technology to map, with high definition, the location of these subtypes in the mouse midbrain, revealing heterogeneity even within neuroanatomical sub-structures. Finally, we demonstrate that in the $LRRK2^{G2019SKI}$ preclinical mouse model of PD, subtype organization and proportions are preserved. Transcriptional alterations occur in many subtypes including those localized to the ventral tier SNc, where differential expression is observed in synaptic pathways, which might account for previously described DA release deficits in this model. Our work provides an advancement of current taxonomic schemes of DA neuron subtypes, a high-resolution view of their spatial locations, and their alterations in a prodromal mouse model of PD.

Disclosures: C.P. Oram: None. **Z. Gaertner:** None. **A. Schneeweis:** None. **C. Bolduc:** None. **D.A. Dombeck:** None. **L. Parisiadou:** None. **J. Poulin:** None. **R. Awatramani:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.05/C4

Topic: C.03. Parkinson's Disease

Support: Parkinson's Foundation Impact Award
NIH Grant R21-NS108771
William C. Rowland Jr. Parkinson's Research Fund

Title: Dysregulated anti-viral response in LRRK2 mice following infection with H1N1 Influenza induces Parkinsonism

Authors: *K. CROWTHER[1], R. J. SMEYNE[2], E. KOZINA[3];
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Abstract: Parkinson’s Disease (PD) affects approximately 1 million patients in the US. Clinically, PD is characterized by a myriad of both motor and non-motor symptoms. In the CNS, PD is characterized by progressive loss of the DA neurons in the SNpc; however, there is also a clear dysregulation in the immune system. The exact etiology of PD has long been debated, and current theories posit a role for gene x environment interactions. One of the most common PD-linked mutations occur in the LRRK2 gene, accounting for 15% of all cases. The incidence of this mutation suggests that the signaling pathways in LRRK2-mediated PD might be also affected in sporadic PD. LRRK2 is primarily expressed in peripheral immune cells, lungs, and brain. The most common LRKK2 mutation, G2019S, enhances LRRK2’s endogenous kinase activity, which has a multitude of downstream effects including the dysregulation of the immune system. However, LRRK2's low penetrance implies the need for an extra trigger to induce its pathogenicity. Given that viral infections, notably some strains of non-neurotropic Influenza, have sparked PD-like symptoms, we hypothesize that mutant LRRK2’s mode of action may be in the abnormalities in peripheral anti-viral response to these viruses. In this study, we sought to determine if the non-neurotropic 2009 pandemic Influenza virus (H1N1, A/CA/04/2009) was capable of synergizing with mutant LRRK2 to increase development of parkinsonian pathology. Using flow cytometry, immunohistochemistry, and multiplex immunoassays, we analyzed DA cell loss and central/peripheral immune responses in WT and G2019S LRRK2 mice following the infection with pandemic H1N1. We found that H1N1-infected G2019S LRRK2 mice developed: 1) a higher mortality rate and an elevated viral load in the lungs compared to WT mice, 2) a 20% SNpc DA cell loss 7-30 days post-infection (dpi), and 3) elevated neuroinflammation that wasn’t resolved as late as 60 dpi. Examination of the brain during this period of infection did not show any evidence for intraparenchymal viral proteins or immune cell infiltrates. We next used cre/flox technology to selectively knock-out mutant LRRK2 in innate immune cell sub-sets and found that a reduction of LRRK2 kinase activity in Cx3Cr1+ peripheral monocytes during viral infection rescued the SNpc DA neuron loss. The lack of direct viral infection or immune cell invasion suggests a critical importance of the signals that emanate from the peripheral immune system in the initiation of the neuropathological process. The ongoing studies in the lab seek to understand the mechanisms of how mutant LRRK2 influences immune responses secondary to viral infection in experimental PD.

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Poster

PSTR210: Parkinson’s Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.06/C5

Topic: C.03. Parkinson’s Disease

Support: NIH R01 Andrew West Lab

Title: Role of microtubules in the mediation of LRRK2 activity

Authors: *T. MALANKHANOVA[1], E. LEE[2], Z. LIU[2], E. XU[2], H. LI[2], S. STRADER[2], N. BRYANT[2], A. B. WEST[2];
[1]Pharmacol. and cancer Biol., Duke Univ., Durham, NC; [2]Duke Univ., Durham, NC

Abstract: Microtubule dysfunction is associated with several neurodegenerative disorders including Parkinson's disease (PD). Recent reports suggest microtubules may stabilize the kinase-active conformation of the LRRK2 protein kinase. LRRK2 activity is linked to PD risk, possibly through the phosphorylation of Rab protein substrates that can control vesicle and lysosome dynamics. Our studies in macrophages that have high levels of endogenous LRRK2, Rab substrates, and dynamic microtubules, genetic, and complementary pharmacological approaches reveal the underlying mechanisms of microtubule and LRRK2 interactions. Preliminary data suggests stable LRRK2 alignment along microtubules does not occur under physiological conditions and instead requires the over-expression of LRRK2 fused to a bulky N-terminal tag in combination with type II inhibitors. However, fast-scanning proximity ligation proteomics highlights prominent LRRK2 kinase-independent interactions with tubulins, which are not influenced by microtubule polymerization. Forced recruitment of LRRK2 to the outer surface of microtubules using a molecular trapping system does not affect LRRK2 kinase activity, whereas forced recruitment to the lysosome in macrophages strongly upregulates LRRK2 activity. Acute microtubule destabilization abolishes Rab positioning on the membranes and rescues the lysosomal stress-induced increases in LRRK2 activity and phosphorylated Rab10 levels. Restoration of GTP levels under microtubule destabilization conditions allows for normal LRRK2-mediated Rab phosphorylation. Together, these results suggest that microtubule polymerization does not have a direct effect on the mediation of LRRK2 activity and that stable binding to microtubules may be a consequence of over-expression and bulky N-terminal LRRK2 tags.

Disclosures: T. Malankhanova: None. **E. Lee:** None. **Z. Liu:** None. **E. Xu:** None. **H. Li:** None. **S. Strader:** None. **N. Bryant:** None. **A.B. West:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.07/C6

Topic: C.03. Parkinson's Disease

Support: Fonds Nationale de la Recherche Luxembourg
University of Luxembourg

Title: Parkinson disease associated G2019S LRRK2 mutation impairs mitochondrial morphology and contributes to metabolic imbalance in dopaminergic neurons

Authors: *C. LAHR, A. SKUPIN;
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Abstract: The G2019S mutation in the *LRRK2* gene is the most prevalent genetic risk factor in Parkinson's disease (PD). A major hallmark of PD, and specifically for the G2019S mutation, is mitochondrial dysfunction, leading to energetic deficits in dopaminergic neurons and their subsequent degeneration. Mitochondrial function is determined by their morphology, and mitochondria in PD conditions show increased fragmentation and sphericity. However, the underlying mechanisms of mitochondrial dysfunction, and particularly the link to intraorganellar ultrastructure remain elusive. We differentiated patient-derived induced pluripotent stem cells (iPSCs), carrying either the G2019S mutation in the *LRRK2* gene or the healthy control version, into midbrain dopaminergic neurons. We characterized the differentiation dynamics by integrative longitudinal multiomics analyses and immunofluorescence staining to identify PD-associated molecular and morphological impairments. This integration of molecular pathways and image analysis revealed key pathways triggering mitochondrial impairments. Preliminary electron microscopy data inidicate differences in mitochondrial ultrastructure caused by the mutation and provide new insights into the molecular mechanisms underlying the LRRK2-related mitochondrial pathology, which may guide new intervention strategies.

Disclosures: C. Lahr: None. **A. Skupin:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.08/C7

Topic: C.03. Parkinson's Disease

Support: ASAP-025188

Title: Dopamine neuron subtype-specific LRRK2 dysfunction in the nigrostriatal synapse

Authors: *C. CHEN[1], Z. GAERTNER[2], Q. HE[3], D. A. DOMBECK[4], R. AWATRAMANI[2], L. PARISIADOU[1];
[1]Pharmacol., Northwestern university, Chicago, IL; [2]Neurol., Northwestern Univ., Chicago, IL; [3]Northwestern Univ., Evanston, IL, ; [4]Neurobio., Northwestern Univ., Evanston, IL

Abstract: The death of dopamine neurons in the substantia nigra pars compacta (SNc) is the pathological hallmark of Parkinson's disease (PD). Within the SNc, cell loss in the ventral tier is more prominent than in the dorsal tier in post-mortem PD brains. LRRK2 is a common genetic target relevant to both familial and sporadic PD. LRRK2 is a common genetic target relevant to familial and sporadic PD, but the mechanisms by which it leads to dysfunction and death of DA neurons are largely unknown. To address this knowledge gap, the present study examines LRRK2 dysfunction in the most vulnerable DA neuron subtypes in a cell type specific manner.

Our team has developed a high-resolution classification scheme of dopamine neurons by single-nucleus RNA (snRNA) sequencing. We relied on snRNAseq to assess gene expression pattern changes across all DA neurons and within any given DA neuron subtype in the knock-in (KI) mice that express the LRRK2 G2019S pathogenic mutation. Transcriptional alterations were present in each subtype, including vulnerable ventral tier DA neurons where alterations were observed in synaptic pathways. Consistent with this finding, we employed 3D super-resolution microscopy to discover that the LRRK2G2019S mutation impacts the structure of DA release machinery in ventral and not dorsal—resilient dopamine axons. Specifically, we show an increase in the volume of Bassoon clusters (a marker of DA release sites), suggesting disorganization of these sites. This dysfunction is specific to the ventral tier (and not dorsal) dopamine neuron axons and is accompanied by a reduction in dopamine release from the dorsolateral axons in LRRK2 KI mice, as evidenced by fast scan voltammetry in ex vivo striatal slices. We are currently carrying out functional studies using optogenetics and genetically encoded dopamine sensors to investigate the diversity of dopamine release defects in these mice in vivo. Additionally, we are employing cell type-specific proteomics to identify the LRRK2 molecular targets involved in these regulations. Our study investigates the cell-autonomous dysregulations of LRRK2 in the most relevant dopamine neuron subtypes. Interrogating molecular, structural, and functional early and potentially reversibly synaptic deficits with LRRK2 mutations will provide comprehensive mechanistic insights and uncover new targets and pathways that could aid neuroprotective therapies in PD.

Disclosures: C. Chen: None. **Z. Gaertner:** None. **Q. He:** None. **D.A. Dombeck:** None. **R. Awatramani:** None. **L. Parisiadou:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.09/C8

Topic: C.03. Parkinson's Disease

Support: NIH R01 Andrew West

Title: Role of LRRK2 in peripheral immune cells in mediating dopaminergic neurodegeneration in mouse models of Parkinson's disease.

Authors: *S. STRADER[1], R. BODDU[1], A. B. WEST[2];
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Abstract: Role of LRRK2 in peripheral immune cells in mediating dopaminergicneurodegeneration in mouse models of Parkinson's disease.Samuel Strader, Ravindra Boddu, Andrew B. WestDuke Center for Neurodegeneration and Neurotherapeutics, Department ofPharmacology and Cancer Biology, Duke UniversityLRRK2-targeting

therapeutics have advanced into clinical trials in Parkinson's disease(PD). Several pre-clinical studies, mostly in mice and rats, have demonstrated thathyperactivation of LRRK2 kinase activity tends to exacerbate the damaging effects ofPD-associated α-synuclein, whereas LRRK2 repression or ablation may curtail α-synuclein neurotoxicity. With LRRK2 expression and activity in both brain resident cellsand high LRRK2 expression and activity in peripheral pro-inflammatory immune cellsthat include monocytes, macrophages, and neutrophils, it has been unclear whetherLRRK2 primarily exerts a modifying effect for α-synuclein neurotoxicity via expression incells in the brain or potentially contributions from immune cells originating from theperiphery. A better knowledge of the origins and mechanisms of LRRK2 neurotoxicitycould have implications in therapeutic strategies, since LRRK2-targeting antisensetechnology in clinical trials may not affect peripheral LRRK2 activity in the same manneras small molecules that distribute across the body.To help address this knowledge gap, we use a complementation strategy to createmosaic mice that remove *LRRK2* expression only in peripheral immune cells, or thereverse with *LRRK2* knocked out or overexpressed only in brain-resident cells withintact expression of *LRRK2* in peripheral immune cells. After reconstitution of themarrow and a return of the mice to immunological homeostasis, the mice received anintracranial injection with AAV-A53T α-synuclein into the SNpc to initiate progressivedopaminergic neurodegeneration known to be accompanied by neuroinflammation.Neurotoxicity and the infiltration of peripheral immune cells were evaluated in thestriatum and SNpc at both one- and three-months post-injection. Results from thesestudies, which are ongoing, will help clarify the role of both peripheral and centralLRRK2 expression relevant to α-synuclein-related neurodegeneration in mice. Wecontinue to evaluate the molecular pathways altered by LRRK2 activity in the CNS andin resident and peripheral immune cells to help elucidate the relationship betweenLRRK2 activity and α-synuclein-related neurodegeneration.

Disclosures: S. Strader: None. **R. Boddu:** None. **A.B. West:** None.

Poster

PSTR210: Parkinson's Disease: LRRK2 and Mitochondrial Mechanisms, Targets, and Pathways

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR210.10/C9

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: THOME Foundation

Title: Investigation of LRRK2 Activity Changes with Human APOE4 and P301S-tau Expression

Authors: *S. WANG, A. B. WEST;
Pharmacol., Duke Univ., Durham, NC

Abstract: Alzheimer's Disease (AD) is a neurogenerative disorder characterized by aggregation of hyperphosphorylated tau protein and loss of cholinergic neurons in the hippocampus and

piriform cortex. Several recent studies suggest tau aggregation in both AD and other tau pathologies may be correlated with phosphorylated Rab10 protein mediated by the LRRK2 protein kinase. One study identified increased levels of pT73-Rab10 with Tau in AD brains. LRRK2 is the only known kinase to phosphorylate T73 on Rab10, and knockout of LRRK2 eliminates pT73-Rab10 in the brain. Furthermore, hyperphosphorylated tau is common in brains associated with pathogenic LRRK2 mutations that increase pT73-Rab10 levels. LRRK2 is tied to neuronal lysosome function, and its expression is particularly high in certain activated innate immune cells such as macrophages, monocytes, neutrophils, and microglia. Preliminary data in mouse models suggests phosphorylation of Rabs also prevents Rabs from binding EHBP1L, which otherwise blocks interactions with Bin1, a validated susceptibility gene for AD. We predict that LRRK2 phosphorylation of Rabs in these neurons and immune cells in the brain may alter vesicle motility and cargo degradation, thus disturbing anti-inflammatory responses in immune cells and exacerbating damaging tau aggregation and the effects of APOE4 expression. We will utilize 3rd generation anti-sense oligonucleotides and advanced small molecule inhibitors in these models to target LRRK2 activity in attempts to ameliorate the damaging effects of tau aggregation in the brain. Further, the effects of APOE4 and APOE2 expression on LRRK2 activity will be measured to help understand whether APOE allelic differences regulate LRRK2 activity under pathological conditions. These studies will help clarify the potential for emerging LRRK2-targeting therapies for tau-associated neurodegeneration.

Disclosures: S. Wang: None. **A.B. West:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.01/C10

Topic: C.03. Parkinson’s Disease

Support: NIH/NS070577
NIH/OD024622
NIH NINDS/K23NS099380

Title: Exploring the functional roles of host immune responses in cell replacement therapy for Parkinson's disease

Authors: *T.-Y. PARK;
Harvard Med. Sch., Belmont, MA

Abstract: Parkinson’s disease (PD) is a progressive neurodegenerative disorder characterized by the loss of midbrain dopamine neurons in the substantia nigra, leading to pronounced motor symptoms such as tremor, rigidity, and bradykinesia, along with non-motor impairments. Previously, we reported the clinical implantation of patient-derived midbrain dopaminergic

progenitor cells (mDAPs), differentiated in vitro from autologous induced pluripotent stem cells, in a patient with idiopathic PD[1]. Although this study showed that using autologous mDAPs can avoid graft rejection, cell survival and functional recovery in the first patient were modest, suggesting that survival of mDA neurons may have been suboptimal even in autologous implantation. Thus, we investigated this issue and found that the surgical procedure itself, referred to as “needle trauma”, induced innate responses and killed the grafted mDA neurons. So, we developed a safer and more effective method by co-transplanting autologous regulatory T cells intra-striatally along with mDAPs[2]. We found that this novel method not only increased survival of grafted mDA neurons but also modulated the proliferative property of grafted mDAPs. However, the incomplete survival of TH^+-mDA neurons and the suboptimal behavioral recovery prompted us to investigate the mechanisms of action associated with the initial microenvironmental changes caused by needle trauma, which we hypothesized to critically influence cell survival. Our finding confirms that changes in the microenvironment during the initial stages of transplantation, such as the release of damage-associated molecular patterns, inflammatory cytokines, activation of glial cells, and infiltration of T/B cells, are crucial in determining the survival of the transplanted cells. We will discuss our current data to modulate these changes, with the long-term goal of further optimizing the functional outcomes of mDAP transplantation for PD.

Disclosures: T. Park: None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.02/C11

Topic: C.03. Parkinson’s Disease

Support: ANR-21-CE18-0025-01

Title: Nanovectors for lysosome-based therapeutic strategies against neurodegenerative diseases

Authors: R. KINET[1], S. NIETO[2], A. MUTSCHLER[2], A. GAUBERT[3], S. LECOMMANDOUX[2], ***B. DEHAY**[1];
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Abstract: Neurodegenerative disorders like Parkinson's disease (PD) have emerged as a critical health concern with these complex and age-related diseases, which are characterized by a selective neuronal vulnerability, including degeneration in specific brain regions hosting dopaminergic neurons and deposits of misfolded proteins. It has been proposed that dysfunction in the autophagy-lysosomal pathway (ALP) contributes to the buildup of pathogenic elements in

neurodegenerative conditions. Lysosomes play a crucial role in clearing out persistent proteins like α-synuclein and eliminating worn-out or impaired organelles such as mitochondria.Two potential avenues for restoring the ALP function to its natural state can be considered: increasing the quantity of lysosomes or enhancing the functionality of existing lysosomes. First, trehalose emerges as a promising candidate for neuroprotection against various neurodegenerative diseases. This natural disaccharide bears a twofold nature: an mTOR-independent ALP biogenesis enhancer and a chemical chaperone. Second, we demonstrated that using acidic nanoparticles (aNPs) made of poly (acid lactic-co-glycolic) (PLGA) could restore lysosomal pH through the hydrolytic chain scission of PLGA, providing lactic and glycolic acid units and function in several experimental models of lysosomal impairment. In this study, we have pioneered the development of novel nanosystem-based therapies, merging two molecules within a polymersome structure to cross the blood-brain barrier (BBB) and target dopaminergic neurons. We exploited the inherent properties of polymersomes as both nanocarriers and active agents to address acidification defects and potentially restore cellular function coupled with trehalose-based derivatives. The aim is, therefore, to study these dual-targeting nanovectors and their ability to modulate the ALP. In vitro, neuronal cultures will be treated with those NPs to validate their functional effects. In parallel, different routes of administration of these NPs will be tested in vivo (i.e., intracerebral, retro-orbital, and intranasal injections) on mice to assess brain biodistribution and internalization within dopaminergic neurons. These data suggest that strategies enhancing or restoring lysosomal-mediated degradation appear as tantalizing neuroprotective/disease-modifying therapeutic strategies and would be of major therapeutic interest for PD.

Disclosures: R. Kinet: None. **S. Nieto:** None. **A. Mutschler:** None. **A. Gaubert:** None. **S. Lecommandoux:** None. **B. Dehay:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.03/C12

Topic: C.03. Parkinson’s Disease

Title: Bioreactor-produced iPSCs-derived Dopaminergic Neuron-containing Neural Microtissues Innervate and Restore Motor Function in a Dose-dependent Manner in a Parkinson Rat Model

Authors: N. PRUDON[1], L. CORDERO ESPINOZA[1], M. ABARKAN[1], B. V. GURCHENKOV[1], M. LEPLEUX[2], N. PUJOL[3], L. MILVOY[1], F. MONCAUBEIG[4], H. WURTZ[1], L. POINÇOT[1], A. JONCKEAU[3], J. PLETENKA[1], E. LUQUET[1], K. SCHMIT[3], L. PIOUCEAU[1], S. GUILBERT[1], L. MANACHE-ALBERICI[1], G. DABEE[1], T. DUFOURD[5], J. SCHROEDER[1], K. ALESSANDRI[6], ***E. BEZARD**[7], E. FAGGIANI[8];
[1]TreeFrog Therapeut., Canéjan, France; [2]CNRS, Bordeaux, France; [3]Treefrog Therapeut., Pessac, France; [4]R&D, Treefrog Therapeut., pessac, France; [5]Grenoble Inst. of Neurosciences -

INSERM U1216, LA Tronche Cedex, France; [6]US, Treefrog Therapeut., Cambridge, MA; [7]Inst. of Neurodegenerative Dis., Bordeaux, France; [8]Treefrog Therapeut., Bordeaux Cedex, France

Abstract: Objective To demonstrate the efficacy of a ready-to-graft 3D neural microtissue product - manufactured at large scale - as a therapeutically viable option to treat Parkinson's disease. **Background**A breadth of preclinical studies is now supporting the rationale of pluripotent stem cell-derived cell replacement therapies to alleviate motor symptoms in Parkinsonian patients. Replacement of the primary dysfunctional cell population in the disease, i.e. the A9 dopaminergic neurons, is the major focus of these therapies. To achieve this, most therapeutical approaches involve grafting single-cell suspensions of dopaminergic progenitors. However, a considerable number of cells die during the transplantation process, as cells face anoïkis. One potential solution to address this challenge is to graft solid preparations, i.e. adopting a 3D format. Cryopreserving such format remains a major pharmaceutical hurdle and is not exempt from causing delays in the time to effect, as observed with the use of cryopreserved single-cell dopaminergic progenitors. **Methods**We used a high-throughput cell-encapsulation technology coupled with standard bioreactors to provide a 3D culture environment that enables the directed differentiation of iPSCs into neural microtissues fit-for-cryopreservation. The product was characterized using orthogonal methods including flow cytometry, immunofluorescence labelling, RTqPCR and bulkRNAseq. Controlled doses (low, high, maximum feasible dose [MFD]) of microtissues were administered into the striatum of 6-OHDA lesioned nude rats for functional assessment. **Results**We demonstrate a scalable process to generate off-the-shelf cryopreserved iPSC-derived 3D neural microtissues containing a mixture of ventral mesencephalic dopaminergic neurons and dopaminergic progenitors. Upon administration, the neural microtissues innervate the lesioned striatum of hemiparkinsonian rodents with TH+ dopaminergic projections and lead to motor recovery in a dose-dependent manner by 16 weeks (MFD and high dose) and 20 weeks (low dose) respectively. **Conclusion**These data demonstrate proof-of-concept efficacy of the dopaminergic neuron-containing neural microtissue product and support the intention to pursue preclinical studies to assess its safety and efficacy as a cell therapy for Parkinson's disease.

Disclosures: N. Prudon: A. Employment/Salary (full or part-time):; TreeFROG Therapeutics. **L. Cordero Espinoza:** A. Employment/Salary (full or part-time):; TrEEFROG Therapeutics. **M. Abarkan:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **B.V. Gurchenkov:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **M. Lepleux:** None. **N. Pujol:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **L. Milvoy:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **F. Moncaubeig:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **H. Wurtz:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **L. Poinçot:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **A. Jonckeau:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **J. Pletenka:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **E. Luquet:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **K. Schmit:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **L. Piouceau:** A. Employment/Salary (full or part-time):; TrEEFROG Therapeutics. **S. Guilbert:** A. Employment/Salary (full or part-time):; Treefrog therapeutics. **L. Manache-alberici:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **G. Dabee:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **T. Dufourd:** A.

Employment/Salary (full or part-time):; TreeFROG Therapeutics. **J. Schroeder:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. **K. Alessandri:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TREEFROG Therapeutics. **E. Bezard:** A. Employment/Salary (full or part-time):; Motac neuroscience. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motac, Treefrog Therapeutics, SE Therapeutics. **E. Faggiani:** A. Employment/Salary (full or part-time):; TREEFROG Therapeutics.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.04/C13

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation Grant (MJFF-021969)
NIH T32 fellowship (T32 AG044402).

Title: Assessment of the 5HT1F receptor agonist, Lasmiditan, in Parkinsonian mice at early and advanced stages

Authors: *A. ISHII[1], M. J. CORENBLUM[2], J. R. MEREDITH[3], N. MENAKURU[4], P. WENE[5,6], R. SCHNELLMANN[7,8], L. MADHAVAN[2,9,8];
[1]Univ. of Arizona, Tucson, AZ; [2]Neurol., Univ. of Arizona, Tucson, AZ; [3]Undergraduate Program in Mol. Cell. Biol., The Univ. of Arizona, Tucson, AZ; [4]Undergraduate Program in Mol. Cell. Biol., Univ. of Arizona, Tucson, AZ; [5]Col. of Agr. and Life Sci., Univ. of Arizona, Tucson, AZ; [6]Undergraduate Program in Molecular Cellular Biology, University of Arizona, Tucson, AZ; [7]Pharmacol. and Toxicology, Univ. of Arizona, Tucson, AZ; [8]BIO5 Institute, University of Arizona, Tucson, AZ; [9]Pharmacology and Toxicology, University of Arizona, Tucson, AZ

Abstract: Parkinson's disease (PD) is an age-related progressive neurodegenerative disorder characterized by debilitating motor deficits and neurocognitive decline. Mitochondrial dysfunction is known to be key contributor to cellular dysfunction and death in PD. In this context, we have identified 5-HT1F receptors as novel mediators of mitochondrial biogenesis (MB), which is the process of producing new mitochondria. Our previous work in an acute 6-hydroxydopamine (6-OHDA) mouse model, showed that the administration of a specific 5-HT1F receptor agonist LY344864 enhanced MB and improved locomotor activity. The current study focused on assessing the therapeutic potential of the FDA-approved 5-HT1F receptor agonist, Lasmiditan, in a more chronic and progressive mouse model of PD (line 61 Thy1-ASyn mice). After determining the pharmacokinetic and pharmacodynamic (PK/PD) properties of Lasmiditan

in the mice, we studied its ability to counter the progression of PD pathology and symptoms by examining its effects in the context of early and late-stage disease in 4-5.5 months and 10-11.5 months old Thy1-ASyn mice. Specifically, we treated male Thy1-ASyn mice with Lasmiditan (1 mg/kg, IP, administered every other day for 1.5 months) or vehicle, and evaluated several motor and cognitive behaviors followed by tissue level cellular and molecular analyses. Behavioral evaluation in the younger early mice revealed improvements episodic memory (novel object recognition paradigm), spatial working memory (spontaneous alterations in a Y-maze) and reduction in anxiety (center entries in Open Field), but no significant improvements in motor function. Further analysis across multiple brain regions indicated that Lasmiditan amplified mtDNA in striatum, hippocampus, and substantial nigra (SN). Protein level analysis via western blotting showed an increase in some proteins (TFAM, NDIFS1, NDUFB8) but these changes were not uniform in all regions. Additionally, mitochondrial function assessment via the Seahorse oxidative phosphorylation assay showed improvements in several parameters in striatum and cortex. Western blotting and immunohistochemistry also showed a reduction in α-synuclein levels in the SN and cortex, and p-α-synuclein in the cortex. These results indicate Lasmiditan as potentially applicable to improving cognitive function at early stages of PD progression. We are currently analyzing 10-11.5 months old mice treated either daily or every other day with 1 mg/kg, IP Lasmiditan. and also correlating the behavioral phenotypes of individual mice with the cellular and molecular data.

Disclosures: A. Ishii: None. **M.J. Corenblum:** None. **J.R. Meredith:** None. **N. Menakuru:** None. **P. Wene:** None. **R. Schnellmann:** None. **L. Madhavan:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.05/C14

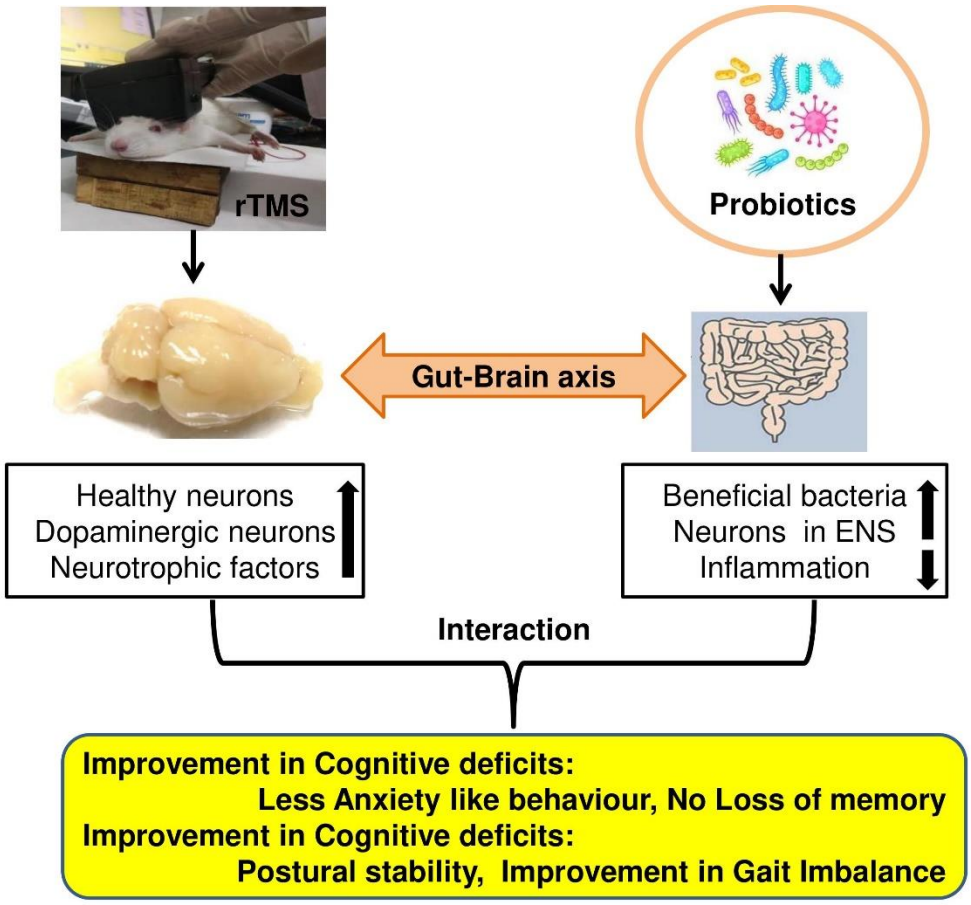
Topic: C.03. Parkinson’s Disease

Support: DSR-13
CSRI

Title: Probiotics and rTMS synergistically alleviate cognitive and motor deficits in 6-OHDA-induced Parkinson's disease rats

Authors: *S. SHARMA[1,2], S. JANGRA[3,2], S. JAIN[9,2], R. CHAUDHRY[1,4], S. DAS[10,2], T. C. NAG[11,5], S. SINGH[5], D. RADHAKRISHNAN[1,6], S. PANDEY[1,7], V. AHUJA[1,8], K. KOCHHAR[1,2];
[2]Physiol., [1]All India Inst. of Med. Sci., New Delhi, India; [3]All India Inst. of Med. Sci., Delhi, India; [4]Microbiology, [5]Anat., [6]Neurol., [7]Biostatistics, [8]Gastroenetrology, All India Inst. of Med. Sci., New Delhi, India; [9]All India Inst. of Med. Sci. Delhi, New Delhi, ; [10]All India Inst. of Med. Sciences, New Delhi, Delhi, ; [11]All India Inst. of Med. Sci. New Delhi, New Delhi.

Abstract: •Introduction: 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 Parkinson's disease (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 cognitive and motor functions, neuronal survival, expression of neurotrophic factors along with morphology of enteric nervous system in bilateral 6-OHDA rat model of PD. **•Material&Methods:** Adult male Wistar rats (280-320gm) were subjected to bilateral i.c.v. 6-OHDA injection. After 1-week rTMS (10 Hz, 20 min/day) and probiotics (270 µL daily) were administered for 4 weeks. Pre and post-intervention motor and cognitive performances, morphology of the brain and gut and expressions of tyrosine hydroxylase (TH), BDNF, GDNF were examined.**•Results:** 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 Hematoxylin & Eosin staining showed increased number of neurons in enteric ganglion after intervention. Expression of all the beneficial factors (BDNF and GDNF) enhanced along with increased TH expression. **•Conclusion:** Our findings indicate that that the administration of probiotics and rTMS improves the motor and cognitive functions of rats with PD. Plausibly, probiotics synergistically with rTMS may facilitate the remodelling of the gut-brain axis thereby improveing the function 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 neuromodulation of the brain-gut axis.



Disclosures: S. Sharma: None. **S. Jangra:** None. **S. Jain:** None. **R. Chaudhry:** None. **S. Das:** None. **T.C. Nag:** None. **S. Singh:** None. **D. Radhakrishnan:** None. **S. Pandey:** None. **V. Ahuja:** None. **K. Kochhar:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.06/C15

Topic: C.03. Parkinson's Disease

Support: Arnold and Mabel Beckman Foundation

Title: Ability of compliance-based treadmill exercise to modulate LID, motor performance and striatal neurochemistry in hemiparkinsonian rats

Authors: *S. VENKATESH[1], N. KINZONZI[2], A. CENTNER[3], H. HOLDEN[4], C. R. BISHOP[3]; [1]Binghamton Univ., Vestal, NY; [2]Binghamton Univ., Otisville, NY; [3]Psychology, Binghamton Univ., Binghamton, NY; [4]Integrative Neurosci., Binghamton Univ., Vestal, NY

Abstract: Parkinson's Disease (PD) is a neurodegenerative disease characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta leading to a hypokinetic state. Symptoms are best controlled with DA replacement therapy using levodopa (LD). Despite its benefits, chronic use of LD causes LD-induced dyskinesia (LID) typified by choreic and dystonic abnormal involuntary movements (AIMs). Preclinical and clinical research suggests that exercise can act as a disease-modifying factor in PD by normalizing DA signaling, thereby improving movement, and slowing LID development. This study evaluated this in a hemi-parkinsonian rat model. Sprague-Dawley rats received a unilateral injection of 6-hydroxydopamine into the left medial forebrain bundle and then were counterbalanced into equally lesioned sedentary and exercise groups using the forepaw adjusting steps (FAS) test. For 4 weeks, exercised animals underwent 35 mins of treadmill-controlled exercise and received a compliance score between 1-4. One-hour post-exercise, all animals were administered 4 mg/kg of LD for 3 weeks. On days 1, 7, 14, and 21 and rated on a scale of 1-4 based on their presentation of axial, limb and orolingual (ALO) AIMs. Additionally, rotarod testing was conducted 24 h prior to or following AIMs testing as an additional measure of motor performance. Following the 4-week testing period, FAS and rotarod were conducted 60 min after LD administration to assess differences in LD efficacy between sedentary and exercise groups. Behavioral results demonstrated that all animals had severe decrements in motor performance following lesion that were not reversed by exercise. Interestingly post-tests on LD revealed enhanced improvement in exercised rats. Moreover, exercise attenuated and delayed the onset of LID development. Post-mortem analyses using high-performance liquid chromatography showed severe DA loss in the lesioned striata of both groups, however only serotonin (5-HT) turnover was significantly higher in exercised vs. sedentary animals. Thus, treadmill exercise optimizes LD while reducing its side effects. While exercise may not restore DA levels, enhanced functional compensation may support its benefits when paired with DA replacement therapy.

Disclosures: S. Venkatesh: None. **N. Kinzonzi:** None. **A. Centner:** None. **H. Holden:** None. **C.R. Bishop:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.07/C16

Topic: C.03. Parkinson's Disease

Support: Instituto de Salud Carlos III FI21/000919
Aligning Science Across Parkinson's ASAP-020505
Instituto de Salud Carlos III PI20/00496

Title: Neuronal expression pattern of AAV9-PHPeB vector in nonhuman primate brain after striatal delivery via BBB opening with focused ultrasound: comparison with intraparenchymal injection case

Authors: *N. ESTEBAN GARCÍA[1,2,3,4], S. CHICA[1], K.-I. INOUE[5], T. BALZANO[1,3,4], A. REINARES SEBASTIÁN[1,3,4], I. TRIGO-DAMAS[1,3,4], M. CIORRAGA[1], N. MERCADO-GARCÍA[1], J. A. PINEDA-PARDO[1], J. A. OBESO[1,3,4], M. TAKADA[6], J. BLESA[1,4,3];
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Abstract: The blood-brain barrier (BBB) has been one of the main obstacles in the development of gene therapy against neurodegenerative diseases. Although the intraparenchymal injection for delivery of therapeutic viral vectors is a so-far-appreciated approach, this involves invasive and risky surgical procedures. Moreover, in brain disorders where treatments may require their simultaneous delivery into several brain regions, the direct intraparenchymal approach is more troublesome. The use of low-intensity transcranial focused ultrasound (tFUS) combined with microbubbles allows transient and focal opening of the BBB to deliver viral vectors into specific brain regions. Recently, we have reported tFUS-induced successful BBB opening and focal delivery of adeno-associated virus serotype 9-related vectors (i.e., AAV9-PHPeB vector) into several brain regions of adult macaque monkeys. However, an axonal transport capacity of the focally infused AAV9-PHPeB vector remains to be elucidated and is an important aspect for gene therapy trials. Here, we performed a detailed post-mortem analysis to examine neuronal expression of AAV9-PHPeB-GFP vector over the whole macaque monkey brain after its striatal delivery via BBB opening. We observed up to 70-fold more GFP+ neurons in the opened putamen compared with the contralateral non-opened putamen. In addition, a small number of GFP+ neurons were also found in cortical areas, bilaterally with an ipsilateral predominance. To evaluate whether the distribution of this remote neuron labeling corresponds to synaptically connected regions or simply to regions with greater permeability to the BBB, we made intraparenchymal injection of the same vector into the putamen of a macaque monkey. In this case, a large number of GFP+ neurons were seen at the injection site and, also, in other distal nuclei directly connected to this region. Still, we found a clear difference between the injection routes, with the intraparenchymally injected animal showing a much higher density of GFP+ neurons throughout all cortical areas and layers. Thus, the present study demonstrates a capacity of axonal transport of the AAV9-PHPeB vector in the macaque monkey brain. It also reinforces the feasibility of viral vector delivery following BBB opening using combined tFUS and microbubble technology. The less-invasive nature of this methodology could facilitate viral vector delivery for gene therapy and might allow early interventions to treat neurodegenerative disorders.

Disclosures: N. Esteban García: None. **S. Chica:** None. **K. Inoue:** None. **T. Balzano:** None. **A. Reinares Sebastián:** None. **I. Trigo-Damas:** None. **M. Ciorraga:** None. **N.**

Mercado-García: None. **J.A. Pineda-Pardo:** None. **J.A. Obeso:** None. **M. Takada:** None. **J. Blesa:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.08/C17

Topic: C.03. Parkinson's Disease

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Veterans Affairs ORD I01BX005015
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Veterans Bio-Medical Research Institute

Title: Moderate treadmill exercise slows dopaminergic neuron loss in a rat model of idiopathic Parkinson's disease

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Abstract: Idiopathic Parkinson's disease (PD) is a common neurodegenerative movement disorder affecting millions of people, beginning in the 6th decade of life. Effective symptom management for PD exists but effective disease modifying treatments that slow or stop progression are not yet available. Moderate exercise has been shown to slow age-related brain atrophy and exercise has also been implicated in the slowing of PD progression. Understanding the biological mechanisms driving this apparent neuroprotection could inform development of future treatments. In rodent and non-human primates, intracranial injection of preformed alpha synuclein fibrils (PFFs) initiates formation of authentic Lewy body-like inclusions (LBs), inflammation, trans-synaptic pathology spread, and neurodegeneration of dopaminergic neurons. This study consisted of 3 groups: monomeric sedentary surgery control, sedentary PFF, and exercise PFF, 10 per group. PFF injection into nigra of male Sprague Dawley rats 10 weeks of age initiated human like transsynaptic spread of pathology through interconnected brain regions. Using this PD model, we and others have previously shown that by 3 months post injection, robust PD-like pathology is achieved with 10-20% neuron loss in nigra and LB deposition in surviving dopaminergic neurons. Treadmill exercise was therefore initiated at 3 months post PFF injection, to determine if moderate exercise can provide meaningful neuroprotection and prevent further neurodegeneration and pathology spread. The experimental exercise group underwent 10 m/min warmup for 5 minutes followed by 30 min of forced run at 20 m/min 5 days a week for a month. Stereological estimation of dopaminergic neurons in nigra, their projections in the striatum, and analysis of p-serin 129 and pTau (AT8) positive LB composition were performed.

20% reduction (one-way ANOVA p = 0.0007) of dopaminergic neuron loss in nigra was achieved by the 6-month endpoint. No differences in LB burden were detected in nigra or striatum. Studies are underway to better understand the mechanisms affecting PD pathology during exercise.

Disclosures: V. Delic: None. **M. Guzman:** None. **A. Sokratian:** None. **A.B. West:** None. **B.A. Citron:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.09/C18

Topic: C.03. Parkinson's Disease

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SRA from Seelos therapeutics
Michael J. Fox Foundation for Parkinson's Research grant MJFF-021362

Title: Snca-targeted epigenome therapy for parkinson's disease: pre-clinical proof of concept in a pd mouse model.

Authors: *B. O'DONOVAN[1], J. RITTINER[2], D. HODGSON[1], B. KANTOR[2], O. CHIBA-FALEK[1];
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Abstract: Elevated SNCA levels are causative in Parkinson's Disease (PD) pathogenesis, while normal physiological levels of SNCA are essential to maintain neuronal function. Patients with SNCA triplication and duplication suffer from familial early onset from of PD, suggesting a therapeutics window of <30%. We aim to translate mechanistic knowledge of SNCA dysregulation towards the development of epigenome therapy for PD targeting SNCA expression. We developed all-in-one lentiviral vector (LV) carrying the deactivated CRISPR/(d)Cas9, a selected gRNA targeted at SNCA-intron1 and synthetic repressor molecules. Previously we provided in vitro proof-of-concept for the efficacy and efficiency of our LV-dCas9-repressor system in human iPSC-derived 'aged' dopaminergic neurons from a PD-patient with the SNCA triplication. We showed downregulation of SNCA-mRNA and protein levels that led to the rescue of disease-related pathological phenotypes including, mitochondrial dysfunction, neuronal-cell death, DNA damage and nuclear deficits. We have now moved forward into in vivo validation studies. Our PD mouse model was generated by inducing expression of human SNCA with an AAV-A53T-human SNCA vector that comprised of the mutated human-SNCA coding sequence fused with its native promoter/intron 1 region. We performed bilateral stereotactic injection of the AAV-A53T-human SNCA vector into the mouse

substantia nigra (SN), the left SN was co-injected with the therapeutic LV-dCas9-repressor and the right SN was co-injected with the control inactive LV-dCas9 vector. Our LV-repressor system successfully reduced human SNCA protein by 46% in the SN. Pathological examinations showed a robust reduction in Ser129-phosphorylated SNCA (71%) and in aggregated SNCA protein (72%). Higher expression of tyrosine hydroxylase in both the SN (1.2 fold) and the striatum (2.6 fold), suggests a greater retention of dopaminergic neurons. Safety studies showed no abnormalities in well-being criterions, no weight loss, normal blood counts, serum chemistry and liver histology, and limited biodistribution. Further, we also show that out LV-repressor system significantly improves motor impairment in the PD mouse model. In conclusion, our novel CRISPR/dCas9-based technology offers the unprecedented tool to modify a particular epigenetic mark resulting in effective fine-tuned reduction of SNCA expression levels sufficient for reversing PD-associated pathological hallmarks and motor impairment. This study provided an in vivo proof-of-concept for advancing our innovative epigenome editing-based system to a clinical trial as the next-generation PD epigenome therapy.

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Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.10/C19

Topic: C.03. Parkinson’s Disease

Support: DoD Grant PD210014
NINDS NS105826

Title: Graft-induced dyskinesia (GID): the unsolved mystery of the curious side effect of dopamine neuron transplantation

Authors: *C. SZAROWICZ[1,2], N. M. MERCADO[3], M. CAULFIELD[2], J. STANCATI[2], M. VANDER WERP[2], C. E. SORTWELL[2], S. BOEZWINKLE[4], T. J. COLLIER[2], K. STEECE-COLLIER[2];
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Abstract: Graft-induced dyskinesia (GID) was an unanticipated side effect described in a subpopulation of individuals with Parkinson's disease (PD) following dopamine (DA) neuron transplantation. Although various theories of the underlying cause have been posited including the role of neuroinflammation, recipient age, and graft cell composition, mechanisms that underlie GID remain unclear and controversial. For the past two decades, our lab has been investigating possible factors implicated in GID development. Currently, we have been interested in the role of genetic risk factors, focusing on a common single nucleotide polymorphism (SNP), rs6265, found in the gene for brain-derived neurotrophic factor (BDNF). Because BDNF is critical for grafted DA neuron maturation/function and host striatal dendritic spine integrity, we hypothesized that decreased BDNF release associated with rs6265 would impair proper maturation and synapse formation of grafted DA neurons, resulting in aberrant GID. Using CRISPR knock-in rs6265 rats, we have demonstrated that homozygous rs6265 rats engrafted with wild-type (WT) DA neurons do uniquely develop GID in contrast to their WT host counterparts. GID in rs6265 rats was correlated with vesicular glutamate transporter 2 (VGLUT2) expression in grafted DA neurons, suggestive of an immature glutamatergic-DA co-transmission phenotype. As a follow-up, we explored whether aging interacts with rs6265 to further exacerbate GIDs since aging is the primary risk factor for PD. Findings from this study indicated that aging was indeed permissive to GID, regardless of genotype, with greatest GID severity still observed in rs6265 rats. We also examined the impact of rs6265 in both host and donor, transplanting WT or rs6265 donor neurons into WT or rs6265 hosts. Curiously, rs6265 with WT DA neurons remained the only host-donor combination to exhibit statistically significant GIDs. Most recently, in studies aimed at mitigating the GID in an environment of decreased extracellular BDNF, we infused exogenous BDNF into rs6265 rats engrafted with WT DA neurons. BDNF infusion, however, increased GIDs in these animals compared to vehicle-infused controls. Moreover, GID was correlated with an increased dopamine transporter (DAT)/tyrosine hydroxylase (TH) ratio, indicative of excess DA in the grafted striatum; a phenomenon that has also been associated with GID in grafted PD patients. Collectively, our studies point to roles of DA/glutamate co-transmission and/or excess DA release in the development of GID. Continuing investigation of GID mechanisms will be critical in optimizing clinical outcomes of cell transplantation as a therapy for PD.

Disclosures: C. Szarowicz: None. **N.M. Mercado:** None. **M. Caulfield:** None. **J. Stancati:** None. **M. Vander Werp:** None. **C.E. Sortwell:** None. **S. Boezwinkle:** None. **T.J. Collier:** None. **K. Steece-Collier:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.11/C20

Topic: C.03. Parkinson's Disease

Title: Improvement of Parkinson's Disease Motor Symptoms and Prevention of Dopaminergic Neuronal Degeneration by CJRB-302, a Live Biotherapeutic Product (LBP), derived from Healthy Human Gut Microbiota

Authors: *N.-R. LEE[1], S. KIM[1], J. IM[1], S. KANG[1], H. KIM[1], H. PARK[1], J. KWON[1], Y. PARK[1], J. LEE[1], S. JO[1], J. KWAK[1], Y. OH[1], Y.-S. KIM[2];
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder clinically characterized by cardinal motor symptoms such as tremor, slowed body movements, and postural instability, along with various prodromal non-motor symptoms. Pathological hallmarks include cytoplasmic inclusions, Lewy bodies, and the degeneration of dopaminergic neurons in the substantia nigra (SN) of the midbrain. Accumulating evidence supports the role of the microbiota-gut-brain axis, a bidirectional pathway, in PD pathogenesis. Clinical fecal microbiota transplantation (FMT) studies have shown 5 to 13 points improvement in UPDRS motor scores. However, FMT presents various disadvantages such as the potential for pathogen transfer and difficulty in reproduction. Thus, in this study, we investigated the therapeutic potential of CJRB-302, a live biotherapeutic product (LBP) identified from healthy human gut microbiota. CJRB-302 reduced alpha-synuclein-induced inflammation response and MPP^+-induced cytotoxicity in differentiated SH-SY5Y cells, and increased expression of dopaminergic neuronal markers such as dopamine transporter and tyrosine hydroxylase . Furthermore, oral administration of CJRB-302 significantly ameliorated dopaminergic neuronal death in the SN of mice injected with1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Accordingly, orally administered CJRB-302 alleviated motor deficits caused by MPTP. Notably, oral administration of CJRB-302 concurrently with MPTP injections, a therapeutic model, significantly attenuated the motor deficit, with motor activity even comparable with that of the control group mice. Ongoing research aims to delve deeper into the mechanisms behind CJRB-302's beneficial effects to elucidate its mode of action in PD pathogenesis. Moreover, clinical trials are imperative to establish the efficacy and safety of CJRB-302 in human PD patients. In conclusion, our study furnishes compelling evidence endorsing CJRB-302 as a promising new drug candidate for the development of innovative PD treatments.

Disclosures: N. Lee: A. Employment/Salary (full or part-time):; CJ Bioscience. **S. Kim:** A. Employment/Salary (full or part-time):; CJ Bioscience. **J. Im:** A. Employment/Salary (full or part-time):; CJ Bioscience. **S. kang:** A. Employment/Salary (full or part-time):; CJ Bioscience. **H. Kim:** A. Employment/Salary (full or part-time):; CJ Bioscience. **H. park:** A. Employment/Salary (full or part-time):; CJ Bioscience. **J. Kwon:** A. Employment/Salary (full or part-time):; CJ Bioscience. **Y. Park:** A. Employment/Salary (full or part-time):; CJ Bioscience. **J. Lee:** A. Employment/Salary (full or part-time):; CJ Bioscience. **S. Jo:** A. Employment/Salary (full or part-time):; CJ Bioscience. **J. kwak:** A. Employment/Salary (full or part-time):; CJ Bioscience. **Y. Oh:** A. Employment/Salary (full or part-time):; CJ Bioscience. **Y. Kim:** F. Consulting Fees (e.g., advisory boards); CJ Bioscience.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.12/C21

Topic: C.03. Parkinson's Disease

Title: Whole-brain 3D quantification of alpha-synuclein spreading in a mouse model of Parkinson's disease.

Authors: *Y. GALLERO-SALAS[1], F. SØRENSEN[2], A. THOMSEN[3], J. HECKSHER-SØRENSEN[4], H. HANSEN[2];
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Abstract: Background & Aim: Progressive spreading of alpha-synuclein (αSyn) aggregates in the brain plays a key role in the prodromal phase of Parkinson's disease (PD). While several preclinical models of synucleinopathies have been developed for studying the neurotoxicity of αSyn aggregate spreading, they remain to be systematically explored with regards to early pathological events that could potentially be targeted to slow down or prevent progression of PD. Using whole-brain light sheet fluorescence microscopy (LSFM), the present study aimed to provide a detailed 3D map of progressive pathological αSyn spreading and tyrosine hydroxylase (TH) expressing neurons and projections in the αSyn pre-formed fibril (PFF) mouse model of PD. **Methods:** 8-weeks old C57BL/6 male mice received two unilateral intrastriatal injections of murine αSyn PFFs (5 µg per injection) in the dorsal striatum. Mice were terminated at 1, 4, 8-, 12-, 16-, or 26-weeks post-injection (wpi), whole-brains were immunolabelled for αSyn phosphorylated at serine-129 (pS129-αSyn, marker of αSyn aggregation) and TH, cleared (iDISCO+) and scanned using lighsheet fluorescence microscopy (LSFM). AI-based computational analysis enabled automated whole-brain mapping and quantification of pS129-αSyn and TH fluorescence intensity across 840 individual brain regions using a custom mouse brain atlas. **Results:** Distinct spatiotemporal phases of endogenous αSyn aggregate spreading observed in αSyn PFF mice over time. The phases included progressive spread of αSyn aggregates to primary seeding regions (amygdala, substantia nigra, and several cortical areas) and secondary seeding regions (entorhinal area and hippocampal formation) based on their interconnectivity to the injection site (striatum). In parallel, TH expression was progressively downregulated in the nigrostriatal pathway, suggesting axonal damage in terminal areas preceding dopaminergic neuronal loss in the αSyn PFF mouse model. **Conclusion:** We here report a complete whole-brain map of αSyn aggregate spreading in an industry-standard αSyn PFF mouse model of PD. The anatomical complexity of αSyn aggregate spreading in the model underscores the unique applicability of whole-brain 3D LSFM imaging to fully capture spatiotemporal dynamics in αSyn and TH expression, making the model highly instrumental for the evaluation of therapeutic modalities that may prevent αSyn aggregate spreading and dopaminergic neuronal loss.

Disclosures: Y. Gallero-Salas: A. Employment/Salary (full or part-time):; Gubra. **F. Sørensen:** A. Employment/Salary (full or part-time):; Gubra. **A. Thomsen:** A. Employment/Salary (full or

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Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

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Topic: C.03. Parkinson's Disease

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Title: Effects of rAAV-based gene delivery on in vitro terminal differentiation of hESC-derived human ventral midbrain dopaminergic progenitors

Authors: *D. H. BELIGALA[1], T. SUBRAMANIAN[2], K. VENKITESWARAN[3]; [1]Dept. of Neurol., Univ. of Toledo, Toledo, OH; [2]Neurology, Neurosci. and Bioengineering, Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH; [3]Dept. of Neurol., Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH

Abstract: Given its potential application as a regenerative therapy for Parkinson's disease, the generation of mesencephalic dopaminergic (mesDA) neurons from human embryonic stem cells (hESCs) has garnered special attention. However, there hasn't been much research done on how dopaminergic grafts affect basal ganglia electrophysiology. Our lab has previously studied the graft-host connectivity and circuit level changes in basal ganglia using mouse fetal ventral mesencephalic (FVM) transplants in parkinsonian rats. Our current focus is to address this question with human ventral midbrain dopaminergic progenitors derived from hESCs using the 16-day protocol published by Sara Nolbrant et al. (2017). This process requires recombinant adeno-associated virus (AAV)-based gene delivery into the mesDA progenitor cells in vitro before the transplantation. The aim of this study is to examine the effects of rAAV-based gene delivery on in vitro terminal differentiation of hESC-derived human ventral midbrain dopaminergic progenitors and to optimize the rAAV transduction protocol. For the terminal maturation of mesDA progenitors, the Nolbrant protocol recommends re-plating the cells at days 16-21 at a density of 155,000 cells/cm2 (i.e., 50,000 cells per well in a 96-well plate) on Lam-111-coated plates in B27 medium supplemented with BDNF (20 ng/ml), AA (0.2 mM), GDNF (10 ng/ml), db-cAMP (500 μM) and DAPT (1 μM). In our study, the cells to be plated in each well were incubated for 45 mins with 0.3 μl of rAAV8/Ef1a-mcherry-IRES-WGA-Cre viral vector with a titer of 1.4 X 1012 vg/ml before plating at 155,000, 232,500, 310,000 and 387,500 cells/cm2 at day 18 for terminal differentiation. Our results indicate that the cell survival is very low (only 12.9% after 24 hrs) when plated at 155,000 cells/cm2 with rAAV transduction. In contrast, an increased cell survival rate was observed with all three higher plating densities. The

percentage cell survival after 24 hrs were 71.3%, 88.2% and 97.1% with 232,500, 310,000 and 387, 500 cells/cm2 cell plating densities, respectively. However, the cells plated at 387,500 cells/cm2 didn't show transgene expression owing to the culture becoming overconfluent by the end of terminal differentiation. Our findings indicate that, it is imperative to increase the cell density of mesDA grafts if the procedure calls for AAV-based gene delivery prior to transplantation into animal models.

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Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

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Topic: C.03. Parkinson's Disease

Support: NIH NS127037
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Postma Family

Title: Motivational state after deep brain stimulation in a 6-OHDA rat model of early Parkinson's disease

Authors: R. ROMAY-TALLON[1], C. M. GONZALEZ[1], A. D. BROWN[1], T. NAPIER[2], ***A. E. KIRBY**[1];
[1]Neurolog. Sci., Rush Univ., Chicago, IL; [2]Psychiatry and Behavioral Sci., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Rationale. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an efficacious treatment for motor deficits in Parkinson's disease (PD), but it remains unclear why STN-DBS disrupts motivation in some patients. Here, we tested the role of STN glutamatergic efferents that engage the limbic (ventral) pallidum (VP) on motivated behaviors in a rat model of early PD using optogenetic stimulation.
Methods. Anesthetized Sprague Dawley male rats (n=9) underwent bilateral injections of (i) 6-OHDA (7.5ug/2uL) into the dorsolateral striatum and (ii) a viral vector that expresses in glutamatergic neurons a channelrhodopsin modified for fast neuronal activation (rAAV5-CamKII-(T159C/E123T)ChR2-EYFP) into the STN; an optic fiber was permanently implanted above each STN. Following recovery, forelimb stepping quantified PD-like akinesia. Motivational state was measured by a sucrose splash test (SP) to quantify self-care, and a novel object recognition test (NOR) to quantify interest in novelty. Behavioral assessments were performed before and after surgery. At post-surgery days 28-35, the rats underwent two DBS sessions (465 nm, 130Hz) per day. On the last study day, rats received DBS and were killed

90min later. Placement of optic fibers tips, and staining for glutamatergic elements (GFP) and activated neurons (cfos) were verified immunohistochemically.
Results. Striatal 6-OHDA decreased forelimb stepping by ~70% and increased latency to first step by 4-fold, confirming a PD-like phenotype. DBS rescued both deficits. In the SP, DBS reduced time spent grooming by 40%, and caused a trend toward decreased time with the novel object in the NOR. To pilot functional specificity of these putative motivational behaviors, we compared cfos staining in the VP between rats with STN opsin staining and fiber placement in the limbic (ventral medial; vm) *versus* basal ganglia (dorsolateral; dl) domains (n=2 each). VP cfos was greater in vmSTN rats, consistent with limbic activation. vmSTN rats also exhibited ~60% greater reduction in NOR and enhanced sensitivity to repeated DBS in the SP test. By contrast, DBS-induced motor improvements were similar between vmSTN and dlSTN rats.
Conclusions. Optogenetic STN-DBS improved PD-like motor deficits and reduced motivated behaviors. Preliminary results indicate that engaging limbic-oriented STN glutamatergic efferents may be particularly relevant to DBS-induced motivational deficits.
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Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

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Topic: C.03. Parkinson's Disease

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Title: Hydrogen-bonded organic framework nanoparticles enabled sono-optogenetics for Parkinsonian rats

Authors: *I. PYATNITSKIY[1,2], W. WANG[2], K. TANG[2], L. E. FENNO[3], S. R. SANTACRUZ[2], H. WANG[2];
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Abstract: Cell-type-specific activation of parvalbumin (PV)-expressing neurons in the external globus pallidus (GPe) through optogenetics has shown promise in facilitating long-lasting

recovery from movement dysfunction in mice with Parkinson's disease (PD). However, its translational potential is hindered by adverse effects stemming from the invasive implantation of optical fibers into the brain. In this study, we developed a non-invasive optogenetics approach utilizing focused ultrasound-triggered mechanoluminescent nanotransducers to enable remote photon delivery deep into the brain for genetically-targeted neuromodulation. These mechanoluminescent nanotransducers consist of sonosensitized hydrogen-bonded frameworks and chemiluminescent L012, serving as a nanoscale light source through ultrasound-induced cascade reactions. This system offers high ultrasound-triggered brightness and long-lasting light emission, facilitating repeatable deep brain stimulation. Our sono-optogenetics technology demonstrated effective modulation in the mouse motor cortex for limb motion control and activation of PV-GPe neurons for rescuing movement dysfunction in dopamine-depleted PD rats. Using a 6-hydroxydopamine (6-OHDA)-induced hemiparkinsonian rat model (four animal groups, n=5 per group), we demonstrated the alleviation of movement dysfunction through cylinder and apomorphine-induced rotation tests. Similar to the effects of optogenetic stimulation in PV-GPe neurons, our sono-optogenetics yielded notable improvements in movement dysfunction and (1) increased paw usage with a sustained increase in contralateral touch percentages lasting over 15 minutes following PV-GPe focused ultrasound stimulation during cylinder test, and (2) a significant reduction in the apomorphine-induced rotation rate. This approach demonstrates a pathway for achieving genetically-targeted and non-invasive neuromodulation for PD treatment, with potential applications in non-human primate models and clinical settings.

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Poster

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Program #/Poster #: PSTR211.16/C25

Topic: C.03. Parkinson’s Disease

Title: Hirsutella sinensis mycelia exhibits anti-neuroinflammatory benefit in MPTP-induced mouse model of Parkinson’s disease

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Abstract: Parkinson's disease (PD) is a neurodegenerative disease characterized by the loss of dopaminergic neurons, often resulting in tremors, bradykinesia, and gait difficulties. Many studies demonstrate that activated microglia trigger neuroinflammation in the brain, leading to the loss of dopaminergic neurons. However, the exact mechanism of neuroinflammation in PD is still unknown. Hirsutella sinensis mycelia extract (HSM) has been found to possess anti-

inflammatory properties by attenuating inflammasome activation and the release of pro-inflammatory cytokines. The therapeutic application and mechanism of HSM in neuroinflammation remain unclear. In the present study, the MPTP-induced mouse model of PD was used to investigate the therapeutic potential of HSM. HSM alleviated inflammation in MPTP-induced mice by decreasing microglia activation, NLRP3 inflammasome and the release of proinflammatory cytokines. Moreover, HSM reduced endoplasmic reticulum stress and the apoptosis pathway. Treatment of HSM decreased the protein level of α-synuclein in the prefrontal cortex of MPTP-induced PD mice. These findings suggest that HSM ameliorates motor impairments and α-synuclein accumulation, endoplasmic reticulum stress, and neuroinflammation in the MPTP-induced mouse model of PD. Our results indicate that HSM may exhibit therapeutic effects for PD.

Disclosures: K. Wu: None. **C. Chiu:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

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Program #/Poster #: PSTR211.17/C26

Topic: C.03. Parkinson's Disease

Support: NIH R61NS112441
NIH U01AG074960-01A1

Title: A Bioengineered Levodopa Live-Bacterial Platform Achieves a Favorable Therapeutic Profile in a Preclinical Model of Progressive Dopaminergic Neurodegeneration of Parkinson's Disease

Authors: *P. PADHI[1], A. OTTO[2], A. GEORGE[3], G. ZENITSKY[4], H. JIN[3], V. ANANTHARAM[1], A. KANTHASAMY[5], G. J. PHILLIPS[6], A. G. KANTHASAMY[5]; [1]Pharmacol. and Physiol., Univ. of Georgia, Athens, GA; [2]Interdepartmental Toxicology, Univ. of Georgia, Athens, GA; [3]Univ. of Georgia, Athens, GA; [4]Pharmacol. and Physiol., Univ. of Georgi, Athens, GA; [5]Physiol. and Pharmacol., Univ. of Georgia, Athens, GA; [6]Dept. of Infectious Dis., Univ. of Georgia, Athens, GA

Abstract: Levodopa (L-DOPA)/benserazide is the gold-standard-of-care (SOC) therapy for Parkinson's disease (PD). However, huge limitations arise from the drug's inherent unfavorable pharmacokinetic properties and non-continuous pulsatile-like delivery, which result in aberrant motor fluctuations. To overcome drug-induced 'ON-OFF' motor complications, we designed and demonstrated the *in vivo* feasibility of a bioengineered human probiotic, *E. coli* Nissle 1917 (EcN), engineered to continuously produce and deliver L-DOPA from L-tyrosine using a synthetic hpaB/C enzymes integrated into the EcN chromosome. We previously demonstrated that our L-DOPA-bacterial live-biotherapeutic (LDBL) system is capable of producing and

delivering L-DOPA in a continuous, sustained manner that achieves a favorable pharmacokinetic and therapeutic profile. Herein, we extend our evaluation of this innovative biological therapeutic in the MitoPark PD rodent model of progressive degeneration following 12 weeks of chronic administration of our LDBL with the aromatic decarboxylase inhibitor benserazide. We present findings from neurochemical, biochemical, and molecular profiling in the brain and observations from the plasma metabolome and fecal metagenome. These results were paired with assessments of motor phenotypes and gastrointestinal function to understand the benefits of our LDBL over SOC. Our findings suggest that the sustained L-DOPA delivery produced by our LDBL platform greatly enhances key motor performance metrics, resulting in elevated dopamine neurochemical levels in the striatum and hippocampus, along with elevated norepinephrine in the frontal cortex. Beneficially, repeated administration of LDBL lowered the abundance of an L-DOPA metabolizing gut bacteria, *Enterococcus faecalis*. Moreover, sustaining elevated L-DOPA levels did not induce microglial or astrocytic activation. Finally, the bacterial metagenome and metabolomic findings revealed unique profiles compared to traditional pharmacological therapy. Together, our results further demonstrate the long-term effectiveness of our LDBL platform as an innovative, non-conventional anti-Parkinsonian therapeutic. (NIH R61NS112441, U01AG074960-01A1). **Disclosure:**VA and AGK have equity in PK Biosciences and Probiome Therapeutics. All other authors have no conflict of interest.

Disclosures: P. Padhi: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiome Therapeutics. **A. Otto:** None. **A. George:** None. **G. Zenitsky:** None. **H. Jin:** None. **V. Anantharam:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiome Therapeutics. **A. Kanthasamy:** None. **G.J. Phillips:** None. **A.G. Kanthasamy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Probiome Therapeutics.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.18/Web Only

Topic: C.03. Parkinson's Disease

Support: STI 2030-Major Projects (2022ZD0208600)
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the National Natural Science Foundation of China (31371001)

Title: Long-term Electrical Motor Cortex Stimulation Alleviates Motor Dysfunction in a Mouse Model of Parkinson's Disease

Authors: R. WANG[1,2], Y. NING[4,2], W. XI[2], H. HE[1], *S. ZHANG[2], *S. ZHANG[3];
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Abstract: Electrical motor cortex stimulation (MCS) has been demonstrated to improve motor dysfunctions in patients with Parkinson's Disease (PD) for over two decades. This suggests that it could be a viable supplement to clinical deep brain stimulation. However, the lack of clarity regarding its mechanisms and the ongoing debate over its efficacy limit its translation into clinical practice. Here, we employed a mouse model of PD to assess the therapeutic impact of long-term MCS on motor dysfunction and to investigate its underlying mechanisms mediated by hyperdirect pathway.Fifteen adult male C57BL/6J mice were utilized in the study, with the PD model being induced through unilateral injection of the neurotoxin 6-hydroxydopamine into the medial forebrain bundle. Post-model establishment, mice were randomly allocated into a sham group, an MCS treatment group, and a hyperdirect pathway inhibition group. MCS treatment for the MCS treatment group and the hyperdirect pathway inhibition group commenced 24 hours after model induction and lasted three weeks, utilizing cathodal, high-frequency biphasic stimulation (130Hz, 400μs, 150μa). Behavioral assessments, including the cylinder test, pole test, and apomorphine-induced rotation test, were conducted at the end of weeks 1, 2, and 3 to evaluate the amelioration of motor impairments. The MCS treatment group exhibited significantly superior performance in the cylinder test ($p = 0.020$), pole test ($p = 0.011$), and apomorphine-induced rotation test ($p = 0.008$) compared to the sham group. Notably, significant enhancements in motor functions were observed as early as 1 or 2 weeks in the cylinder test ($p = 0.028$), pole test ($p = 0.003$), and rotation test ($p = 0.041$). Following inhibition of the hyperdirect pathway with Clozapine N-oxide, mice subjected to long-term MCS showed significantly less behavioral improvement in the cylinder test ($p = 0.039$) and pole test ($p = 0.041$) than those in the MCS treatment group. Our results suggest that long-term MCS effectively alleviates motor dysfunction across various behavioural assays in a PD mouse model. This intervention markedly enhances motor capabilities at the early stage of PD progression. Furthermore, the activation of the hyperdirect pathway contributes to the long-term protective effects observed with MCS. This study provides evidence that MCS is an efficacious therapeutic approach for motor dysfunctions in PD, offering insights into the mechanisms underlying its treatment.

Disclosures: R. Wang: None. **Y. Ning:** None. **W. Xi:** None. **H. He:** None. **S. Zhang:** None. **S. Zhang:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.19/C27

Topic: C.03. Parkinson’s Disease

Support: Aligning Science Across Parkinson´s [ASAP 020505]

Title: Effect of focused ultrasound subthalamotomy in non-human primate mptp model

Authors: *A. REINARES SEBASTIÁN[1], R. RODRIGUEZ-ROJAS[2], J. A. PINEDA-PARDO[1], J. BLESA[2], I. TRIGO DAMAS[1], J. A. OBESO[2];
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Abstract: Focused ultrasound (FUS) is an imaging-guided method for creating therapeutic lesions in deep-brain structures. In the case of Parkinson´s disease (PD), the decision of when to intervene can be conceptualized within different time frames being the prodromal stage the ideal option to maximize the possible impact of an effective disease modifying therapy. Thus, we intend to evaluate the effect of the early subthalamotomy guided by FUS in a non-human primate MPTP model. Two monkeys (*macaca fascicularis*) were used in this study. They were treated with one dose of MPTP (0,5mg/kg) to cause mild dopaminergic depletion before unilateral FUS subthalamotomy. Subsequently, they were submitted weekly to one dose of MPTP until motor manifestations appeared. Monkeys were monitored with behavioral test and Kurlan scale along with progressive dopaminergic depletion produced by MPTP. The animals were euthanized, the brain tissue was analyzed through immunohistochemical procedures and the number of TH+ neurons was quantified in the substantia nigra by stereology. After subthalamotomy procedure one of the animals developed choreic dyskinesia on the treated hand suggesting a correct location of the lesion in the subthalamic nucleus. The second animal experienced improvement in the behavioral test on the treated side, which continued throughout the intoxication period. The immunohistochemical analysis corroborates the correct location of the lesions in both animals. The number of TH+ neurons of substantia nigra shows no changes comparing the treated hemisphere and the non-treated one. The subthalamotomy guided by FUS implies a therapeutic advance in comparison with traditional surgical procedures by its incisionless nature. We observed similar motor effects and benefits as the ones observed in clinical trials. The approach can allow to design monkey studies aiming to show a putative benefit of early correction of basal ganglia abnormalities associated with dopaminergic depletion, including neuronal survival in the substantia nigra pars compacta.

Disclosures: A. Reinares Sebastián: None. **R. Rodriguez-Rojas:** None. **J.A. Pineda-Pardo:** None. **J. Blesa:** None. **I. Trigo Damas:** None. **J.A. Obeso:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.20/C28

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: SMASH Dementia
Program of Excellence: Iowa Neuroscience Institute/ Roy J. Carver Charitable Trust

Title: The impact of alpha-synuclein pathology in locus coeruleus on cell morphology and arousal behavior of mice.

Authors: *K. GUPTA[1], K. DENIZ[2], R. THANGAVEL[3], B. KREITLOW[4], Y. LIU[4], C. KRUSEMARK[6], A. M. SLOAN[7], D. PRUITT[8], J. C. GEERLING[9], G. M. ALDRIDGE[5]; [1]Dept. of Neurology, Univ. of Iowa, Iowa city, IA; [2]Univ. of Iowa, Iowa City, IA; [3]Univ. of Iowa, Iowa city, IA; [4]Univ. of Iowa, Iowa City, IA, ; [5]Neurol., Univ. of Iowa, Iowa City, IA; [6]university of Iowa, Fairmont, MN; [7]Vulintus, Westminster, CO; [8]Univ. of Texas at Dallas, Richardson, TX; [9]Neurol., Univ. of Iowa (Hospital and Clinics), Iowa City, IA

Abstract: Cognitive fluctuations are a defining feature of Dementia with Lewy Body (DLB) and are marked by unpredictable shifts in cognitive abilities and alertness. Understanding these fluctuations is crucial for accurate diagnosis, tailored treatment, and enhancing quality of life. DLB and Parkinson's disease dementia (PDD) share a common pathology: aggregated alpha-synuclein (α-syn), found in regions linked to attention and arousal, like the locus coeruleus (LC). LC neuron loss and α-syn-positive inclusions are observed in PD, PDD, and DLB, but it's unclear if early pathology in arousal network contributes to or precedes attention and arousal deficits. We hypothesized that α-syn aggregates in the LC and associated network nuclei lead to dysfunction and aberrant activity of LC neurons. To test this, we compared two α-syn pathology models in the LC: 1) viral overexpression of human α-syn and 2) mouse pre-formed α-syn fibrils (PFFs), assessing their impact on neuronal morphology and behavior. First, we assessed the regional spread to connected arousal nuclei in PFF injected mice and compared it to mice injected with a control viral-induced fluorescent marker. We observed p-syn positive inclusions within the LC at 14 days post-injection with PFF, spreading by 3 months to key arousal nuclei (dorsal raphe, parabrachial, VTA, etc.) and cortical/subcortical regions involved in attention and arousal (prelimbic, cingulate, basal forebrain, hypothalamus, thalamus). Next, we explored how α-syn affects arousal levels across different times of day and environmental conditions in mice injected with PFFs or viral α-syn overexpression compared to controls. Both treatment groups showed increased activity after encountering a novel object and/or new environments during their sleep phase. Mice with α-syn overexpression showed delayed return to sleep in contrast to PFF mice that exhibited normal return to sleep based on activity tracking estimates. We also evaluated anxiety-like behavior using the elevated plus maze and found a trend towards increased exploration in the open arm in PFF injected mice. Intriguingly, all behaviors showed increased variability in outcomes within the PFF-treated group, necessitating further exploration of pathological correlates like aggregate location and neuronal loss. Moreover, to better understand cognitive fluctuations we are working with Vulintus to adapt our STOP (Select side To Obtain Prize) task to a novel home cage automated tracking system, called the STOP-homecage. This setup dispenses water rewards to freely moving mice upon successful trials and is designed to allow frequent measurements of timing and behavioral flexibility.

Disclosures: K. Gupta: None. **K. Deniz:** None. **R. Thangavel:** None. **B. Kreitlow:** None. **Y. Liu:** None. **C. Krusemark:** None. **A.M. Sloan:** None. **D. Pruitt:** None. **J.C. Geerling:** None. **G.M. Aldridge:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.21/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Grant (PJT-169197)

Title: Lipidomic analyses in a pre-tangle rat model to develop a peripheral blood biomarker of disease progression

Authors: *A. M. JANES[1], C. FLYNN[1], R. BROWN[2], Q. YUAN[1];
[1]Div. of BioMedical Sci., [2]Dept. of Biochem., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Alzheimer's Disease (AD), a chronic neurodegenerative condition, is characterized by memory loss, behavioural impairments, and personality changes. AD commonly involves the hyperphosphorylation of tau proteins inside neurons, giving rise to "pre-tangle" formations that evolve into insoluble neurofibrillary tangles (NFTs). This tau pathology is linked to progressive cognitive decline, with pre-tangles commonly emerging in the locus coeruleus (LC) of the brainstem by age 20. Consequently, impeding the progression from pre-tangle tau pathology to NFTs represents a significant window for disease intervention. One novel therapy in AD is the manipulation of the gut-brain axis by probiotic supplementation. AD patients may exhibit gut dysbiosis: an imbalance in gut microbial species. In conjunction with blood-brain barrier impairments in AD, the result is the passage of bacterial metabolites (e.g. lipopolysaccharides, neuroinflammatory cytokines) into the brain, promoting disease through neuroinflammation. By fostering a healthy gut microbiome, probiotics can decrease tau hyperphosphorylation, improve intestinal and blood-brain barrier integrities, and improve AD-associated blood lipid dysregulations. It is not known, however, whether probiotics could exert their beneficial effects in pre-clinical AD, potentially delaying AD progression. Currently, AD diagnosis is dependent on clinical symptoms and the detection of biomarkers in the cerebrospinal fluid. The latter, however, involves an invasive and inaccessible collection procedure. Here, we aim to develop peripheral blood biomarkers capable of detecting pre-clinical signs and monitoring treatment effects. Blood lipidomics, which offer insights into lipid metabolism associated with various aspects of disease progression, hold promise in the development of these biomarkers. Our laboratory recently established the first pre-tangle tau rat model which closely recapitulates the progression of pre-clinical AD pathology as outlined by Braak, by seeding a human pre-tangle tau mimic in the rat LC. Upon development of pre-tangle pathology, the rats were administered Probiotic-4, a mixture of *Bifidobacterium* and *Lactobacillus*, via their drinking water. Blood lipidomic analyses were performed by serum lipid extractions, followed by electrospray ionization-mass spectrometry. Preliminary findings suggest increased inflammation in pre-tangle animals as well as altered cholesterol metabolism, with possible probiotic repair of the latter.

Moving forward, we aim to use this method to correlate brain and blood lipids and suggest peripheral biomarkers of disease progression.

Disclosures: A.M. Janes: None. **C. Flynn:** None. **R. Brown:** None. **Q. Yuan:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.22/C30

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 1R35 GM118182
NIH Grant 1R01AR083159-01

Title: Crh-positive neurons in the bnst control il1-mediated inflammatory responses.

Authors: *O. HASHIMOTO[1], T. HEPLER[1], A. TYNAN[1], K. J. TRACEY[1,2], S. S. CHAVAN[1,2];
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Abstract: The brain maintains homeostatic conditions, including an inflammatory condition, in the body through neuronal communication with peripheral organs. The bed nuclei of the stria terminalis (BNST), comprised of 16 subnuclei, plays a crucial role in maintaining homeostasis by integrating descending cortical information with ascending interoceptive and exteroceptive information. However, whether and how the BNST regulates immune responses is unclear. Here we describe the role of the BNST in regulation of IL-1β-mediated inflammatory responses. 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 identified BNST neurons that respond to IL-1β challenge. Chemogenetic reactivation of these IL-1β-responsive neuronal subsets in the BNST is sufficient to broadly retrieve the IL-1β -induced responses including tachycardia and systemic inflammation. These results suggest that 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

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.23/C31

Topic: D.03. The Chemical Senses

Title: Preconditioning of the zebrafish immune system confers protection after olfactory sensory neuron lesion

Authors: *B. E. EBENDICK[1], C. A. BYRD-JACOBS[2];
[1]Western Michigan Univ., Kalamazoo, MI, MI; [2]Dept Biolog Sci., Western Michigan Univ., Kalamazoo, MI

Abstract: Phagocytic microglia are the main mediators of immune function in the brain and are activated by damage. They are stimulated into a proinflammatory state to clear debris, then anti-inflammatory microglia promote rebuilding. Less injurious, transient stimuli can also affect immune function in a way that may confer protection against future damage, a phenomenon called preconditioning. Most preconditioning evidence relates to cardio-protective effects after transient ischemic events but also has been noted in other organ systems and various animal models. This study used saline injections into the skull as a potential immune stimulus to determine whether the olfactory bulb response to lesioning of the olfactory epithelium (OE) would be reduced or enhanced. Adult zebrafish were injected with sterile saline into the telencephalic ventricle and/or lesioned with detergent infusion of the OE. Preconditioning saline injections were given 1 day prior to lesioning, and concurrent saline injections were given during the same surgery as the lesion. Damage was visible in the olfactory bulb following death of sensory neurons in the OE; target glomeruli became less organized as they lost innervation. Morphological damage to 3 different glomeruli was rated on a 9-point scale. The quality and quantity of fibers in conserved glomerular structures were assessed at 1-, 4-, and 7-days post-lesion (dpl) by observing antibody-labeled axons in whole brains using confocal microscopy. Changes in amount of damage and time to recover to untreated control morphology were compared between lesion-only controls and saline-treated fish. Consistent with previous work from our lab, lesion-only fish exhibited high levels of damage early that resolved by 7 dpl. The saline concurrent group exhibited more damage compared to control at each time point, but still moved toward recovery by 7 dpl. Saline preconditioning resulted in less damage overall compared to control or concurrent injections. Zebrafish offer a unique insight into damage and recovery of neurons and may also be a useful model elucidating the protective effects of preconditioning in an unexplored organ system. Further analysis will include exploration of molecular pathways and effects of immune-modulating drug injections. This research may provide insight into mitigating neural damage, leading to novel approaches in medicine and adding to our understanding of preconditioning as a protective mechanism.

Disclosures: B.E. Ebendick: None. **C.A. Byrd-Jacobs:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.24/C32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant K08NS093130
NIH Grant R21EB029139
Whitehall Foundation
McKnight Foundation

Title: Recombinant Adeno-associated virus (AAV) infection induces DNA damage and neurodegeneration in the murine and marmoset brain

Authors: *D. ENTERRIA-MORALES[1], A. DOAN[1], A. KARVE[1], A. P. GONZALEZ[1], A. J. MARTINEZ[1], M. C. JOYA[1], E. DUONG[1], X. YU[1], B. L. TSE[1], N. SOUTIPAN[1], M. WILLARDSON[1], T. SARRAF SABEDOT[4], J. JONES[4], A. LEFEVRE[2], V. SINGH[2], J. LAGERWALL[1], J. SCHLACHETZKI[3], J. MERTENS[1], C. T. MILLER[2], F. H. GAGE[4], M. SHTRAHMAN[1];
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Abstract: Recombinant adeno-associated virus (AAV) has been widely used as a viral vector to study mammalian biology and for human gene therapy. However, a number of recent animal and clinical studies have reported significant AAV toxicity, particularly in the central nervous system (CNS). In this work, we demonstrate atrophy and neurodegeneration in the mouse hippocampus resulting from chronic AAV infection. Injection of AAV, but not saline or empty AAV capsid, results in dose-dependent neuronal toxicity at experimentally relevant viral titers as low as $3E10^{12}$ GC/mL. AAV-induced neurodegeneration is progressive, with loss of $NeuN^{+}$ cells observed beginning at 6 weeks post-injection and severe atrophy and cell loss in multiple subfields observed at 12 weeks post-injection. Similar neurodegenerative changes are observed in the marmoset brain in response to AAV injection at these doses. In addition, we show that AAV infection induces a marked increase in expression of the DNA damage repair markers gamma-H2AX and 53BP1 in hippocampal neurons starting at 4 weeks post-injection. This matches the timeline for the AAV-induced inflammatory response, which includes activation of microglia, astrogliosis, and infiltration of peripheral T-cells. We also characterize the effect of AAV toxicity on synaptic plasticity and hippocampal-mediated learning and memory in these mice. Together, these findings suggest that AAV is an intriguing and tractable model system to study viral-induced DNA damage and neurodegeneration and expands on recent reports in both experimental and clinical contexts describing AAV-induced toxicity in the CNS.

Disclosures: D. Enterria-Morales: None. **A. Doan:** None. **A. Karve:** None. **A.P. Gonzalez:** None. **A.J. Martinez:** None. **M.C. Joya:** None. **E. Duong:** None. **X. Yu:** None. **B.L. Tse:** None. **N. Soutipan:** None. **M. Willardson:** None. **T. Sarraf Sabedot:** None. **J. Jones:** None. **A. Lefevre:** None. **V. Singh:** None. **J. Lagerwall:** None. **J. Schlachetzki:** None. **J. Mertens:** None. **C.T. Miller:** None. **F.H. Gage:** None. **M. Shtrahman:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.25/C33

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Modeling long-covid and neurological changes with mouse-adapted sars-cov-2

Authors: *Y. QU[1], Z. ZHAO[2];
[1]USC, LA, CA; [2]Physiol. and Neurosci., USC, Los Angeles, CA

Abstract: Alzheimer’s disease (AD) is a common progressive dementia, the neurological condition is represented by amyloid-β deposits and neurofibrillary tangles. The progression of AD is related to microglia activation and neuroinflammatory state. Post-acute Sequelae of COVID-19 (PASC), or long COVID, still troubles patients even after several months post infection, the neurological impacts are much more severe in AD patients, which calls for in-depth mechanistic studies. Here, we develop a long COVID model in mice with the mouse adapted (MA) strain of SARS-CoV-2, allowing us to examine the impact of SARS-CoV-2 on neurological changes over time, particularly neuroinflammation and microglia dysfunction. We demonstrated that high level of accessary protein ORF3a can enter the mouse brain even in the absence of viral invasion, which is highly consistent with the ability of ORF3a in hijacking the host exosomes for the long-distance communication of viral protein. In addition, we found that ORF3a, once being up-taken, can activate microglia and disrupt its phagocytosis and homeostasis, represented by the increase of amoeboid-like microglia in SARS-CoV-2^{MA} infected mouse model. The lipid content of microglia was altered, including elevated number and size of intracellular lipid droplets, which subsequently increase the production of proinflammatory cytokines such as TNF-α, IL-1β and IL-6. In addition, we utilized adeno-associated viral vector for lung-specific expression of ORF3a with intratracheal intubation method and found AAV5-ORF3a induced neuroinflammation and behavioral changes in mice at 2 weeks post-infection. Some mice showed depressive-like behaviors, which were worsened between overtime. Therefore, our data showed that ORF3a and host exosome system play an important role in neurological changes associated with long-COVID.

Disclosures: Y. Qu: None. **Z. Zhao:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.26/C34

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RS-2022-00141392

Title: Implantable miniaturized liquid crystal polymer stimulator for deep brain stimulation in the ventral posterolateral nucleus (VPL)

Authors: *W. MUN[1], C. KOH[1], S.-H. AHN[2], J. JEONG[3], H. JUNG[1];
[1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Electric and Computer Engin., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Pusan Natl. Univ., Yangsan, Korea, Republic of

Abstract: Introduction: Deep brain simulation (DBS) is a technic that uses electrical pulse to modulate the specific brain area associated with diseases. In the case of intractable pain, deep brain stimulation (DBS) is one of the promising treatment options for those with high resistance to medication or other neurosurgery attempts. In this study, we present a miniaturized head-mounted DBS based on liquid crystal polymer (LCP). The key feature of the LCP electrode is its thin profile, which is only one-tenth of the thickness of conventional DBS electrodes. A miniaturized and fully implantable DBS system that allows untethered small animals to move freely while receiving electrical stimulation would open up a series of possibilities in neural engineering.
Methods: To evaluate the effect of the proposed DBS system, we used an in vivo behavioral test on a rat model of neuropathic pain model called spared nerve injury (SNI) method which is reliable and highly responsive pain model. For pain relief, we inserted electrode at ventral posterolateral nucleus (VPL). We varied the stimulation amplitudes at 130 Hz and 60 μs and observed differences in the threshold values for each amplitude.
Results: Mechanical threshold was 1.297 ± 0.2546 g before the DBS was applied. However, when a current stimulation of 100 μA was applied, the threshold increased up to 5.996 ± 1.413 g (* $p < 0.05$). When a current stimulation of 500 μA was applied, the threshold rose to 10.27 ± 1.236 g (*** $p < 0.001$). When 1 mA amplitude was applied, the threshold was similar to that on stimulation with a current of 500 μA (10.24 ± 1.236 g; *** $p < 0.001$).
Conclusion: This study presents a fully implantable miniaturized DBS device for rat that uses biocompatible LCP film as a substrate and packaging material. It enables compact and lightweight miniaturization, and compatibility with standard micromanufacturing processes. But we need several challenges to successfully translate to the clinical stage. First, migration to larger wafers (>8 inches in diameter) is required to accommodate for the human models. Second, integration of recording functionality to monitor brain signals in real-time during implantation surgery is expected to ensure accurate electrode placement.

Disclosures: W. mun: None. **C. Koh:** None. **S. Ahn:** None. **J. Jeong:** None. **H. Jung:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.27/C35

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Vision Restoration Initiative - Gilbert Family Foundation

Title: Retinal ganglion cell survival, axon degeneration, and tumor formation in a Neurofibromatosis type 1 mouse model: foundation for preclinical tests.

Authors: *S. DE LIMA[1], A. MONAVARFESHANI[2], S. YU[3], P. CASEY-CAPLAN[4], G. DUQUE SALAZAR[5], T. ZHANG[3], Y. ZHU[6], D. H. GUTMANN[7], J. R. SANES[8], Z. HE[9], L. I. BENOWITZ[10];
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Abstract: Neurofibromatosis Type 1 (NF1) is an autosomal dominant disorder that predisposes individuals to tumor formation in both the peripheral and central nervous systems (PNS and CNS). One of the most common tumors in NF1 occurs along the optic pathway causing vision loss. The mechanisms by which optic pathway glioma (OPG) cells affect axons from retinal ganglion cells (RGCs) are still unknown. In the present study, we performed a longitudinal characterization in a mouse model for Neurofibromatosis type 1 optic pathway glioma (NF1-OPG) and confirmed earlier reports of pre-chiasmatic tumor formation, however, we demonstrated that there is a susceptibility for the glioma to be formed in the periphery of the optic nerve and discovered that this results in a loss of RGCs in the periphery of the retina. The rate of peripheral RGC loss varied from 28-47%. Surprisingly, we also identified similar levels of RGC loss in animals that were haploinsufficient for Neurofibromin (Nf1 flx/ko). Both NF1-OPG and NF1 flx/ko presented with tumor formation, axon degeneration, and decreased visual evoked potential (VEP) responses. Further investigation into the glial populations of the glioma, through immunohistochemistry and snRNA Seq showed an increase in the population of oligodendrocyte precursor cells (OPC), astrocytes, mature oligodendrocytes, and microglia in animals from both genotypes. The g ratio analysis showed a defect in myelination, with axons showing both demyelination and thick/decompaction of myelin, as well as axons with normal myelin profiles. We show here that the onset of RGC death is slow compared to tumor formation and loss of signal conduction (VEP), with significant cell death only happening in 32-week-old mice, ca. 20 weeks after the appearance of the glioma. In most of the cases studied here, we observed that the formation of the optic glioma is asymmetric, with normal optic nerves, retinas, and VEPs on the unaffected side. These findings are critical for future studies that will investigate potential therapies for NF1-OPG and support the decision-making for the proper time window to target tumor formation, RGC survival, oligodendroglia changes in the glioma, and myelination defects.

Disclosures: S. de Lima: None. **A. Monavarfeshani:** None. **S. Yu:** None. **P. Casey-Caplan:** None. **G. Duque Salazar:** None. **T. Zhang:** None. **Y. Zhu:** None. **D.H. Gutmann:** None. **J.R. Sanes:** None. **Z. He:** None. **L.I. Benowitz:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.28/C36

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: ISF Grant 2034/16

Title: Developmental trajectories of neuroinflammatory, neuroanatomical and behavioral abnormalities in female and male rats exposed to maternal immune activation in lactation reveal persistent neuroinflammation that may mediate the link between immune alteration in neonatal brain and the late emerging neural and behavioral phenotypes.

Authors: N. S. ALBELDA[1], R. BEN YEHUDA[2], E. N. SHAMIR[3], Y. PIONTKEWITZ[3], ***I. WEINER**[3,4];
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Abstract: Neuroinflammation is considered a pivotal developmental pathway to adult-onset neuropsychiatric disorders such as schizophrenia and depression as well as to abnormalities in maternal immune activation (MIA) models. However, it remains unclear whether it has a causal role in the etiopathogenesis of these disorders. One reason for this is a scarcity of developmental studies in preclinical research. Here we delineated developmental trajectories of neuroinflammatory, neuroanatomical and behavioral abnormalities in female and male offspring of nursing dams that were injected with the viral mimic poly-I:C (4mg/kg) or saline on PND4 (Arad et al 2017), with the aim of exploring whether neuroinflammation may mediate the link between MIA exposure and late-emerging neural and behavioral phenotypes. We used quantification of cells immunoreactive for Iba1 to examine microglia density in hippocampus (HIP) and prefrontal cortex (PFC) from MIA exposure till adulthood (PNDs 4,5,21,33,48,70,90). We used in vivo longitudinal imaging to examine the volumes of whole brain (WB), striatum (STR), HIP and PFC, from adolescence to adulthood (PNDs 35, 50, 70 and 90), in parallel to behavioral testing. Since lactational MIA produces sex-dimorphic deficits (Arad et al 2017), we tested latent inhibition (LI) in males and forced swim test [FST] in females. We also tested whether the anti-inflammatory drugs N-acetylcysteine (NAC) and minocycline (MINO) co-injected with poly-IC prevent the effects of poly-I:C. We found that poly-I:C increased IL-6 in the dam's blood and milk and in the offspring HIP. Longitudinal imaging revealed that in both

sexes gray matter loss began in all the regions post-puberty, at PND 50, preceding the emergence of behavioral abnormalities (persistent LI and increased immobility in FST) at PND 70, recapitulating the clinical prodromes of SCZ and DPS. Microglia density in poly-I:C offspring was higher than controls' at each/most (in different experiments) of the assessed PNDs, preceding gray matter loss. Co-injection of NAC or MINO normalized IL-6 levels in dams' blood and milk, and prevented microglial, volumetric and behavioral abnormalities in the offspring of both sexes. To our knowledge these results provide the first demonstration that MIA-induced immune alteration in neonatal brains persists across offspring's lifespan and precedes gray matter loss, suggesting that developmental neuroinflammation may be a mechanism underlying the disruption of normal neuromaturation that leads to the onset of "symptoms". If neuroinflammation precedes and predicts changes in gray matter, this has implications for biomarkers and early intervention.

Disclosures: N.S. Albelda: None. **R. Ben Yehuda:** None. **E.N. Shamir:** None. **Y. Piontkewitz:** None. **I. Weiner:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.29/C37

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Children's Brain Diseases Foundation

Title: Transient choroid plexus response to onset of neurodegeneration in CLN2 mouse model

Authors: *M. DOMOWICZ, C. S. LAI, N. A. PEREZ CATALAN, N. B. SCHWARTZ; Univ. of Chicago, Chicago, IL

Abstract: Neuronal ceroid lipofuscinoses (NCLs) are among the most common neurodegenerative diseases in the pediatric population, with an incidence estimated at 2-4 per 100,000 live births. In particular, the classical late-infantile NCL (cLINCL/CLN2) in humans is caused by mutations in the CLN2/TPP1 gene. As all NCLs, CLN2 is characterized by lysosomal accumulation of fluorescent storage material (lipofuscin) and profound neurodegeneration that leads to a progressive visual deterioration, intellectual and motor decline, epilepsy, ataxia, spasticity, and premature death. Using global RNAseq analysis in cerebellum and forebrain of a well characterized mouse model of CLN2 we have stablished a transient increase in transcripts expressed in the choroid plexus (CP) during asymptomatic stages of the disease (1- to 2-month-old) compared to controls. We confirmed increases of secreted proteins, transporters, integral components of secretory epithelium membrane and cilia components in RNA purified from 2mo-old *Tpp1*-/- CP by qPCR. Interestingly, the CP epithelium in the lateral and fourth ventricle also exhibit the largest accumulation of lipofuscin, detected by mitochondrial ATP synthase subunit

C antibody (SMACS) at 2mo. To understand if these CP-associated-changes may lead to protection strategies or disease-associated decline of brain function, we analyzed morphological changes in CP and studied how the CP is associated with the initial inflammatory response in *Tpp1* KO. Staining for the cell membrane components (Aqp1, ATPase1A1) and F-actin highlight increased images of apical membrane protrusions, a classical display of apocrine secretion, in *Tpp1* KO CP. Furthermore, these apical membrane protrusions contain SMACS, suggesting that this increased apocrine secretion may be a mechanism to alleviate some of the aggregate buildup during the early stages of CLN2. Increase levels of CD68, and CD4 were detected in *Tpp1*-/- CP by qPCR. By immunohistochemistry, an increased number of borders associated IBA1+, CD68+ macrophages were also detected in mutant CP, supporting an increased inflammatory response possibly driven in part by the increased apocrine secretion and global functional activation of the mutant CP. This study highlights the CP as a dynamic and complex tissue with a highly regulated immune-secretory response tightly link to the neurodegenerative processes.

Disclosures: M. Domowicz: None. **C.S. Lai:** None. **N.A. Perez Catalan:** None. **N.B. Schwartz:** None.

Poster

PSTR211: Neurodegeneration and Neuroinflammation Therapeutic Strategies: Preclinical Animal Models II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR211.30/

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Conacyt :712328

Title: Blue corn tortilla influences hippocampal cell density at an early age in rat offspring exposed to lipopolysaccharide

Authors: *P. F. GONZÁLEZ NIETO[1], M. ALVARADO[2], R. I. GUZMÁN-GERÓNIMO[3], L. T. HERNANDEZ SALAZAR[2], J. F. RODRÍGUEZ-LANDA[2];
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Abstract: Prenatal infections may be related to neurodevelopmental disorders in the early ages of the offspring, because, in these infections, the presence of lipopolysaccharide (LPS) endotoxin present in some enterobacteria, causes the production of molecules involved in the infectious process, which may alter neurodevelopment, since they have been related to the decreased expression of enzymes important for placental function. However, some foods have anti-inflammatory properties due to their compounds, such as blue corn tortilla, due to its anthocyanin content. Therefore, the objective of this work was to evaluate the effect of blue corn tortilla on the hippocampal cell density of offspring at the age of 7 days (P7) exposed to LPS during gestation. Eighteen male offspring were analyzed, which were distributed in 3 groups: control

(CTL), LPS-G (offspring of mothers exposed to LPS during gestation) and LPS-G TNM (offspring of mothers exposed to LPS during gestation and consumption of tortilla). The tortilla was administered the whole gestation period, administering an equivalent of 6 mg/kg of anthocyanins and 10 mg/kg of polyphenols. The nixtamalization process was carried out in a microwave with the addition of gallic acid, to improve the retention of anthocyanins and their biological function. LPS (50 µg/kg) was administered on embryonic days 10, 12 and 14. Cell density was evaluated in the different areas of the hippocampus: dentate gyrus (GD), Cornu ammonis (CA1, CA2 and CA3), for which Nissl stain was performed. Cell counting was performed using "ImageJ" software and was expressed as the number of cells per unit area (cells/mm 2). In the results obtained, the most affected areas of the hippocampus were the dentate gyrus and the CA2 zone, showing in the GD a significant decrease of 21% in the LPS-G group compared to the CTL group and a decrease of 28% in relation to with LPS-G TNM. On the other hand, in the CA2 zone a decrease of 50% was obtained in the LPS-G group compared to CTL and a 29% in the LPS-G TNM group compared to CTL. It is important to highlight that the decrease in cell density in the DG has a greater impact on memory consolidation processes, because it is an important area for the neuron formation processes (neurogenesis), besides being the main point of cortical sensory input, essential for memory and learning processes, while CA2 has been mostly related to social interaction. Therefore, it can be said that the administration of blue corn tortilla processed by microwave and added with gallic acid restores the damage caused by LPS in the DG at early ages. CONAHCYT :712328.

Disclosures: P.F. González Nieto: None. **M. Alvarado:** None. **R.I. Guzmán-Gerónimo:** None. **L.T. Hernandez Salazar:** None. **J.F. Rodríguez-Landa:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.01/C38

Topic: E.09. Motor Neurons and Muscle

Support: RO1AG067758
RO1AG078129

Title: Refining a progressive resistance exercise model and examining its short-term effects in mice

Authors: *C. D. BRENNAN[1], Z. WILLIARD[1], W. ARNOLD[2], F. B. DARVISHI[3], A. ROSHANI DASHTMIAN[4];
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Abstract: Sarcopenia, or age-related loss of muscle mass and strength, is a major contributor to loss of independence in older adults. Traditionally, sarcopenia has been considered a primary failure of skeletal muscle function and atrophy, but growing evidence suggests that both muscle and neurological factors contribute. Sarcopenia not only significantly impairs physical function but also contributes to increased mortality. While progressive resistance exercise (PRE) is a key management strategy, its effectiveness varies among older adults. Limited research has explored resistance exercise in preclinical models, which are essential for understanding aging mechanisms and improving interventions. The two aims of this project were: Experiment 1) Refine a previously described resistance training model and Experiment 2) Investigate neural and muscular impacts of short-term PRE in mice. In experiment 1, two devices for PRE exercise interventions, a weighted cart on an incline and weighted pulley system, were designed, fabricated, and compared for reliability across 10 young male C57BL/6 mice. Both systems employed a weight that applied an equal force of resistance to the mouse undergoing testing. In experiment 2, adult mice were randomized into exercise and control groups. The results of Experiment 1 found that the Weighted Cart system produced less variability and required fewer prompts compared to the Pulley system. Accordingly, for the PRE intervention of Experiment 2, the Weighted Cart System was used. Exercised mice underwent progressive loading and were paired with a control mouse (equal repetitions with an unweighted cart). Mice in experiment 2 underwent a battery of assessments to assess the short-term impact of PRE including measures of muscle excitation following nerve stimulation (compound muscle action potential), neuromuscular junction transmission (repetitive nerve stimulation quantifying compound muscle action potential amplitude decrement), motor unit size and number, body composition, and wet muscle weight. Motor power was increased by 30% in exercised mice versus controls. NMJ transmission significantly improved in exercised versus control mice. Otherwise, other parameters of muscle and neural excitability showed no significant changes. Our work highlights a novel way to implement PRE in mouse models and demonstrates significant improvement of neuromuscular junction transmission in adult mice. This Weighted Cart approach can be applied to investigate the effects of PRE in the contexts of health, aging, and disease.

Disclosures: C.D. Brennan: None. **Z. Williard:** None. **W. Arnold:** None. **F. B. Darvishi:** None. **A. Roshani Dashtmian:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.02/C39

Topic: C.06. Neuromuscular Diseases

Title: Myopathies in the tropical environment: about 50 cases followed at the fitima guinea center and the ignace deen university hospital in conakry

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Deen, BROOKLYN, NY; [3]Pediatrics, Hosp. Natl. Ignace Deen, Conakry, Guinea; [4]Neurol., Hosp. Natl. Ignace Deen of Conakry, CONAKRY, Guinea; [5]Pediatrics, Clinique Privée Santé de la Famille, Conakry, Guinea

Abstract: Introduction: The challenges in identifying motor disorders within the broad group of myopathies, the associations of these disorders with the onset, distribution, and clinical course allowing differentiation of the type of muscle disease, and the need for appropriate medical and social assistance for these patients make myopathies one of the most important problems in modern myology. The objective was to evaluate the follow-up of patients with myopathy in tropical regions.**Result:** This situation is found in under-equipped countries, and through the analysis of records of 50 patients identified at the FITIMA center and Ignace Deen University Hospital in Conakry, we aimed to determine both the clinical manifestations structure of myopathies and to make a diagnostic approach among the 50 patients identified while reviewing the impact of physical exercise on muscle function. Late management led to varying degrees of muscle deterioration.**Conclusion:** This study shows a stereotyped clinical picture in a context of worsening neuromuscular signs related to delayed multidisciplinary care. These data are used for clinical, evolutionary, and prognostic evaluation.

Disclosures: S. Diallo: None. **M. Diallo:** None. **M. Diallo:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.03/C40

Topic: C.06. Neuromuscular Diseases

Support: NIH R01 NS112291-01
Myotonic Dystrophy Foundation Pre-doctoral Fellowship

Title: Molecular and Behavioral Characterization of Neuron-Specific Mouse Model of Myotonic Dystrophy type 1 (DM1)

Authors: *B. A. BEKELE[1], J. D. ARBOLEDA[2], J. P. SCHROEDER[3], E. T. WANG[4], J. JIANG[5], G. J. BASSELL[6];
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Abstract: Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy affecting 1 in 2,300 people worldwide. The cause of the disease is known to be a trinucleotide cytosine-thymine-guanine (CTG) repeat expansion in the *dystrophia myotonica protein kinase* (DMPK) gene. The expanded RNA forms toxic intranuclear foci disrupting processes such as the alternative splicing, a highly regulated process that results in multiple protein isoforms with

distinct functions. In DM1, RNA-binding proteins of the Muscleblind-like (MBNL) family are sequestered to RNA foci in the nucleus leading to dysregulated splicing of target RNAs. Loss of functional MBNL proteins in different tissues of the body has been linked to various disease phenotypes including muscle weakness, immune system abnormalities and central nervous system (CNS) deficits. While mechanisms of DM1 have been extensively studied in muscle, CNS manifestations of the disease have only recently gained attention. Here we developed and characterized a new transgenic mouse model for studying the effect of CTG expansion in the brain. We crossed mice expressing the Tet-On transactivator (tTA) under the control of calcium-calmodulin-dependent kinase II (CaMKIIα) with mice containing 960 interrupted CTG repeats in the context of human DMPK gene (TREDT960I) to drive expression of the toxic RNA in neurons expressing CaMKIIα. Using fluorescence *in-situ* hybridization (FISH) and immunofluorescence (IF) we assessed nuclear CUG foci and sequestration of MBNL protein. We found that RNA foci accumulate in the nuclei of double transgenic mice forebrain regions as early as 1.5-month-old and that cytoplasmic MBNL1 and MBNL2 proteins levels are reduced due to nuclear sequestration by CUG foci. Using RNA-sequencing, we identified several mis-splicing events in the prefrontal cortex of mice expressing CTG repeats including previously reported genes, *Mbnl1*and *Gabrg2,* which we confirmed by Real-Time Polymerase Chain Reaction (RT-PCR). We also performed a range of behavioral assays to determine if this DM1 mouse model display behavioral phenotypes relevant to CNS symptoms in DM1. We found that mice expressing CTG repeats have normal performance in open-field, grip and rotarod assays but showed deficits in associative learning and displayed anhedonia-like phenotype. By further characterizing the molecular and behavioral phenotypes of this mouse model, we will further assess the role of RNA-toxicity driven mechanisms in driving CNS deficits in DM1.

Disclosures: B.A. Bekele: None. **J.D. Arboleda:** None. **J.P. Schroeder:** None. **E.T. Wang:** None. **J. Jiang:** None. **G.J. Bassell:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.04/C41

Topic: C.06. Neuromuscular Diseases

Support: AFM-Telethon 2022 SR Grant
University Claude Bernard Lyon 1
Inserm
Institut de Myologie
Institut du Cerveau
CNRS

Title: Alterations in hippocampus signaling and quality of sleep were found in a mouse model of myotonic dystrophy type 1 disease

Authors: *S. PARROT[1], L. LALLEMANT[2], L. DAUSSY[2], S. MARTY[3], B. POTIER[4], S. ARTHAUD[1], G. GOURDON[2], C. PEYRON[5], M. GOMES-PEREIRA[6];
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Abstract: Myotonic dystrophy type 1 (DM1) is a multisystemic disorder caused by a non-coding CTG repeat expansion in the DMPK gene. The debilitating neurological manifestations are characterized by varying degrees of cognitive impairment and behavioral changes. Excessive day time sleepiness is prevalent across patients of all ages, impacting daily life. Disease pathogenesis is driven by nuclear accumulation of mutant RNA foci, and dysregulation of RNA processing across multiple cell types. However, the disease mechanisms in the brain remain elusive, hampering the development of molecular therapies. In this study we took advantage of a transgenic mouse model of DM1: DMSXL mice exhibit relevant molecular hallmarks of disease and offer a valuable tool to explore the disease neurobiology. Our research has focused on the hippocampus, which is involved in the major CNS functions affected in DM1. The DMSXL hippocampus display regional RNA foci accumulation, splicing defects, and alterations in the excitation (glutamate) and inhibition (GABA) balance. Therefore, we sought to investigate the impact of the detected unbalance on brain function and specific brain networks. Ex vivo electrophysiological experiments on hippocampal slices revealed a net increase in GABAergic evoked currents in the dentate gyrus (DG) of DMSXL mice. EEG recordings demonstrated significant hypersomnolence in DMSXL mice, primarily during the dark phase (rodent active phase), consistent with excessive daytime sleepiness in DM1 patients. We then explored cellular abnormalities behind these phenotypes. Electron and confocal microscopy analyses showed no significant changes in the density of excitatory synapses or the surface occupied by inhibitory synapses in the DG and CA1 regions. These findings strongly suggest relevant functional defects associated with the imbalance between excitatory and inhibitory signaling, without major ultrastructural changes in synaptic organization in the DMSXL hippocampus. Ongoing investigations aim to further elucidate the functional changes and the molecular mechanisms underlying these phenotypes, opening avenues to the development of molecular therapies for some the most debilitating symptoms of DM1.

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Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.05/C42

Topic: E.09. Motor Neurons and Muscle

Support: The Nemours Foundation
The Swank Foundation
The Nemours Cerebral Palsy Center

Title: A Single Cell RNA-Seq Study of Skeletal Muscle Gene Expression in Spastic Cerebral Palsy

Authors: K. ROBINSON[1], S. K. LEE[1], J. HICKS[3], M. SHRADER[4], ***R. E. AKINS**[2]; [2]Biomed. Res., [1]Nemours Children's Hlth. Syst., Wilmington, DE; [3]Bioinformatics & Computat. Biol., Univ. of Delaware, Newark, DE; [4]Cerebral Palsy Program, Nemours Children's Hosp. - Delaware, Wilmington, DE

Abstract: Cerebral palsy (CP) is a set of static encephalopathies characterized by damage to the developing brain that results in neuromotor dysfunction. CP is a leading cause of permanent physical disability arising in children. Spastic CP, the most common type, is typified by muscle spasticity, poor coordination, and weakness. Individuals with spastic CP have impaired longitudinal skeletal muscle growth and contractures that contribute to impaired motor control, muscle atrophy, and increased need for clinical care or surgery. CP muscle is characterized by decreased numbers of satellite cells (SC; muscle stem cells), and SCs isolated from CP muscle exhibit reduced myotube formation in vitro. It is unclear how SCs contribute to these differential effects or to overall muscle dysfunction in CP. We used single cell RNA-sequencing (scRNA-seq) to investigate differences in SC subpopulations using primary cells isolated from participants with spastic CP and controls. Surgical explants of skeletal muscle were obtained from subjects undergoing spinal fusion surgery at the Nemours Children's Hospital, Delaware, following IRB approval and informed consent/assent. Four subjects with spastic CP (average age=13.8 ± 3.8) and 5 control subjects (average age 14.8 ± 0.8) met inclusionary criteria. Satellite cells were isolated by double immunomagnetic selection for CXCR4 and NCAM1. SC phenotype was confirmed by MYF5 immunofluorescence. Single cells were isolated from proliferating satellite cell-derived myoblast (SC-MB) cultures and lysed to capture RNA molecules. Barcoded next-generation sequencing (NGS) cDNA libraries were prepared and sequenced on a NextSeq 2000. scRNA-seq profiles from CP and control cohorts were compared using the R package *Seurat*. The cohorts displayed similar overall profiles, and cells segregated into the same cluster types, which could be represented by different phases of the cell cycle and different stages of myogenesis. Within each cluster, differentially expressed genes (DEGs) were identified between the CP and non-CP cohorts using Wilcoxon Rank Sum tests. Enrichment analysis of DEGs demonstrated significant differences in the GO biological processes of cell division, cell adhesion, extracellular matrix assembly, positive regulation of transcription from RNA polymerase II promoter, and response to hypoxia. In conclusion, SC-MBs isolated from subjects with CP comprised similar SC subpopulations as those isolated from control subjects but exhibited differentially expressed genes within each subpopulation. Overall, these results suggest that spastic CP is associated with altered gene expression programming of SCs in muscle.

Disclosures: K. Robinson: None. **S.K. Lee:** None. **J. Hicks:** None. **M. Shrader:** None. **R.E. Akins:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.06/C43

Topic: E.09. Motor Neurons and Muscle

Title: Genetic Landscape of Duchenne Muscular Dystrophy in the Nepalese Population: Insights from a Case Series

Authors: *S. THAPA;
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Abstract: Duchenne Muscular Dystrophy (DMD) is a progressive neuromuscular disorder characterized by the absence of dystrophin, leading to muscle degeneration and weakness. Understanding the genetic landscape of DMD within specific populations aids in diagnosis, prognosis, and potential therapeutic interventions.
This study presents a case series of individuals from the Nepalese population diagnosed with DMD, highlighting the spectrum of genetic mutations observed in these patients. The prevalence and genetic characteristics of DMD in Nepal remain underexplored, warranting investigation to better inform clinical management strategies.
Using Multiplex Ligation-dependent Probe Amplification (MLPA) analysis, genetic screening was conducted on a cohort of individuals suspected of DMD. The MLPA technique allows for the detection of deletions or duplications within the 79 exons of the dystrophin gene. However, this assay does not detect point mutations or changes that lie outside the target region of the probes. Inversions/translocations too will not be detected.
Analysis by sex revealed a predominance of males, consistent with DMD's X-linked inheritance pattern. Age distribution indicated a wide range, with patients ranging from 5 to 34 years old, emphasizing the variability in disease onset and progression. Genetic results unveiled a spectrum of mutations, including deletions spanning single or multiple exons, with notable variations in affected exons across individuals. In males, deletions frequently involved exons 48-50, while exon 45 deletions were also notable. In contrast, females exhibited heterozygous deletions, often involving exons 8 to 28, reflecting carrier status. These findings underscore the complexity of DMD genetics and its manifestation within the Nepalese population.
This case series underscores the genetic diversity of DMD mutations in the Nepalese population. Understanding the specific genetic alterations associated with DMD in this population is crucial for accurate diagnosis, genetic counseling, and the development of targeted therapies. Further research is warranted to elucidate the full spectrum of DMD mutations in Nepal and their clinical implications.

Disclosures: S. Thapa: None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.07/C44

Topic: C.06. Neuromuscular Diseases

Support: DoD Grant #PR170326

Title: The ERG1A potassium channel increases sodium current amplitude in cultured skeletal myotubes

Authors: *A. L. POND[1], S. GUHA[1], G. H. HOCKERMAN[2];
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Abstract: Skeletal muscle atrophy occurs in response to denervation, muscle disuse, some systemic diseases, and with age. It is characterized by the loss of muscle size and strength, resulting from an imbalance in protein synthesis and degradation. Another characteristic of atrophic muscle is an increase in fast sodium current, potentially leading to reduced resistance to muscle fatigue. We have reported that the ERG1A K^+ channel is upregulated in mouse skeletal muscle atrophying in response to denervation, disuse, and cancer cachexia and have shown that proteolysis increases while cell size decreases in both mouse muscle and cultured C_2C_{12} muscle myotubes over-expressing ERG1A. Interestingly, using whole cell patch clamp electrophysiology, our laboratory has also observed a significant ~64% ($p<0.05$) elevation of fast sodium current amplitude in C_2C_{12}myotubes over-expressing ERG1A. We hypothesized that this increased sodium current likely results from modulation of the skeletal muscle voltage-gated Nav1.4 channel. Indeed, we found that the increased fast Na^+ current was blocked by 1μM tetrodotoxin and inhibited by a 20 msec conditioning pulse to -50 mV in both control and HERG over-expressing myotubes, which strongly suggests that the source of the HERG-enhanced sodium current is the Nav1.4 sodium channel. To explore this further, we performed qPCR to evaluate Nav1.4 gene expression levels in C_2C_{12} myotubes transduced with either a control or ERG1A-encoded adenovirus. Our results reveal that, at 48 hours after viral transduction, there is no statistically significant difference in expression of the SCNA4 gene (which encodes the Nav1.4 channel) in cells over-expressing ERG1A compared to control cells. This reveals that ERG1A is not affecting SCNA4 gene expression at 48 hours post transduction. In conclusion, our study demonstrates that ERG1A increases current amplitude of the Nav1.4 channel in myotubes, but likely does not accomplish this through modulation of SCNA4 gene expression. However, it is possible that ERG1A may affect gene expression at a later time point. It is also possible that ERG1A is modulating Nav1.4 channel protein abundance and/or kinetics. Thus, in the future, we will study the effect of ERG1A over-expression on SCNA4 gene expression and Nav1.4 protein abundance overtime. Also, we will study the effect of ERG1A on Nav1.4 channel kinetics. Our work highlights the potential involvement of K^+ channels in regulating sodium channel currents in myotubes. We hope that further work will unveil novel therapeutic targets for combating muscle wasting disorders like denervation atrophy.

Disclosures: A.L. Pond: None. **S. Guha:** None. **G.H. Hockerman:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.08/C45

Topic: C.06. Neuromuscular Diseases

Support: DoD Grant #PR170326

Title: Erg1a potassium channel modulates calcium signaling in skeletal muscle atrophy

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[4]Anat., Southern Illinois Univ., Carbondale, IL

Abstract: Skeletal muscle can undergo protein loss and/or decreased protein synthesis in response to denervation, disuse, aging, cancer or other diseases; and individuals can experience a significant loss in muscle mass referred to as atrophy. Careful control of calcium (Ca^{2+}) signaling is required to avoid atrophy by facilitating muscle growth, development, repair, and function. Thus, perturbation of regulated calcium signaling in muscle cells can lead to atrophy. We reported upregulation of the ERG1A K^+ channel in the skeletal muscle of mice after denervation and further demonstrated that over-expression of the human homolog, HERG1A, in the C_2C_{12} skeletal muscle cell line yields increased intracellular Ca^{2+} concentration and calpain enzyme-mediated proteolysis. However, the mechanism by which this Ca^{2+} concentration is increased was not known. We hypothesized that ERG1A might be modulating known calcium pathways. To study this, we evaluated intracellular Ca^{2+} concentration in C_2C_{12} myotubes using cell-based assays and the ratiometric calcium dye, Fura-2. With this dye, we assessed the relative differences in the intracellular Ca^{2+} concentration of C_2C_{12} myotubes transduced with either a control adenovirus or one encoding the HERG1A channel. We observed that HERG1A overexpression enhances the activity of a major extracellular Ca^{2+} entry pathway called store operated calcium entry (SOCE; 36.8%, $p=0.0275$) to increase intracellular Ca^{2+}. Further, we also showed by immunoblot that the Ca^{2+}-buffering protein, Calsequestrin1 (CSQ1), which at high levels inhibits SOCE, is reduced 89.7% ($p=0.012$) in response to HERG1A over-expression in C_2C_{12} myotubes. Additionally, RT-qPCR showed reduced CSQ1 gene expression (0.83 fold; $p<0.05$) in cells over-expressing HERG1A. The data indicate that HERG1A increases intracellular calcium by decreasing expression of the CSQ1 gene. Thus, abundance of the CSQ1 protein which sequesters Ca^{2+} is decreased, allowing for greater levels of calcium to be released from the sarcoplasmic reticulum into the cytosol in response to stimulation. Presently, there are no truly effective therapies for skeletal muscle atrophy, thus deciphering new regulatory mechanisms such as this may reveal potential novel targets for therapeutics.

Disclosures: S. Guha: None. **J. Koran:** None. **G.H. Hockerman:** None. **A.L. Pond:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.09/C46

Topic: E.09. Motor Neurons and Muscle

Support: Project NODES grant agreement no. ECS00000036
MIUR national project "Dipartimenti di Eccellenza 2018-2022 and 2023-2027"

Title: The biological ActR-Fc-nLG3, synergistically acting on myostatin and agrin pathways, reinforces neuromuscular stability and increases motor endurance in old mice

Authors: *M. BOIDO[1], R. SCHELLINO[1], J. VRIJBLOED[2], R. FARIELLO[2], A. VERCELLI[1]; [1]Neurosci. Inst. Cavalieri Ottolenghi, Dept. of Neuroscience, Univ. of Turin, Orbassano (TO), Italy; [2]Pharmafox, Möhlin, Switzerland

Abstract: Sarcopenia is the primary cause of motor disability in the elderly being also responsible for increased morbidity and mortality. The current prevailing approach to ameliorate muscle function is the inhibition of the myostatin system: this treatment (ActR-Fc) increases muscle mass and partially strength, but is unable to ameliorate endurance. A dual action directed at both muscle and nerve (particularly targeting the neuromuscular junctions, NMJs) seems necessary for correcting the performance deficit characterizing sarcopenia. To this aim, we developed ActR-Fc-nLG3 that combines the nLG3 domain from the C-terminus of human agrin with the potent myostatin inhibitor ActR-Fc, via the constant region of an Igg1 monoclonal antibody. We administered for 8 weeks ActR-Fc-nLG3, ActR-Fc or PBS to C57BL/6J male mice, 2 years old. To evaluate the treatment effects on motor endurance and muscle strength, the animals underwent behavioral tests (treadmill and grip strength). Then, we sacrificed the animals, we collected triceps, gastrocnemius and quadriceps skeletal muscles, and we performed histological and morphometric analyses on muscle fibers and NMJs. ActR-Fc-nLG3 administration increased in a sustained way both motor endurance and muscle strength, compared with ActR-Fc and PBS (ANOVA for repeated measures, $p<0.001$). Histological data demonstrated that the treatment improved neuromuscular stability and fiber innervation maintenance, preventing muscle fiber atrophy and inducing only moderate hypertrophy. In particular, by quantifying the innervated/denervated/fragmented NMJs, we observed a reduction in denervation in the Actr-Fc-nLG3 group ($p<0.01$), also confirmed by the analysis with the α-NMJ Macro ImageJ plugin. In addition a significant increase in AChR clusters compared to vehicle was observed for ActR-Fc-nLG3 treated NMJs (one-way ANOVA; $p= 0.0024$). Interestingly, only in ActR-Fc-nLG3-treated NMJs we observed that the surface of the synaptic cleft results amplified, indicating sites of new outgrowth and AChR clustering. These data can explain the enhanced motor endurance observed. In conclusion, we suggest that ActR-Fc-nLG3 may become a valid option for treating sarcopenia and possibly other disorders of striatal muscles.

Disclosures: M. Boido: None. **R. Schellino:** None. **J. Vrijbloed:** None. **R. Fariello:** None. **A. Vercelli:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.10/C47

Topic: C.06. Neuromuscular Diseases

Title: Electrophysiological characterization of human CLC-1 and CLC-2 ion channels stably expressed in HEK cells using the SyncroPatch 384i automated electrophysiology platform.

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SB Drug Discovery, Glasgow, United Kingdom

Abstract: The CLC family comprises voltage-dependant cell surface chloride (Cl^-) ion channels and intracellular Cl^-/H^+ transporters which perform an array of physiological functions. Two members of the CLC family, CLC-1 and CLC-2, are inward-rectifying ion channels with crucial roles in physiology. CLC-1 is predominantly expressed in skeletal muscle and performs a vital role in homeostasis of muscle excitability by repolarisation of skeletal muscle in response to depolarisation of an action potential. Variants of the CLCN1 gene are associated with a rare disorder of muscle membrane hyperexcitability, myotonia congenita. In contrast, the widely expressed CLC-2 is activated upon hyperpolarisation of the membrane and is involved in many physiological functions, including epithelial transport. Variants of the CLCN2 gene have been linked to rare disorders such as leukodystrophy and hyperaldosteronism. Despite the channelopathies caused by CLC channel dysfunction, only a small number of selective CLC channel modulators have been reported, and the pharmacology of these channels is poorly characterized compared to cation or other ligand-gated Cl^- channels. The non-selective nature of currently defined Cl- channel modulators provide an obstacle in developing a therapeutic strategy targeting CLC channelopathies. This highlights the importance of developing novel routes to identify selective compounds in pursuit of therapeutic remedies to CLC channel dysfunction. Advancements in 384-well automated high throughput electrophysiology allows accelerated evaluation of large numbers of molecules against ion channel targets such as CLC-1 and CLC-2. We have developed robust, high quality recombinant cell lines expressing wild-type CLC-1 and CLC-2 ion channels. Electrophysiological characterization of these ion channels, including current-voltage relationship and assessment of known CLC modulators was performed using automated voltage-clamp electrophysiology. Reproducible and selective inhibition of 9-AC for CLC-1 (~1 µM IC_{50}) over CLC-2 (no effect) and $CdCl_2$ inhibition of CLC-2 (~55 µM IC_{50}) currents over CLC-1 (~350 µM IC_{50}), as well as inhibition with known Cl^- channel blocker $ZnCl_2$ confirm that a successful assay has been developed, capable of rapidly identifying and characterizing novel pharmaceutical tools targeting CLC-1 and CLC-2 channels. This should

allow further investigation into the role of these exciting targets in physiological and pathological conditions.

Disclosures: A. Dickson: None. **M. Kras:** None. **K. Arthur:** None. **D. Young:** None. **R. Long:** None. **L. mcarthur:** None. **C. Brown:** None. **L. Hutchison:** None. **D. Dalrymple:** None. **D. Pau:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.11/C48

Topic: E.09. Motor Neurons and Muscle

Support: PAPIIT UNAM: IN211720
CONAHCyT: ERB1007567

Title: Association between inflammatory and regenerative responses in pelvic floor muscles of nulliparous and multiparous rabbits

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Abstract: Multiparity is one of the main factors associated with urogynecological disorders such as urinary incontinence that is highly prevalent among adult women. Repeated deliveries may exacerbate the pelvic floor muscles damage associated to childbirth, wich may be influenced by the triggered inflammation response. The objective of this study was to correlate the pro- and anti-inflammatory reponse with the presence of Pax7 and myogenin in pelvic floor muscles at postpartum days 3 and 20. Bulbospongiosus (Bsm) and pubococcygeus muscles (Pcm) of age-matched nulliparous and multiparous chinchilla rabbits were excised and processed for histological analyses, hematoxylin-eosin staining and immunohistochemistry anti-HLA-DR, anti-CD206, anti-Pax7, and anti-myogenin. Significant differences among the groups were considered at a $P<0.05$, which were investigated with one-way ANOVA or Kruskal-Wallis tests, and Spearman tests. Data indicated an increased in the immunostaining anti-HLA-DR (M1 macrophages) and Pax7 for both muscles when evaluated three days postpartum. In addition, the anti-CD206 and myogenin were found to be elevated on the postpartum day 20. Correlation analyses showed different responses in bulbospongiosus and pubococcygeus muscles. Present findings suggest inflammation and satellite cell activation are conjointed at postpartum in multiparous rabbits. Moreover, anti-inflammatory processes seem run along muscle later regenerative stages in pelvic floor muscles of multiparous rabbits.

Disclosures: E. Rodriguez-Benitez: None. **N. Xelhuantzi:** None. **D.L. Corona Quintanilla:** None. **C.H. Bonilla:** None. **F. Castelán:** None. **M. Martinez-Gomez:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.12/C49

Topic: E.09. Motor Neurons and Muscle

Support: CONAHCyT 322333 to FC
CONAHCyT 1003610 to MXN

Title: Effects of the XT1 neurotrophic peptide administration on pelvic floor muscle damage at early postpartum in rats.

Authors: *M. XOCHITEMOL NAVA[1], M. CARRASCO RUIZ[2], E. CUEVAS[3], J. AGUILERA[4], F. CASTELÁN[5];
[1]Biochem. & tissue Biol., Ctr. Tlaxcala Biologia de la Conducta, Tlaxcala, Mexico; [2]Facultad de Odontología, Univ. Autonoma De Tlaxcala, Tlaxcala, Mexico; [3]Ctr. Tlaxcala De Biología De La Conducta, Ctr. Tlaxcala De Biología De La Conducta/Universidad Autónoma De Tlaxcala, Tlaxcala, Mexico; [4]Inst. de Neurociències, Univ. Autònoma de Barcelona, Cerdanyola del Vallès (Barcelona), Spain; [5]Inst. de Investigaciones Biomedicas, UNAM, Tlaxcala, Mexico

Abstract: Effects of the XT1 neurotrophic peptide administration on pelvic floor muscle damage at early postpartum in ratsAuthors:Xochitemol Nava Mayra [1,2], Carrasco-Ruiz María de los Ángeles [3], Cuevas-Romero Estela [1,], Aguilera Ávila José [5], Castelán Francisco [1,4]
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AbstractVaginal childbirth weakens the female pelvic floor muscles causing muscular distension. This predisposes to damage of these structures and the development of pathologies such as stress urinary incontinence. After the damage process, the muscle undergoes a process of degeneration and regeneration coupled to an inflammatory response. The interest in understanding the mechanisms related to these lesions has led our group to investigate in models such as the primiparous rat. In a previous approach we observed that the neurotrophic peptide XT1 has the capacity to reduce pelvic muscle damage. In an effort to learn more about the probable mechanism and anti-inflammatory effect, the aim of this project was to evaluate some morphometric, biochemical, histological and electrophoresis variables in the ilio- and pubococcygeus muscles of primiparous rats treated for three days with XT1. Findings indicated that XT1 is implicated in the inflammatory process, which was associated with the levels of muscle damage and muscle weight.

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Disclosures: M. Xochitemol Nava: None. **M. Carrasco Ruiz:** None. **E. Cuevas:** None. **J. Aguilera:** None. **F. Castelán:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.13/C50

Topic: C.06. Neuromuscular Diseases

Support: Forschungskommission of the Medical Faculty of the Heinrich Heine University Düsseldorf

Title: ITIH3: a serum biomarker for disease activity in myasthenia gravis

Authors: *C. B. SCHROETER[1], C. NELKE[1], F. STASCHEIT[2], C. PREUSSE[3], V. DOBELMANN[1], H.-P. HARTUNG[1], A. ROOS[1], W. STENZEL[3], A. G. MEISEL[4], S. MEUTH[1], T. RUCK[1];
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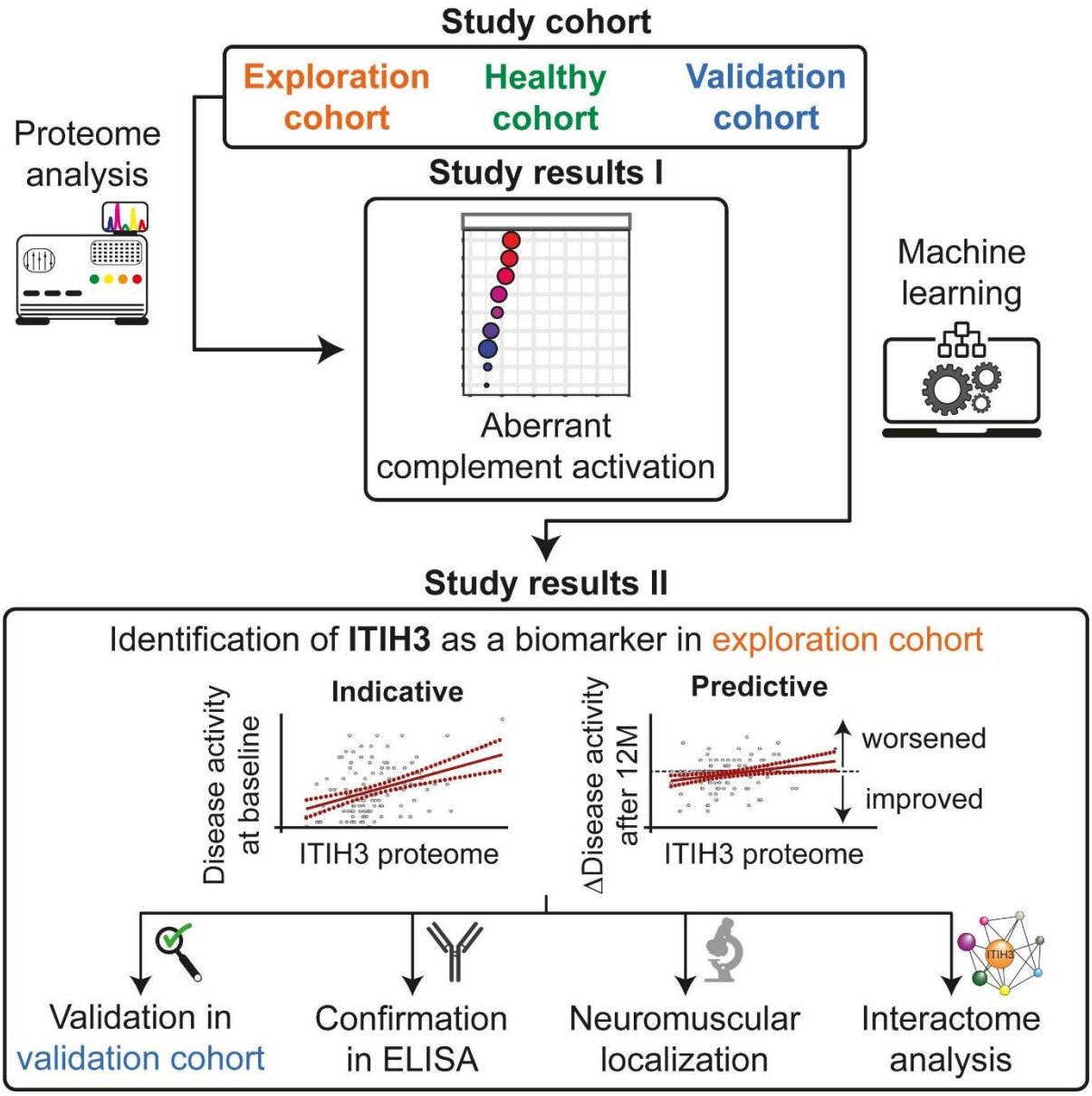
Abstract: Myasthenia gravis is a chronic antibody-mediated autoimmune disease disrupting neuromuscular synaptic transmission. Informative biomarkers remain an unmet need to stratify patients with active disease requiring intensified monitoring and therapy; their identification is the primary objective of this study.
We applied mass spectrometry-based proteomic serum profiling for biomarker discovery. We studied an exploration and a prospective validation cohort consisting of 114 and 140 anti-acetylcholine receptor-antibody (AChR-Ab)-positive myasthenia gravis patients, respectively. For downstream analysis, we applied a machine learning approach. Protein expression levels were confirmed by ELISA and compared to other myasthenic cohorts. Anti-AChR-Ab levels were determined by a radio receptor assay. Immunohistochemistry and immunofluorescence of intercostal muscle biopsies were employed for validation in addition to interactome studies of inter-alpha-trypsin inhibitor heavy chain H3 (ITIH3).
Machine learning identified ITIH3 as potential serum biomarker reflective of disease activity. Serum levels correlated with disease activity scores in the exploration and validation cohort and were confirmed by ELISA. Lack of correlation between anti-AChR-Ab levels and clinical scores underlined the need for biomarkers. In a subgroup analysis, ITIH3 was indicative of treatment responses. Immunostaining of muscle specimens from these patients demonstrated ITIH3 localization at the neuromuscular endplates in myasthenia gravis but not in controls, thus providing a structural equivalent for our serological findings. Immunoprecipitation of ITH3 lead

to identification of its interaction partners playing crucial roles in neuromuscular transmission. This study provides data on ITIH3 as a potential pathophysiological-relevant biomarker of disease activity in myasthenia gravis. Future studies are required to facilitate translation into clinical practice.



Disclosures: C.B. Schroeter: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent at the European Patent Office (EPO) relating to the use of ITIH3 as a biomarker for (assessing) disease activity in myasthenia gravis patients. **C. Nelke:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent at the European Patent Office (EPO) relating to the use of ITIH3 as a biomarker for (assessing) disease activity in myasthenia gravis patients.. **F. Stascheit:** None. **C. Preusse:**

None. **V. Dobelmann:** None. **H. Hartung:** None. **A. Roos:** None. **W. Stenzel:** None. **A.G. Meisel:** None. **S. Meuth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent at the European Patent Office (EPO) relating to the use of ITIH3 as a biomarker for (assessing) disease activity in myasthenia gravis patients. **T. Ruck:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent at the European Patent Office (EPO) relating to the use of ITIH3 as a biomarker for (assessing) disease activity in myasthenia gravis patients..

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.14/C51

Topic: C.06. Neuromuscular Diseases

Support: Kansas City Consortium on Musculoskeletal Diseases

Title: Effects of whole-body electrical muscle stimulation exercise on motor unit activity in adults with myasthenia gravis: a preliminary analysis

Authors: *K. M. KELLY[1], A. KETABFOROUSH[2], D. VORKINK[3], C. PARKS[4], W. ARNOLD[2];
[1]Physical Med. and Rehabil., [2]NextGen Precision Hlth., [4]Hlth. Sci., [3]Univ. of Missouri, Columbia, MO

Abstract: Myasthenia Gravis (MG) is an autoimmune neuromuscular disorder that results in loss of neuromuscular junction transmission. Patients with MG experience fatigable muscle weakness that impacts participation in daily activities and quality of life. Exercise can improve physical function in MG but may be difficult to tolerate due to symptoms of fatigue. Improved approaches to exercise and mechanistic understanding of the effects of exercise are needed to fully realize the benefits of exercise for this population. Enrolled participants complete a supervised whole-body electrical muscle stimulation exercise intervention, which consists of 20 minutes of exercise twice per week for 4 weeks. At baseline and post-intervention, decomposition electromyography (dEMG) is performed, including a maximal voluntary isometric contraction (MVIC) strength assessment. Surface electromyographic interference patterns are captured at the vastus lateralis and middle deltoid during a ramped 50% MVIC force-matching task completed pre- and post- a fatiguing protocol. Individual motor units are identified for analysis of the following parameters: max motor unit (MU) action potential amplitude, peak MU firing rate, average interpulse interval, and interpulse interval coefficient of variation. Recruitment and data collection are ongoing. Analysis of one participant has been completed (female, age 22 years). At baseline, the vastus lateralis yielded 3 MUs pre-fatigue and 10 MUs post-fatigue; the middle deltoid yielded 20 MUs pre-fatigue and 16 MUs post-fatigue. Post-intervention, the vastus lateralis yielded 7 MUs pre-fatigue and 8 MUs post-fatigue; the middle deltoid yielded 29 MUs

pre-fatigue and 27 MUs post-fatigue. Preliminary data suggest an intervention-dependent muscle-specific response in MU activity. The vastus lateralis demonstrated a trend toward increased MU excitation during pre-fatigue assessment after the intervention and ability to maintain this excitation post-fatigue. Conversely, the middle deltoid demonstrated a trend toward decreased excitation in the post-fatigue parameters after the intervention. MVIC values increased by 38.5% and 46.3% for the vastus lateralis and middle deltoid, respectively. Updated results will be presented. Preliminary analyses suggest that whole-body electrical muscle stimulation exercise alters MU activity as potential mechanisms of motor functional improvements in adults with myasthenia gravis. The muscle-specific response may have implications for: (1) use of dEMG as a monitoring biomarker, and (2) development of therapies that consider the potential diverging MU physiology of different muscle groups.

Disclosures: K.M. Kelly: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Katalyst Interactive, Inc.. **A. Ketabforoush:** None. **D. Vorkink:** None. **C. Parks:** None. **W. Arnold:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.15/C52

Topic: C.06. Neuromuscular Diseases

Title: Phenotyping of a quick passive transfer model of myasthenia gravis

Authors: J. TOIVANEN[1], T. BOLKVADZE[1], L. RAUHALA[1], *S. BÄCK[2];
[1]Charles River Discovery Res. Services, Kuopio, Finland; [2]Charles River Discovery Reserach Services, Kuopio, Finland

Abstract: Myasthenia gravis is an autoimmune disease and the most common form is characterized by antibody (Ab) and complement targeting the acetylcholine receptor (AChR) and the motor end plate in the neuromuscular junction (NMJ). The disease often leads to severe decrease in the quality of life due to weakness and abnormal fatiguability of skeletal muscles. Our aim was to characterize the in vivo phenotype in a rat passive transfer model with an Ab dose likely to cause a quick and severe disease. Our second aim was to investigate if titration of the Ab would result in a less severe phenotype.
The animal work was conducted in accordance with European Union directive 2010/63 and approved by the national Project Authorization Board. In total 40 female Lewis rats were administered with a single i.p. dose of vehicle or monoclonal rat IgG1 mAb35 binding to the major immunogenic region of the AChR α subunits (1, 0.3 or 0.03 mg/kg; n=10/group). Weakness was assessed by clinical scoring and body weights measured twice per day. At approximately 33-35 h after mAb35 dosing, CMAP responses were measured in the gastrocnemius muscle. The sciatic nerve was stimulated witht 4 separate trains of 10 supramaximal (12 mA) stimulations at 5, 10, 20 and 40 Hz. The animals were followed until 6

days post mAb35 dosing or until reaching a humane end point criterion (HEP). Tibialis anterior muscles were collected for future motor end plate and complement activation analysis by IHC. An mAb35 dose of 1 mg/kg resulted in observable weakness (tail tonus loss, abnormal walking) already 24-26 h after injection in 9 out 10 rats. By 36h, there was approximately 7% weight loss and increased weakness (chin down, hunched position), with the rats fulfilling HEP criteria by 38-46 hThree (3) rats dosed at 0.03 mg/kg and 2 rats at 0.3 mg/kg showed occasional tail weakness, but no consistent phenotype was observed. Fatiguability was evident in the gastrocnemius muscle in the 1 mg/kg group when the 10^{th} response peak-to-peak amplitude was compared to the response of the 1^{st} stimulation. The reduction in this group was 11.5% at 5 Hz, 11.8% at 10 Hz and 11.6% at 20 Hz whereas there was a slight increase in the amplitude in the sham and mAb35 lower dose groups.
In conclusion, the 1 mg/kg mAb35 dose resulted in rapidly progressing and severe myasthenia gravis-like disease with evident muscle fatiguability that can be utilized to test the effects of potential therapies e.g., targeting the autoantibodies, the complement or making the AChR more available in the NMJ. Lower mAb35 doses did not lead to a significant disease phenotype suggesting an “all or nothing” induction mechanism.

Disclosures: J. Toivanen: A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **T. Bolkvadze:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **L. Rauhala:** A. Employment/Salary (full or part-time):; Charles River Discovery Research Services. **S. Bäck:** A. Employment/Salary (full or part-time):; Charles River Discovery Services.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.16/C53

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant AG051510
VA Gant BX005642
CWRU funds

Title: Muscular Swedish mutant APP in the development of AD-related muscle weakness and neuro-muscular junction (NMJ) deficit

Authors: *M.-Y. WU, W.-J. ZOU, L. MEI, W.-C. XIONG;
Neurosci., Case western reserve Univ., Cleveland, OH

Abstract: Alzheimer’s disease (AD) is a slow progressive brain degenerative disorder. Interestingly, recent studies have suggested alterations in peripheral tissues and organs, with a notable focus on muscle weakness, that could occur in the preclinical phase of AD. Intriguingly, muscle weakness evolves into severe sarcopenia in the late stages of AD. However, the

connection between muscle weakness and AD, as well as its contribution to AD development remain elusive. Here, we provide evidence for an early NMJ deficit in mice selectively expressing Swedish mutant APP in skeletal muscles, $TgAPP_{swe}^{HSA\text{-}Cre}$. The NMJ deficits included impaired neuromuscular transmission by compound nerve action potential (CMAP), decreased frequency of miniature end-plate potential (mEPP) by electrophysiological recording of diaphragm, and reduced nerve terminal innervation at the NMJs by co-immunostaining analysis. Interestingly, these NMJ deficits were age-dependent, muscle type-selective, and more severe at the presynaptic terminals. We are currently investigating the underlying molecular mechanisms, and their contribution(s) to AD progression.

Disclosures: M. Wu: None. **W. Zou:** None. **L. Mei:** None. **W. Xiong:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.17/C54

Topic: E.09. Motor Neurons and Muscle

Title: Presentation of shed acetylcholine receptors from damaged muscles triggers myasthenia gravis in a rodent model

Authors: *L. C. KUZMA[1], B. S. LAWSON[2], A. PHAM[2], J. LIN[3], A. W. BORBI[1], Y. WU[2], Q. XU[4], O. A. GARDEN[2], H. WANG[3], J. LUO[1];
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Abstract: In myasthenia gravis (MG) and experimental autoimmune MG (EAMG), pathogenic autoantibodies target the extracellular epitopes of the muscle nicotinic acetylcholine receptor (AChR), thereby impairing neuromuscular transmission. The pathogenic mechanisms that initiate and sustain the autoimmune response to the AChR remain poorly understood. Conventional EAMG models rely on a transient immune response elicited by immunization with exogenous immunogens, such as AChRs purified from fish electric organ. We developed a self-sustaining EAMG model by immunizing rats with chimeric ACh binding proteins (AChBPs) that contain either the human or syngeneic main immunogenic region (MIR)—a group of conformation-dependent, overlapping B cell epitopes on the extracellular domain of the AChR α1 subunit. Immunization with the chimeras induced a strong primary antibody response to these immunogens, followed by a secondary autoimmune response to rat muscle AChRs, as reflected by the distinct antibody response dynamics and isotype profiles. The production of autoantibodies to the AChR cytoplasmic domain, which is absent in these immunogens, suggests that an internal source of muscle AChRs initiated and sustained the secondary immune response. A small fraction of the antibodies elicited by the immunogens cross-reacted with rat muscle

AChRs, commencing the complement-mediated focal lysis of the postsynaptic membrane and leading to the shedding of AChR-enriched membrane fragments. The presentation of shed AChRs subsequently initiated and sustained the autoimmune response to AChRs, which is supported further by the passive transfer of monoclonal antibodies to the AChR in healthy rats generating an autoimmune response to rat muscle AChR. The feedforward loop of autoimmune stimulation observed in these rats sheds light on pathological mechanisms that induce and sustain MG in patients. By faithfully mimicking MG, this novel EAMG model enables the rigorous investigation of immunosuppressive therapies' effectiveness in suppressing the self-sustaining autoimmune response.

Disclosures: L.C. Kuzma: None. **B.S. Lawson:** None. **A. Pham:** None. **J. Lin:** None. **A.W. Borbi:** None. **Y. Wu:** None. **Q. Xu:** None. **O.A. Garden:** None. **H. Wang:** None. **J. Luo:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.18/C55

Topic: E.09. Motor Neurons and Muscle

Support: An Accelerator grant from the Chan-Zuckerberg Biohub Chicago, LLC
NSF NRT-UtB 1735252

Title: Age-dependent changes in responses to circadian disruption-induced inflammation within the neuromuscular junction.

Authors: *S. ASIF[1], Y. AN[2], J. W. MITCHELL[3], H. KONG[2], M. U. GILLETTE[1]; [1]Neurosci., Univ. of Illinois Urbana-Champaign, Urbana, IL; [2]Chem. & Biomolecular Engin., Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Dept. of Cell and Developmental Biol., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: Circadian rhythms regulate daily physiological patterns, including sleep, eating, hormonal cycles, and immune function. Disruption of these rhythms can lead to chronic health issues such as neurological disease, cancer, and metabolic dysfunction, often accompanied by increased inflammation. An understudied area is how circadian-dependent inflammation affects the neuromuscular junction (NMJ). Here, we aim to elucidate this relationship, hypothesizing that circadian disruption-induced stress increases pro-inflammatory cytokines and alters NMJ morphology. Young (2 months, n=10) and aged (1 year, n=10) rats were divided into control or CRD groups. After 10 days of CRD, the gastrocnemius muscle was dissected for analysis 2 h after lights-on. The control group was maintained under a 12-h light/12-h dark cycle (LD12:12; lights on at 07:00, lights off at 19:00). The CRD group was maintained under a 10-h light/10-h dark cycle, advancing 4 hours each day. We used quantitative polymerase chain reaction (qPCR) to examine the expression of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis

factor-alpha (TNF-α) in NMJs subjected to circadian disruption-induced stress compared to control. Additionally, we used immunohistochemistry (IHC) to visualize and analyze morphological changes within the NMJ. To achieve this, we wrote a MATLAB script to facilitate automated and accurate quantification of NMJ morphology parameters. We analyzed segmented NMJ structures, allowing for measurements of area and perimeter to assess NMJ size and shape. Furthermore, compactness, a measure of the NMJ's structural density, and fragmentation, was used to quantify NMJ integrity. Additionally, we evaluated colocalization between pre- and post-synaptic markers of the NMJ to measure the degree of innervation. We found a significant decrease in IL-6 gene expression in gastrocnemius accompanied by alterations in NMJ morphology between young and aged rats. Aged rats have more fragmented NMJ compared to young rats, pointing to age-dependent differences in the response to circadian disruption-induced inflammation within the NMJ. These findings highlight the intricate relationship between circadian rhythms, age, and neuromuscular inflammation, and provide insights into potential therapeutic targets.

Disclosures: S. Asif: None. **Y. An:** None. **J.W. Mitchell:** None. **H. Kong:** None. **M.U. Gillette:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.19/C56

Topic: E.09. Motor Neurons and Muscle

Title: Advancing Neuromuscular Junction Modeling with Compartmentalized Microfluidic Platforms Coupled with MEA Functional Analysis

Authors: *B. MAISONNEUVE;
NETRI, Lyon, France

Abstract: Neuromuscular disorders present persistent challenges in therapeutic intervention, largely due to the absence of reliable predictive models for studying the neuromuscular junction (NMJ). Enhancing the predictive capacity of these models holds immense potential for elucidating the mechanisms underlying NMJ development, maintenance, and pathology. Induced pluripotent stem cells (iPSCs) represent a promising avenue for generating human NMJ models, facilitating investigations into synaptic development, maturation, and disease pathogenesis, as well as drug discovery endeavors. Leveraging iPSCs alongside innovative technological platforms is instrumental in crafting robust models for therapeutic exploration and development. Within the dynamic landscape of the neuromuscular junction, precise regulation of assembly and plasticity is orchestrated by intricate communication among motor nerve endings, muscle fibers, and glial cells. Dysregulated intercellular signaling within this milieu is implicated in age-related physiological alterations and pathological conditions, such as Amyotrophic Lateral Sclerosis (ALS), where mutations in C9ORF72 stand as a prevalent genetic cause. In this collaborative

effort between NETRI and AXOL, we introduce a pioneering approach combining NETRI's Dualink microfluidic technology with iPSC-derived motor neurons (MNs) and skeletal muscle (myotubes). This synergy integrates compartmentalized microfluidic architecture with multi-electrode array (MEA) technology, enabling precise measurement of neuronal hyperexcitability and NMJ dynamics in vitro, notably in the context of C9orf72 mutation ALS iPSC models. NETRI's microfluidic devices offer fluidically isolated compartments, fostering specificity in compound treatments, while facilitating the quantification of muscle contractility and neuronal firing patterns. This integration underscores the potential of NETRI's platforms in advancing the fidelity and translational relevance of NMJ models, thereby catalyzing breakthroughs in therapeutic discovery and development.

Disclosures: B. Maisonneuve: None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.20/C57

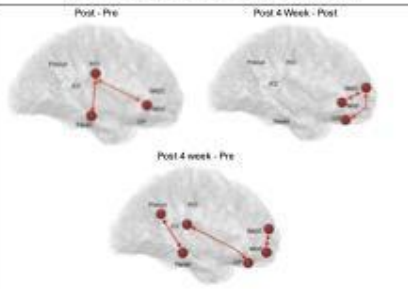
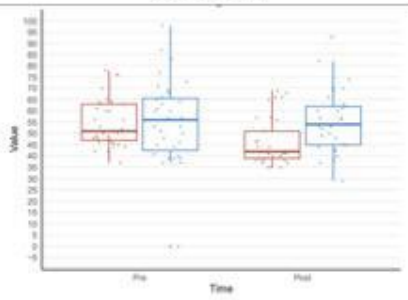
Topic: C.06. Neuromuscular Diseases

Title: Multimodal Impact of Chiropractic care on Chronic Low Back Pain:Neurophysiological,Activity, and Quality of Life Outcomes

Authors: *I. NIAZI[1], I. AMJAD[2], N. KUMARI[1], U. GHANI[1], M. ASHFAQUE[3], U. RASHID[1], M. NAVID[1], E. KAMAVUAKO[4], K. HOLT[1], A. CADE[1], A. N. PUJARI[3], H. HAAVIK[1]; [1]Ctr. for Chiropractic Res., New Zealand Col. of Chiropractic, Auckland, New Zealand; [2]Riphah Intl. Univ., Islamabad, Pakistan; [3]Engin., Univ. of Hertfordshire, Hertfordshire, United Kingdom; [4]Kings Col. London, London, United Kingdom

Abstract: Several clinical studies have reported positive outcomes in treating chronic low back pain with chiropractic care. However, the neural mechanisms that drive these effects are still not fully understood. To investigate this, a study was conducted to explore the immediate and prolonged effects of chiropractic intervention on neurophysiological (EEG) and patient-reported subjective measures (PROMIS-29). Sixty-seven people with chronic low back pain (mean age ± SD: 43±13 years, 33F) were randomly divided into usual care control and chiropractic + usual care groups. Neurophysiological assessments and subjective measures were collected before (pre), immediately after the first session (post-session 1), and after four weeks of intervention or control (post-4 weeks). Neurophysiological assessments included EEG spectral power and source-level analysis of somatosensory evoked potentials (SEPs) and resting EEG. PROMIS-29 was also recorded at the mentioned time points. The chiropractic group exhibited a significant increase in alpha band power post and post-4week comparison. SEP source analysis within the Default Mode Network (DMN) revealed increased alpha activity and decreased beta activity in specific brain regions within the chiropractic group compared to the control group. The chiropractic group also demonstrated enhanced overall quality of life measured by PROMIS-29,

with **minimal clinically important differences (MCIDs) of 9** post-4 weeks. In conclusion, chiropractic intervention positively affected neurophysiological parameters, particularly in alpha band power and DMN activity, and overall quality of life. These findings contribute valuable insights into the comprehensive impact of chiropractic care on various aspects of health.

EEG alpha band connectivity	PROMIS 29
	

Disclosures: I. Niazi: None. **I. Amjad:** None. **N. Kumari:** None. **U. Ghani:** None. **M. Ashfaque:** None. **U. Rashid:** None. **M. Navid:** None. **E. Kamavuako:** None. **K. Holt:** None. **A. Cade:** None. **A.N. Pujari:** None. **H. Haavik:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.21/C58

Topic: E.09. Motor Neurons and Muscle

Title: Redundant nerve roots on MRI can predict ongoing denervations in lumbar spinal stenosis patients

Authors: *K. KIM;
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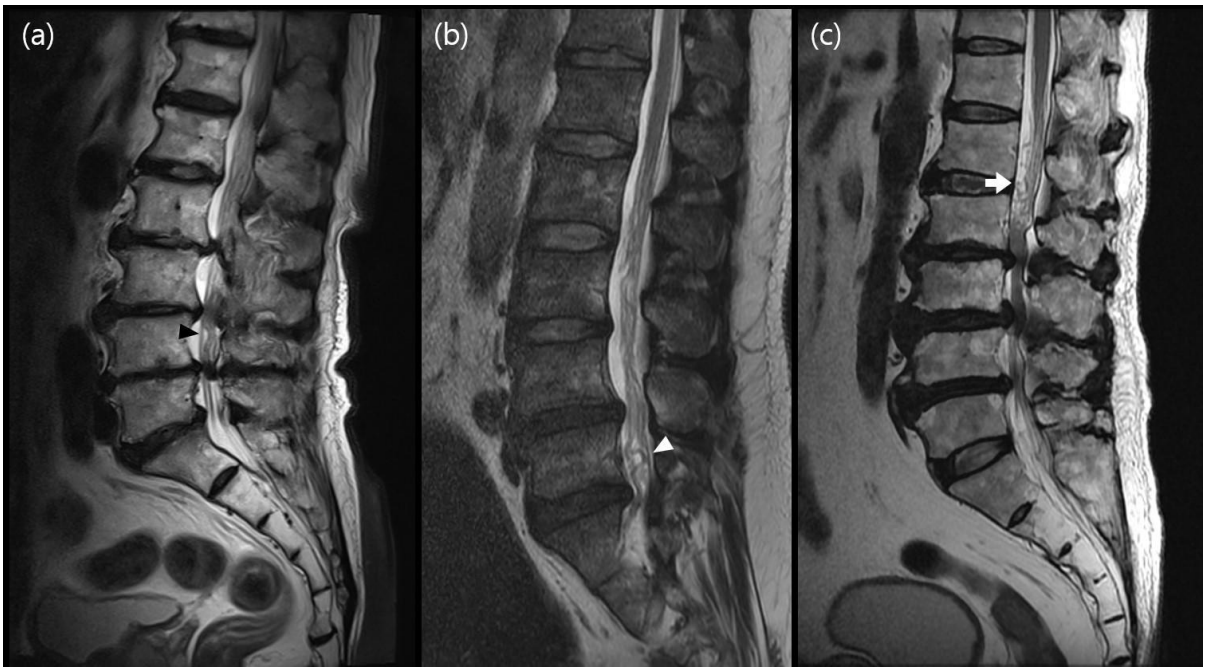
Abstract: Background Redundant nerve roots (RNRs), which are abnormally elongated and tortuous nerve roots, can be developed secondary to degenerative spinal stenosis. It has been reported that RNRs are associated with worse clinical outcome after decompressive surgery. However, there are limited studies about clinical significance of RNR. This study aims to investigate association of RNRs and denervation potentials observed in electromyographic study (EMG). **Methods** Among 2003 patients who underwent electrodiagnostic study of lower extremities from January 2020 to March 2023, 193 patients were included demonstrating lumbar spinal stenosis on their spinal MRI. Their clinical information and image findings including presence and of RNRs were investigated. In the EMG study, presence of abnormal spontaneous activity (ASA) was collected. Statistical analysis was performed to compare the difference between patients with and without RNRs. Multivariate logistic regression analysis was conducted to find out factors associated with development of ASA. **Results** RNRs were associated with advanced age ($p<0.001$), longer symptom duration ($p=0.009$), narrower CSA ($p<0.001$) and higher frequency of ASA ($p<0.001$). Higher probability of ASA was correlated to the increasing redundancy of nerve ($p<0.001$). Multiple logistic regression analysis showed that occurrence of ASA was associated with narrower CSA, multiple stenotic sites, severe RNR. **Conclusion** Presence of RNR, especially severe RNR, is a significant risk factor for development of denervation potentials in electromyographic study. It may help physicians to predict the prognosis of spinal stenosis patients.



Disclosures: K. Kim: None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.22/C59

Topic: C.06. Neuromuscular Diseases

Support: Carter Foundation for Neurological Research
National Institutes of Health
Massachusetts General Hospital

Title: LXR agonist mitigates SPG3A pathology in patient induced pluripotent stem cell derived neurons

Authors: *G. THAKUR[1,2], R. DHANUKATE[3], Y. MOU[3], Z. CHEN[3,4], C. BLACKSTONE[5], X.-J. LI[3,4];
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Abstract: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurological disorders caused by a length-dependent axonopathy of long corticospinal projection neurons.

SPG3A, the most prevalent early-onset form of HSP, is caused by the mutations in the *ATL1* gene that encodes the Atlastin-1 protein. Patient specific stem cell neurons were produced to model SPG3A axonal degeneration and identify potential therapies. We generated patient-specific induced pluripotent stem cells (iPSCs) from two SPG3A patients with distinct mutations (p.P342S and p.M408T). This was achieved by introducing pluripotent factors into the patients' fibroblast cells. After confirming the iPSCs' characteristics, we differentiated them into cortical projection neurons, which are the cells most affected in SPG3A patients. To understand axonal degeneration in this iPSC-based SPG3A model, we employed a multi-step approach. First, RNA sequencing provided insights into regulated pathways and potentially affected genes. Next, guided by this data, we evaluated axonal swellings, synapsin levels, phosphorylated neurofilament heavy chain (pNFH) concentrations, caspase activity, lipid, and cholesterol gene expression. We observed significant reductions of synaptic genes and proteins in cortical projection neurons from both SPG3A-P342S and SPG3A-M408T patient iPSCs, revealing synaptic dysfunction in SPG3A. To further test the role of these processes in HSP, we treated cells with an oral active liver-X-receptor (LXR) agonist that can regulate lipid metabolism and transfer. LXR-agonist significantly mitigated the reduced synaptic proteins, followed by the rescue of axonal degeneration and apoptosis in SPG3A cortical neurons. Furthermore, analyses of the lipid and synaptic genes revealed that LXR-agonist treatment effectively restored these pathways in SPG3A patient neurons, which is correlated with the rescue of nerve degeneration. Taken together, this study reveals the role of synaptic dysfunction in the degeneration of SPG3A neurons, and identifies the therapeutic potential of LXR-agonist in mitigating human cortical neuron degeneration in HSP. This comprehensive strategy allowed us to precisely assess the extent of axonal degeneration and identify the underlying mechanisms.
Key Words: Hereditary spastic paraplegia, SPG3A, Axonal degeneration, iPSCs, Cortical projection neurons, Liver-X-receptor agonist

Disclosures: G. Thakur: None. **R. Dhanukate:** None. **Y. Mou:** None. **Z. Chen:** None. **C. Blackstone:** None. **X. Li:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.23/C60

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Carter Foundation
Blazer Foundation
Medical Biotechnology Program, UIC-Rockford

Title: Elucidating the role of BMP signaling in hereditary spastic paraplegia type 3A neurons

Authors: *P. KUNHIRAMAN[1], G. THAKUR[2], Z. CHEN[3], X.-J. LI[4];
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IL; [3]Univ. of Illinois at Chicago, Rockford, IL; [4]Dept. of Biomed. Sci., Univ. of Illinois, Rockford, IL

Abstract: Hereditary spastic paraplegia (HSP) is a group of neurological disorders characterized by the degeneration of cortical motor neuron axons. Based on the causative gene and its loci, HSP is classified into 80 genetic types. Spastic paraplegia type 3A (SPG3A) is an early-onset autosomal dominant form of HSP caused by mutations in the *ATL1* gene that codes for atlastin 1 protein. Atlastin 1 protein is enriched in brain and spinal cord, particularly in neurons. Earlier studies in zebrafish models showed that atlastin 1 knockdown led to increased BMP signaling and axonal abnormalities. Interestingly, inhibition of BMP signaling restored axonal defects. Very little studies were carried out in understanding how BMP signaling is altered in SPG3A patients' neurons. Herein, we have used SPG3A patient induced pluripotent stem cell (iPSC)-derived neurons to investigate how BMP signaling is altered in SPG3A and whether targeting BMP signaling can mitigate the degeneration of SPG3A neurons. First, patient specific iPSCs were generated by reprogramming SPG3A patient fibroblast cells. These stem cells were then differentiated into cortical projection neurons using the protocol established in the lab, which led to efficient generation of CTIP2- and TBR1-positive neurons. Interestingly, increased pSMAD1/5/8 levels were observed in SPG3A neurons compared to control neurons, implying the increased BMP signaling in SPG3A. To further evaluate the role of BMP signaling, cortical neurons were treated with BMP inhibitors and assessed for axonal degeneration and caspase activity. There was a significant increase in apoptosis levels (as indicated by Caspase 3/7 activity) in SPG3A neurons, which was mitigated by the treatment of BMP inhibitor. Furthermore, the increased release of pNFH (a biomarker for degeneration) was significantly reduced by the BMP inhibitor. Taken together, our data suggests that BMP signaling is dysregulated in SPG3A patient-derived neurons and targeting BMP signaling serves as a potential approach to mitigate the pathological changes in SPG3A. Further studies will be carried out to understand the molecular mechanisms underlying the dysregulated BMP signaling and how this impairment results in the disease phenotypes in SPG3A patient-derived neurons.

Disclosures: P. Kunhiraman: None. **G. Thakur:** None. **Z. Chen:** None. **X. Li:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.24/C61

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01NS118066
MBT, UIC

Title: Unveiling the role of astrocytes in the pathogenesis of SPG3A

Authors: *R. HAPASE[1], G. THAKUR[2], X.-J. LI[3,4];
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Abstract: Spastic Paraplegia Type 3A (SPG3A) is a rare hereditary neurodegenerative disease having an early onset of 4 years on average and is inherited in an autosomal dominant manner. It is characterized by lower body stiffness, spasms, and paralysis due to axonal degeneration of the upper motor neurons. SPG3A is caused by the mutations in the *ATL1* gene that encodes for the Atlastin-1 protein which is known to be involved in the tubule formation in Endoplasmic Reticulum (ER). These mutations lead to loss of function in Atlastin-1 protein which affects the cellular processes like cholesterol transport, protein synthesis, and tubule formation in ER. Neuroinflammation, induced by glial cells such as astrocytes, is recognized as a significant contributor to the disease's pathogenesis. Nevertheless, the underlying mechanisms of neuroinflammation are still not fully elucidated. Our study investigates the induction of neuroinflammation using synthetic dsRNA Polyinosinic-polycytidylic acid (PIC), and compare the differences between SPG3A and control astrocytes. Astrocytes are generated from human pluripotent stem cells using the established protocol in our lab. The cells were treated with the LXR agonists followed by PIC and the cell culture supernatant was collected to observe different cytokine secretions using ELISA. Using immunocytochemistry we analyzed the protein expression of GFAP (Glial Fibrillary Acidic-like Protein), an astrocytic marker, in the cells treated with PIC. We observed an increase in proinflammatory cytokines such as IL-6 and IL-8, along with elevated expression of GFAP following stimulation with PIC. Interestingly, treatment with LXR agonists (which have been shown to mitigate nerve degeneration in SPG3A) led to a significant rise in anti-inflammatory cytokines like CXCL10 and IFN-γ, along with a decrease in proinflammatory cytokines, implying that the LXR agonists might be able to alleviate the inflammation caused by astrocytes. In the future, we will construct and analyze co-culture models of SPG3A neurons and astrocytes aimed at facilitating a deeper exploration of their characteristics and thereby advancing our understanding of SPG3A pathogenesis.

Disclosures: R. Hapase: None. **G. Thakur:** None. **X. Li:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.25/C62

Topic: C.06. Neuromuscular Diseases

Support: Telethon #GGP20040
AFM-Telethon #24904
MUR-PRIN #2022WYCLTR
EU funding within the MUR PNRR “National Center for Gene Therapy and Drugs based on 8 RNA Technology” (# CN00000041 CN3 RNA)

Title: Inhibition of cullin 4 ring ubiquitin ligase complex recovers spastin protein levels and reduces defects in preclinical drosophila melanogaster models for Hereditary Spastic Paraplegia type 4

Authors: *C. CARSETTI[1,2], F. SARDINA[3], L. GIORGINI[3], G. FATTORINI[1,2], G. CESTRA[3,4], C. RINALDO[3];
[1]Univ. of Rome Sapienza, Dept. of Biol. and Biotech., Rome, Italy; [2]Institute of Molecular Biology and Pathology (IBPM), National Research Council (CNR), c/o University of Rome Sapienza, Rome, Italy; [3]Inst. of Mol. Biol. and Pathology (IBPM), Natl. Res. Council (CNR), c/o Univ. of Rome Sapienza, Rome, Italy; [4]Fondazione Santa Lucia IRCCS, c/o CERC, Rome, Italy

Abstract: Hereditary spastic paraplegias (HSPs) are a group of motor neuron disorders characterized by progressive weakness and spasticity in the lower limbs due to axonal degeneration. Spastic paraplegia type 4 (SPG4) is the most common form, it has an autosomal dominant inheritance pattern. Haploinsufficiency is the most plausible pathogenic mechanism behind the disease onset. SPG4 encodes spastin, an enzyme involved in microtubule remodeling and dynamics, which directly affects axonal trafficking. There is no cure for SPG4, although spastin-elevating strategies are emerging as promising therapeutic approaches. Our study aims to identify the molecular pathway regulating the turnover of spastin protein. It was already established that spastin levels are finely regulated by polyubiquitylation and proteasomal-mediated degradation, in a neddylation-dependent manner. Here, we show that the DDB1-Cullin-4-Ring ubiquitin ligase complex (CRL4) regulates spastin stability. Inhibition of CRL4 increases spastin levels by preventing its poly-ubiquitination and degradation. We have generated a *Drosophila melanogaster* model of SPG4 (Dm-SPG4), in which we achieved RNAi-mediated downregulation of spastin in different fly tissues. Genetic inhibition of the CRL4 complex, highly conserved in Drosophila, significantly affects several SPG4-like phenotypes in flies. We observed that RNAi-mediated downregulation of spastin in Drosophila causes defects in the locomotory activity, which is caused by alterations of neuromuscular junction morphology and function. Here we show that the RNAi-mediated downregulation of Cul4 rescues the alteration of synapse morphology and function, and the locomotor defects observed in the Dm-SPG4 model. Further, silencing Cullin 4 rescues spastin protein levels in the same fly model. As a proof of concept, we have pharmacologically inhibited the CRL4 complex with NSC1892. NSC1892 elevates spastin levels and rescues pathological phenotypes in the fly model and patient-derived cells. To find a more exclusive way to inhibit CRL4-mediated degradation of spastin, we are screening candidate DDB1-CUL4 associated factors (DCAFs) to find those specifically involved in spastin turnover. Chromatin assembly factor 1/ p55 subunit (CAF1-55) is one of the candidates DCAFs that has caught our attention. The RNAi-mediated downregulation of CAF1-55 in Dm-SPG4 model partially rescued spastin protein levels and retina degeneration due to spastin downregulation. Taken together, these findings show CRL4 involvement in spastin turnover and suggest the possibility of novel therapeutic interventions through the modulation of CRL4 activity.

Disclosures: C. Carsetti: None. **F. Sardina:** None. **L. Giorgini:** None. **G. Fattorini:** None. **G. Cestra:** None. **C. Rinaldo:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.26/C63

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS118066
MBT
Blazer Foundation

Title: Utilizing human iPSCs for SPG15 modeling: Investigating the impact of mitochondrial fission and lipid homeostasis on neuronal degeneration

Authors: *B. TAMILSELVAM[1], G. THAKUR[1], Z. CHEN[1], X.-J. LI[1], C. BLACKSTONE[2]; [1]Biomed. sciences, Univ. of Illinois Chicago, Col. of Med., Rockford, IL; [2]Neurol., Massachusetts Gen. Hosp., Charlestown, MA

Abstract: Hereditary Spastic Paraplegia (HSP) is characterized by progressive spasticity, stiffness, and weakness of the lower limbs resulting from axonal degeneration of corticospinal tract neurons. SPG15, a complicated form of HSP, is also the second-most prevalent autosomal recessive HSP. Mutations in the *ZFYVE26* gene result in the loss of function of the spastizin protein, leading to SPG15 pathogenesis. Spastizin has been found to localize to mitochondria, and lysosomes suggesting a potential role in mitochondrial and lysosomal function. The objective of our study was to investigate whether the loss of spastizin causes mitochondrial aberrations, leading to impaired ATP production and defective lipid homeostasis, ultimately resulting in defective anterograde axonal transport. To explore this possibility and investigate potential treatments for the neurodegeneration caused by spastizin deficiency, SPG15 patient-specific induced pluripotent stem cells (iPSCs) were generated from patient fibroblasts. The pluripotency of these SPG15 iPSCs was validated through immunocytochemical detection of pluripotency marker proteins. Subsequently, the SPG15 iPSCs were differentiated into cortical projection neurons using our established protocols. The resulting SPG15 iPSC-derived neurons recapitulated disease-relevant phenotypes, such as increased axonal swellings, a hallmark of the disease. Compared to healthy control neurons, the SPG15 neurons exhibited reduced ATP production, implying mitochondrial dysfunction in spastizin-deficient neurons. Furthermore, these neurons displayed slower axonal transport, which led to an accumulation of axonal swellings. To address these pathological features, we employed small molecules targeting specific pathways. Firstly, we utilized inhibitors of excessive mitochondrial fission, which increased ATP production in the SPG15 neurons. Secondly, we employed compounds that facilitated cholesterol efflux, thereby improving axonal transport in the SPG15 neurons. By targeting both mitochondrial dysfunction and impaired lipid homeostasis, our approach aimed to ameliorate the axonal pathology observed in this disease model.

Disclosures: B. Tamilselvam: None. **G. Thakur:** None. **Z. Chen:** None. **X. Li:** None. **C. Blackstone:** None.

Poster

PSTR212: Muscle, NMJ, HSP and Other Spinal Cord Diseases

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR212.27/C64

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Science Foundation award 2244127
University of Delaware Research Foundation award
Delaware Bioscience Center for Advanced Technology
American Academy for Cerebral Palsy and Developmental Medicine
Pedal with Pete Foundation award
programmatic funding from Nemours

Title: Epigenetic regulation of Spastic Cerebral Palsy regulatory circular RNAs

Authors: B. ROMERO CARPIO[1], P. HOQUE[2], K. ROBINSON[3], S. K. YEAGER[4], V. PARASHAR[1], R. E. AKINS[5], ***M. BATISH**[1];
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Abstract: Spastic cerebral palsy (CP) is the most common motor disability in childhood, affecting about 1 in 134 children in the U.S. Unfortunately, the underlying molecular mechanisms responsible for motor dysfunction in individuals with spastic cerebral palsy are not well understood. Recent studies suggest that regulatory RNAs could help explain major functional differences in the CP muscle. Specifically, circular RNAs (circRNAs) have been shown to play important roles in musculoskeletal disorders, however, their role in CP is not fully explored. This project aims for functional characterization of circRNAs in satellite cells-derived myoblasts (SC-MBs) and differentiated myotubes (SC-MTs).Differentially regulated circRNAs were identified from existing total RNAseq data obtained from RNA isolated from SC-MBs, SC-MTs, and erector spinae muscle of 9 control and CP subjects. SC-MBs were isolated from skeletal muscle biopsies of individuals diagnosed with spastic CP and control subjects (CN). The expression of reference markers PAX7 and MYF5 was used to validate the isolated SC-MBs. SC-MBs were cultured and differentiated into myotubes. Total RNA was isolated and qRT-PCR analysis using divergent primers was performed to identify and validate circular RNAs that were differentially expressed. The circFISH was used to image selected circRNAs to determine copy number and cellular localization. RNA binding proteins interacting with selected circRNAs were identified using the CRISPR-assisted detection of RNA-protein detection method.42 differentially expressed circRNAs were identified in SC-MBs, 106 in SC-MTs, and 227 in tissue sections. Among these, 11 circRNAs showed amplification and differential expression between control and CP groups. Particularly, we observed that the circRNA, Nuclear factor IX, (circNFIX), is significantly downregulated in muscle cells from CP as compared to control subjects. Using circFISH, we found circNFIX to be highly abundant and predominantly cytoplasmic. YBX-1 protein was validated to be interacting with circNFIX.Future work is aimed at validating the role and mechanism of action of circNFIX so that it can be used as a potential

therapeutic target. Additionally, we aim to perform targeted RNA seq specific for circRNAs to identify and characterize other dysregulated circRNAs for their function and potential for therapeutic targets. circRNAs are highly enriched into extracellular vesicles and thus this study opens the potential to identify dysregulated circRNAs that can be used as biomarkers for early, specific and non-invasive diagnosis for CP to improve outcomes of therapy and quality of life of CP patients.

Disclosures: B. Romero Carpio: None. **P. Hoque:** None. **K. Robinson:** None. **S.K. Yeager:** None. **V. Parashar:** None. **R.E. Akins:** None. **M. Batish:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.01/C65

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH SBIR Phase I

Title: Bridging the Gap: a complex, reproducible, multicellular, glia-containing cerebral organoid system for the study of neuroinflammation in pre-clinical screening and beyond

Authors: *N. Y. YUAN, W. RICHARDS, K. PARHAM, K. GREUEL, S. VISURI, R. GORDON, C. LEBAKKEN;
Stem Pharm, Inc., Madison, WI

Abstract: The role of neuroinflammation in neurodegeneration has been increasingly implicated as a major driver of disease development and progression. As the resident immune cells of the CNS, glia play a critical role in the production and propagation of inflammatory mediators such as cytokines, chemokines, reactive oxygen species, and bioactive lipids. The study of the activation, resolution, and failure of resolution of neuroinflammation by glial cells is of significant interest to the field and elucidating this process will further our understanding of neurodegenerative disease and potential treatments and therapeutics thereof. Most past research on neurodegenerative disorder takes a neuro-centric approach to modeling the disease, focusing on neuroprotection with most research performed in in-vitro systems having only neural lineage cells in either 2D or 3D systems. Recent advances in differentiation of human induced pluripotent stem cells (hiPSCs) has allowed great strides in generating models with greater recapitulation of the multi-cellular components present in the human brain. Stem Pharm's cerebral organoids containing neurons, astrocytes, endothelial cells, and microglia is ready for screening within 21 days. Bulk and single cell RNA-seq analysis of our organoids demonstrates high intraclass correlation and low coefficients of variation between biological replicates. Stem Pharm's organoid system eliminates much of the inherent variability in organoids by leveraging our proprietary synthetic hydrogel platform to form reproducible glia-containing organoids with

responsive astrocytes and microglia in residence. Herein, we characterize and describe the neuroimmunologicalresponse of our organoids with both PAMP and DAMPs; including pathogen derived (LPS) and host associated stimuli (amyloid beta oligomers and apoptotic neurons) with immunofluorescence staining, cytokine and biomarker quantification, and transcriptional analysis. Complex multicellular neural organoid systems bridge the gap between the in vitro to in vivo and can provide crucial and fundamental information on therapeutic development with high translational relevance.

Disclosures: N.Y. Yuan: A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **W. Richards:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **K. Parham:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **K. Greuel:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **S. Visuri:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **R. Gordon:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated. **C. Lebakken:** A. Employment/Salary (full or part-time):; Stem Pharm, Incorporated.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.02/C66

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: An iPSC derived neuroinflammation in vitro model of neurons and glial cells

Authors: *M. BSIBSI[1], A. POPALZIJ[1], M. ZANELLA[1], L. GEERTS[1], M. MUSTERS[1], S. KOSTENSE[1], I. FERREIRA[3], M. VLAMING[1], D. F. FISCHER[2];
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Abstract: Neuroinflammation occurs in most, if not all, neurodegenerative and inflammatory diseases. Mono *in vitro* cultures of astrocytes and microglia are powerful tools to study specific molecular pathways involved in neuroinflammation. However, more complex neuronal *in vitro* models are required to capture the effects of cellular communication on neuroinflammation in a human-based model which reduces animal testing in the early stages of drug discovery. We have established a complex *in vitro* culture model of neurons, astrocytes, microglia and oligodendrocytes, for which we used a variety of iPSC-derived neuronal and glial cells (ioGlutamatergic Neurons™, ioMicroglia™, ioOligodendrocyte-like cells™, iCell Astrocytes). We evaluated and validated the co-cultures by immunocytochemistry, multi-parametric high content imaging and multi-variate data analysis and the release of cytokine TNF-α as read-outs for neuroinflammation. Additionally, we triggered neuroinflammation and neurotoxicity in this model with LPS, nigericin and beta amyloid fibrils, followed by electrochemiluminescence-based detection to measure Neurofilament light chain (NfL), an established biomarker associated

with neurotoxicity and neurodegeneration. Co-cultures of neurons, astrocytes and oligodendrocytes increased branching of neuronal dendrites and astrocytes process. Maturation of oligodendrocytes, however, seems to be reduced in the presence of astrocytes, treatment with Tasin-1 (i.e., a well-known inducer of oligodendrocyte differentiation and myelination) promoted the maturation and myelin-binding protein (MBP) production in oligodendrocytes. Adding microglia to the cultures could inhibit maturation and differentiation of neurons and oligodendrocytes. Treatment with LPS, nigericin or beta amyloid fibrils leads to damage of neurons and oligodendrocytes as quantified by high content imaging and by the release of TNF-α and NfL. Taken together, we successfully established and characterized a complex *in vitro* culture model using iPSC derived neuronal and glial cells which we consider as a highly valuable tool for modeling neurodegenerative and inflammatory disease in drug discovery programs.

Disclosures: M. Bsibsi: None. **A. Popalzij:** None. **M. Zanella:** None. **L. Geerts:** None. **M. Musters:** None. **S. Kostense:** None. **I. Ferreira:** None. **M. Vlaming:** None. **D.F. Fischer:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.03/C67

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Deciphering neuroinflammation in neurodegeneration: Insights from a human iPSC-derived model

Authors: *T.-Y. HO[1], T. PAULUS[1], E. VOLFINZON[1], R. E. YANG[1], D. J. STONE[2], S. BARDEHLE[1], S. GYONEVA[1];
[2]Genet., [1]Cerevel Therapeut., Cambridge, MA

Abstract: Neuroinflammation represents a hallmark feature of various neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease. The modulation of inflammatory responses presents a promising strategy for therapeutic intervention against these debilitating conditions. In this study, we present a human-relevant model aimed at elucidating the roles of inflammation in neurodegeneration. First, we developed an *in vitro* ALS disease model by utilizing motor neurons derived from ALS patient induced pluripotent stem cells (iPSCs). Notably, we observed a reduction in TDP-43 nuclear intensity and a decrease in neurite length in motor neurons carrying the ALS C9ORF72 hexanucleotide repeat expansion. Furthermore, our analysis uncovered an upregulation of interferon-stimulated genes (ISGs) across different ALS patient iPSC-derived motor neurons. While we detected a cell-autonomous increase in inflammation marker expression within diseased neurons, overt neuronal death was not evident in neuron-only cultures. Thus, we hypothesized that

neuroimmune interactions, particularly with microglia, may exert significant influence on neurodegeneration. To dissect the impact of inflammation on neurodegeneration further, we established a human iPSC-derived microglia-neuron co-culture model. Upon stimulation with lipopolysaccharide (LPS), iPSC-derived microglia underwent activation and exhibited increased expression of proinflammatory cytokines and ISGs. In our preliminary findings, LPS treatment induced a reduction in the number of MAP2-positive neurons within microglia-neuron co-cultures, supporting the concept of inflammation-induced neurodegeneration. In summary, our study highlights the value of human iPSC-derived models for investigating neuroimmune interactions and neurodegeneration, and this strategy holds promise for assessing the efficacy of therapeutic interventions aimed at mitigating inflammation in neurodegenerative disorders.

Disclosures: T. Ho: None. **T. Paulus:** None. **E. Volfinzon:** None. **R.E. Yang:** None. **D.J. Stone:** None. **S. Bardehle:** None. **S. Gyoneva:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.04/C68

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: TGen Foundation

Title: Exploring Rare Childhood Diseases through Neural Organoid Generation

Authors: *L. NICHOLSON, M. MOSQUEDA CRESPO, A. KAMZINA, F. TAGUINOD, S. BERES, I. S. PIRAS, M. J. HUENTELMAN;
Translational Genomics Res. Inst., Phoenix, AZ

Abstract: Rare neurological disorders in children are inadequately diagnosed and treated resulting in quality of life decrease through symptoms such as developmental delay and reduced cognitive and motor abilities. Improving both the diagnostic process, as well as focusing on a personalized drug testing model would significantly increase the quality of life for these children and their families. Organoid generation is a novel technique that allows for reproducible and versatile modeling of neurodevelopmental processes and associated disorders. In this study we used neural organoids generated from iPSCs with patient-specific genetic mutations to study genotype-phenotype relationship *in vitro*. We generated patterned forebrain, dorsal and ventral, neural organoids with CRISPR-introduced clinically-validated disease-causing point mutations identified in children with rare disorders (*DOCK3, GABRA1*, *HNRNPH2*, *MTFMT*, *WDR45*) and wildtype control (WT). Organoids were collected over 100-days. Immunofluorescence, RT-qPCR and scSeq was performed. RT-qPCR of *SOX* and *PAX1* confirmed expected neurodifferentiation and maturation of organoids during the optimization stage. The presence of different cell types, including astrocytes, inhibitory and excitatory neurons, were seen in all five

mutant and wildtype organoids. Cell proportions showed differences across mutation, region, and timepoint, such as astrocytes being predominantly present in dorsal organoids compared to ventral and excitatory neurons having a higher proportion in WT dorsal/ventral on day 50 compared to all mutants except *HNRNPH2* ventral organoids (Fig.1 A). Immunofluorescence of organoids confirmed mature neurons via beta-tubulin III, as well as region specific brain structures (Fig.1 B). We successfully used neural organoids generated from cells containing patient-specific mutations to investigate phenotypic changes. Organoids represent an efficient and reproducible *in vitro* disease model for neurodegenerative diseases, specifically due to their ability to be generated from mutation-carrying iPSCs.

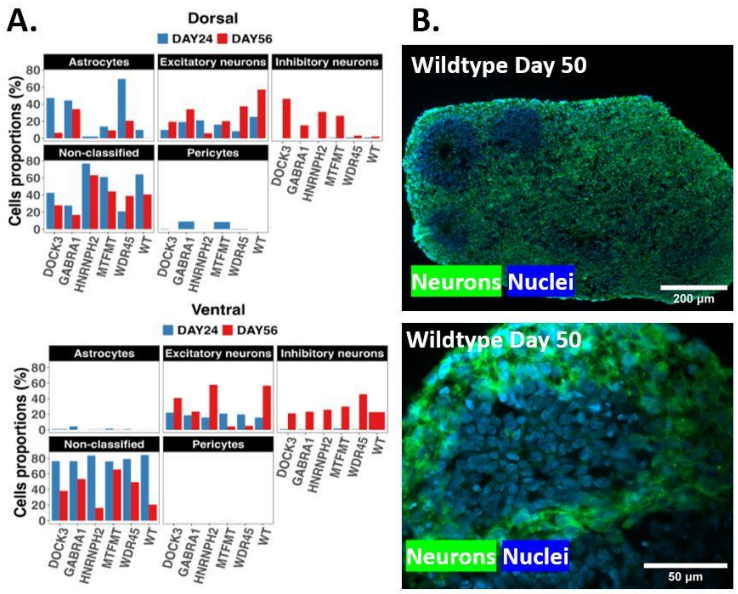

Figure 1. **A**. Single cell sequencing results showing cell type proportions between five mutant organoids (*DOCK3, GABRA1, HNRNPH2, MTFMT, WD45*) and control (WT). Comparisons are divided by forebrain region (ventral and dorsal) and timepoints (day 24 and day 56). **B.** Immunofluorescence staining of beta-tubulin III (neurons) in a representative WT organoid on day 50. Mature neurons and ventricle-like structures can be seen.

Disclosures: L. Nicholson: None. **M. Mosqueda Crespo:** None. **A. Kamzina:** None. **F. Taguinod:** None. **S. Beres:** None. **I.S. Piras:** None. **M.J. Huentelman:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

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Program #/Poster #: PSTR213.05/C69

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: This work was supported by the National Research Foundation of Korea (NRF-2021R1F1A1049191), project for Industry-University-Research Institute platform

cooperation R&D funded Korea Ministry of SMEs and Startups in 2022 (S3309832), the National Resea

Title: Quantitative Analysis and Drug Screening Potential with Tissue Clearing Using Tumoroids

Authors: *S. PARK;
Korea Inst. of Toxicology, Daejeon, Korea, Republic of

Abstract: Glioblastoma is one of the brain cancers known for its highly aggressive and rapidly growing characteristics. Due to its structural features, diagnosis and treatment are exceptionally challenging, leading to a low survival rate. Research for more effective treatments is still actively ongoing. Recently, various disease models using organoids have been established for drug development. Brain organoids derived from human cells are 3D structures that mimic human organs, and they have gained attention as a technology that can replace animal experiments because of limitations in evaluating chemicals and drugs responses-based species differences between human and animal. Here, we generated organoids derived from glioblastoma patients and utilized them for quantitative analyses. To comprehensively analyze the organoids with a 3D structure, we introduced tissue clearing technique, minimizing the loss of information and developing an accurate method for toxicity assessment. The cleared organoids allowed observation of the internal tissue structure and enabled morphological analysis using various types of biomarkers. Moreover, the treatment with Temozolomide (TMZ), one of the commonly used chemotherapeutic agents for brain cancer, was employed to compare and identify differences with patient-derived organoids. This approach confirmed its suitability as a screening method for potential drug candidates.

Disclosures: S. Park: None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.06/C70

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI Grant Number 21K05443

Title: Neuroinflammation and gut microbiota dysbiosis in Alzheimer’s disease

Authors: *K. YAMASHIRO;
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Abstract: Neuroinflammation plays a key role in the pathogenesis of Alzheimer’s disease (AD). With the recent advances in diffusion magnetic resonance imaging techniques, free water (FW)

imaging has been used as a potential biomarker of neuroinflammation. Gut microbiota affect human health by modulating the host's immune system and regulating host physiology. Gut microbiota dysbiosis has been linked to the pathogenesis of neurological diseases including AD. However, the relationship between neuroinflammation and gut microbiota in AD is not well known. The aim of the present study was to examine the relationship between FW imaging and gut microbiota in patients with AD and mild cognitive impairment (MCI). Fifty-six participants underwent neuropsychological assessments, FW imaging, and gut microbiota analysis targeting the bacterial 16S rRNA genes. They were categorized into the cognitively normal control (NC) (n=19), MCI (n=19), and AD (n=18) groups. The correlations among FW values, neuropsychological assessment scores, and the relative abundance of gut microbiota were analyzed. FW values were higher in several white matter tracts and in gray matter regions, predominantly the frontal, temporal, limbic, and paralimbic regions, in the AD and MCI groups than in the NC group. In the combined AD + MCI group, higher FW values in the temporal (superior temporal and temporal pole), limbic, and paralimbic (insula, hippocampus, and amygdala) regions were the most associated with worse neuropsychological assessment scores. In addition, increased FW values in these gray matter regions were correlated with decreased relative abundances of butyrate-producing genera *Anaerostipes*, Lachnospiraceae UCG-004, and [*Ruminococcus*]*gnavus* group, in the AD + MCI group. Butyrate is one of the most abundant short-chain fatty acids produced by gut microbial fermentation of dietary fibers. Butyrate is a multi-functional molecule that has several neuroprotective effects, including modulating microglial functions, maintaining blood-brain barrier integrity, and preventing the formation of toxic soluble amyloid β aggregates. The present findings suggest an association between neuroinflammation and decreased levels of butyrate, which has potentially beneficial roles in brain homeostasis.

Disclosures: K. Yamashiro: None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.07/C71

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Cerebral amyloid angiopathy-related inflammation: comparison with cerebral amyloid vasculitis associated with anti-amyloid beta therapy

Authors: *R. J. CASTELLANI, P. JAMSHIDI;
Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Background. Cerebral amyloid angiopathy-related inflammatory disease (CAA-RI) is a rare form of vasculitis characterized clinically by diverse neurological signs, radiographically by T2/FLAIR hyperintensities, and pathologically by CAA vasculitis. In recent years, it has

become apparent that anti-Aβ immunotherapy is often complicated by an imaging and symptom complex (i.e., ARIA) that shares pathogenic similarities with CAA-RI. We previously reported the first case in the literature of a fatal complication of anti-Aβ-related immunotherapy, and described the primary pathological findings as a necrotizing histiocytic CAA vasculitis, plaque phagocytosis, and subtotal clearing of Aβ plaques. Here, we report the biopsy findings in a patient with CAA-RI, and compare the findings with emerging anti-Aβ antibody-associated pathology. Methods. This case was submitted to the institutional review board and exempted. A 75-year-old woman presented with new right-sided weakness and was diagnosed with left occipital hemorrhagic stroke. Initial MRI showed complicated occipital FLAIR hyperintensity with leptomeningeal enhancement. A left parietal craniotomy with open biopsy was performed. Results. Histopathology demonstrated CAA with widespread granulomatous vasculitis without a specific necrotizing component. In addition, there were robust perivascular lymphoplasmacytic infiltrates, including B and T lymphocytes. Focal phagocytic involvement of amyloid plaque cores was noted. Conclusion. This prototypical case of CAA-RI showed remarkably similar vasculitis to anti-Aβ antibody-associated vasculitis (i.e., ARIA), but differed with its robust lymphoplasmacytic perivascular infiltrates (consistent with the literature on this entity) and absence of a necrotizing component, compared to the pure histiocytic inflammation and prominent fibrinoid degeneration of blood vessel walls in our case of anti-Aβ antibody-associated vasculitis. Examination of more cases anti-Aβ antibody-associated vasculitis is needed to determine whether the monomorphic inflammation and marked fibrinoid change represent distinguishing features of this condition, distinct from CAA-RI.

Disclosures: R.J. Castellani: None. **P. Jamshidi:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

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Program #/Poster #: PSTR213.08/C72

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Deanship of Research, Jordan University of Science and Technology, grant number: 20220388

Title: The potential of programmed cell death protein-1 (PD-1) and its ligands PD-L1 and PD-L2 as biomarkers for multiple sclerosis

Authors: *M. S. ALKEILANI[1], W. ALSHARAA[3], S. JARADAT[2];
[1]Clin. Pharm., [2]Jordan Univ. of Sci. and Technol., Irbid, Jordan; [3]Jordan university of science and technology, Irbid, Jordan

Abstract: Background: Multiple sclerosis (MS) is a chronic neurodegenerative disease that affects the central nervous system. Programmed cell death protein (PD-1) and its ligands; PD-L1

and PD-L2 play critical roles in regulating the immune response with a suggested implication in MS.**Objectives**: The present study aimed to identify the serum levels of PD-1, PD-L1, and PD-L2 as prognostic and diagnostic biomarkers in MS. Moreover, we wanted to explore the genetic association of programmed cell death gene 1 (PDCD1) variants with the susceptibility of MS in the Jordanian population.**Methods**: A case-control study was conducted on Jordanian MS patients and healthy subjects. The protein serum levels were measured using enzyme-linked immunosorbent assay (ELISA) technique for 88 MS patients and 82 age- and gender-matched healthy controls. PDCD1 variants in the five exonic regions were genotyped in 95 MS patients and 97 healthy controls using the automated Sanger sequencing method. All statistical analyses were done using Statistical Package for the Social Sciences software.**Results**: PD-L2 levels were significantly lower in MS patients compared to healthy subjects (368.683 pg/ml vs. 404.96 pg/ml, respectively; p=0.004). Receiver operating characteristics analysis (ROC) suggested that PD-L2 serum level can be used to discriminate between MS patients and healthy controls with a cut-ff value of 379.4405pg/ml. No significant associations were found between PD-1 and PD-L1 levels and MS susceptibility. In MS patients, associations were found between PD-L1 levels with the EDSS score (p=0.036), and PD-L2 levels with the presence of comorbidities (p-value = 0.007). Negative correlations were found between PD-L2 levels with BMI (r= -0.268, p=0.013), and PD-L1 levels (r=-0.259, p-value=0.016), and between PD-L1 and PD-1 levels was found (r= -0.357, p=0.001). A positive correlation was revealed between PD-L2 and PD-1 levels (r =0.434, p<0.001).
We found one SNP in exon five (rs2227981; c.804 T>C). The frequencies of CC and CT genotypes were equal but were higher than the frequency of TT genotypes among all participants. But there were no significant differences in the distribution of those genotypes between MS patients and healthy controls (p= 0.869), also there were no significant differences in the distribution of C and T alleles between them.**Conclusion**: PD-L2 serum level was significantly lower in MS patients compared to healthy controls, suggesting PD-L2 as a diagnostic biomarker for MS. Further studies with larger sample sizes are required to confirm our results.

Disclosures: M.S. Alkeilani: None. **W. Alsharaa:** None. **S. Jaradat:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.09/C73

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Socs differential expression in MS patients

Authors: *V. SEDEÑO MONGE[1], G. SANTOS-LOPEZ[2], E. BAUTISTA RODRÍGUEZ[3], N. ROSAS MURRIETA[4];
[1]Med. Sci., Univ. Popular Autonoma De Estado De Puebla, Atlixco, Mexico; [2]Inst. MEXICANO

DEL SEGURO SOCIAL, ATLIXCO, PUEBLA, Mexico; [3]Univ. Popular Autónoma de Puebla, PUEBLA, PUEBLA, Mexico; [4]CIENCIAS QUIMICAS, BENEMERITA Univ. AUTONOMA DEL ESTADO DE PUEBLA, Puebla, Mexico

Abstract: Socs differential expression in MS patients Sedeño Monge Virginia, Rosas Murrieta Nora Hilda, Santos López Gerardo, Bautista Rodríguez Elizabeth**Abstract**
Multiple sclerosis is a chronic and demyelinating disease of the central nervous system, environmental, nutritional, and genetic factors have been implicated in the susceptibility. As an autoimmune disease, an exacerbated response is found, some proinflammatory interleukins are regulated by suppressors of cytokine signaling (SOCS) proteins. This study aimed to identify the association among four members of the socs family (cis, socs2, socs4, and socs6), and IL23 and IL4 interleukins in the pathophysiology of MS. Blood samples were obtained from thirty MS patients, RNA was extracted and the expression level of *cis, socs2, socs4, and socs6* genes by RTqPCR was quantified, and plasmatic levels of IL23 and IL4 were measured through the ELISA method. A significant reduction (p=0.03) in Socs2 expression was observed in MS patients in comparison with the healthy group. Between the MS patients, *socs2* expression was the most reduced (0.694 fold) and least significant compared to *cis* and *socs4* (p=0, p=0.00002 respectively), while *socs4* RNAm presented the highest and most significant (1.011 fold, p= 0) expression compared to the other socs. According to the genus, in MS women, a reduction in *socs2* and *cis* expression (0.726 and 0.655 fold respectively) was observed as compared to healthy women (1.00 and 1.01 fold), significant only for socs2 (p=0.03), and a reduction in *socs2* expression (0.698 fold) in MS men as compared to healthy men. MS patients treated with IFN or GA had the lowest RNAm expression for socs2 (p=0.05) 0.679 and 0.737 respectively compared to other socs. Significantly higher levels of IL4 and IL23 were found in the MS group in comparison to the control group (p=0.015, p=0.001 respectively), finding significantly higher levels of IL23 (69.35 mg/dl) compared to IL4 (5.44 mg/dl) (p=0.0000) in the MS group. Patients treated with glatiramer acetate presented higher levels of IL4 and IL23 (6.03 and 73.92 mg/dl) than patients treated with IFN (5.22 and 67.64 mg/dl). A significant and relatively weak negative correlation between Cis expression and plasmatic levels of IL23 was observed. Results presented show a reduction in the regulation of immune response that could contribute to the immunopathophysiology of MS.
Keywords multiple sclerosis, socs, interleukins

Disclosures: V. Sedeño MONGE: None. **G. Santos-Lopez:** None. **E. Bautista Rodríguez:** None. **N. Rosas Murrieta:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.10/C74

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R21 NS123546
NINDS R01 NS123532

Title: M6a methylation is associated with hippocampal pathology in multiple sclerosis patients

Authors: *N. SRINIVASAN, H. MENDRIES, T. D. NIEPOKNY, R. DUTTA;
Neurosci., Lerner Res. Inst., Cleveland Clin., Cleveland, OH

Abstract: Introduction: Among the plethora of neurological issues memory impairment occurs in majority of MS patients. Pathological analysis estimates ~60% of MS hippocampus to be demyelinated and associated with decrease in genes controlling memory and synaptic functions. Recent studies have found m6A-methyladenosine (m6A) modification playing a role in multiple processes of the CNS including development and biological functions in neurodegenerative disorders. **Objectives/Aims:** We investigated the possibility of m6A modification playing a major role in altering hippocampal gene expression and memory function in MS patients. **Methods:** Using a combination of RNA expression analysis and immunohistochemistry measurements we localized m6A methylation pathway members in MS hippocampus. Using MeChIP array studies we identified several key genes that are differentially methylated in MS hippocampus following demyelination. **Results:** Through RNA analysis of MS hippocampus, we found altered mRNA levels of key genes associated with m6A methylation, m6A proof reading, and m6A methylation erasing pathways. Increased m6A methylation suggested by increased levels of mRNA encoding methyltransferase “writer” METTL3 (+1.38 fold, p=0.0317), accessory component WTAP (+3.79 fold, p=0.0079) as well as the m6A “reader” YTHDC2 (+1.37 fold, p=0.0340) was detected. Conversely, downregulation of the demethylase “eraser” FTO (-1.29 fold, p=0.0159) possibly implicated a lack of removing methylation marks in demyelinated MS hippocampus. These key m6A pathway genes were localized primarily to CA1, CA3 and DG regions in MS brains. Comparative analysis identified several genes with hypo- and hyper methylated marks in demyelinated MS hippocampus previously identified to be involved in MS pathogenesis. **Conclusion:** This work identifies m6A methylation as an epigenetic mechanism that may be operative in MS hippocampus and alter memory function following demyelination.

Disclosures: N. Srinivasan: None. **H. Mendries:** None. **T.D. Niepokny:** None. **R. Dutta:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

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Title: Phase I trial of intracerebroventricular transplantation of allogeneic neural stem cells in people with progressive multiple sclerosis: further development and phase II planning

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Abstract: We have completed an open-label, first-in-human, dose-escalation Phase I study (ClinicalTrials.gov: NCT03282760, EudraCT2015-004855-37) involving the transplantation of allogeneic human neural stem cells (hNSCs, derived from a single donor) into 15 patients (9 females and 6 males) suffering from secondary progressive multiple sclerosis. Study participants were divided into 4 groups and received different escalating dosages of hNSCs (5- 10- 16-million, with 3 patients per group, or 24-million cells, with 6 patients per group) administered via intracerebroventricular injection, along with a systemic immunosuppressive regimen. No deaths, serious adverse events, or withdrawals were observed. Laboratory and clinical outcomes (EDSS and MSCF scales), as well as lesion load and brain activity (MRI), did not exhibit significant changes throughout the 1-year follow-up. Longitudinal metabolomic and lipidomic analysis of cerebrospinal fluid and serum revealed time- and dose-dependent responses, with increased levels of acyl-carnitines and fatty acids in the cerebrospinal fluid. Our work demonstrates that the treatment is feasible, safe, and well-tolerated, even at the highest dosage, representing a significant milestone for the safe translation of stem cells into regenerative medicine. We will present the new study design for the follow-up Phase II study, in which we aim to increase the cell dosage, introduce a placebo arm, and enhance the metabolomic and lipidomic analysis of cerebrospinal fluid. Also on behalf of hNSC-SPMS[2] group: Paola Crociani, Giada D'Aloisio, Pietro Di Viesti, Danilo Fogli, Andrea Fontana, Antonio Laborante, Giuliana PIacentino, Teresa Popolizio, Michele Zarrelli.

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of CITC Ltd. F. Consulting Fees (e.g., advisory boards); Chair of the Scientific Advisory Board at ReNeuron plc..

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.12/Web Only

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: UNAM-DGAPA IN213423

Title: Detection of white degeneration of the visual pathway via MRI diffusion images

Authors: *D. COUTIÑO[1], M. GARCIA GUILLEN[1], J. GUERRERO ZAVALA[2], L. CONCHA[1];
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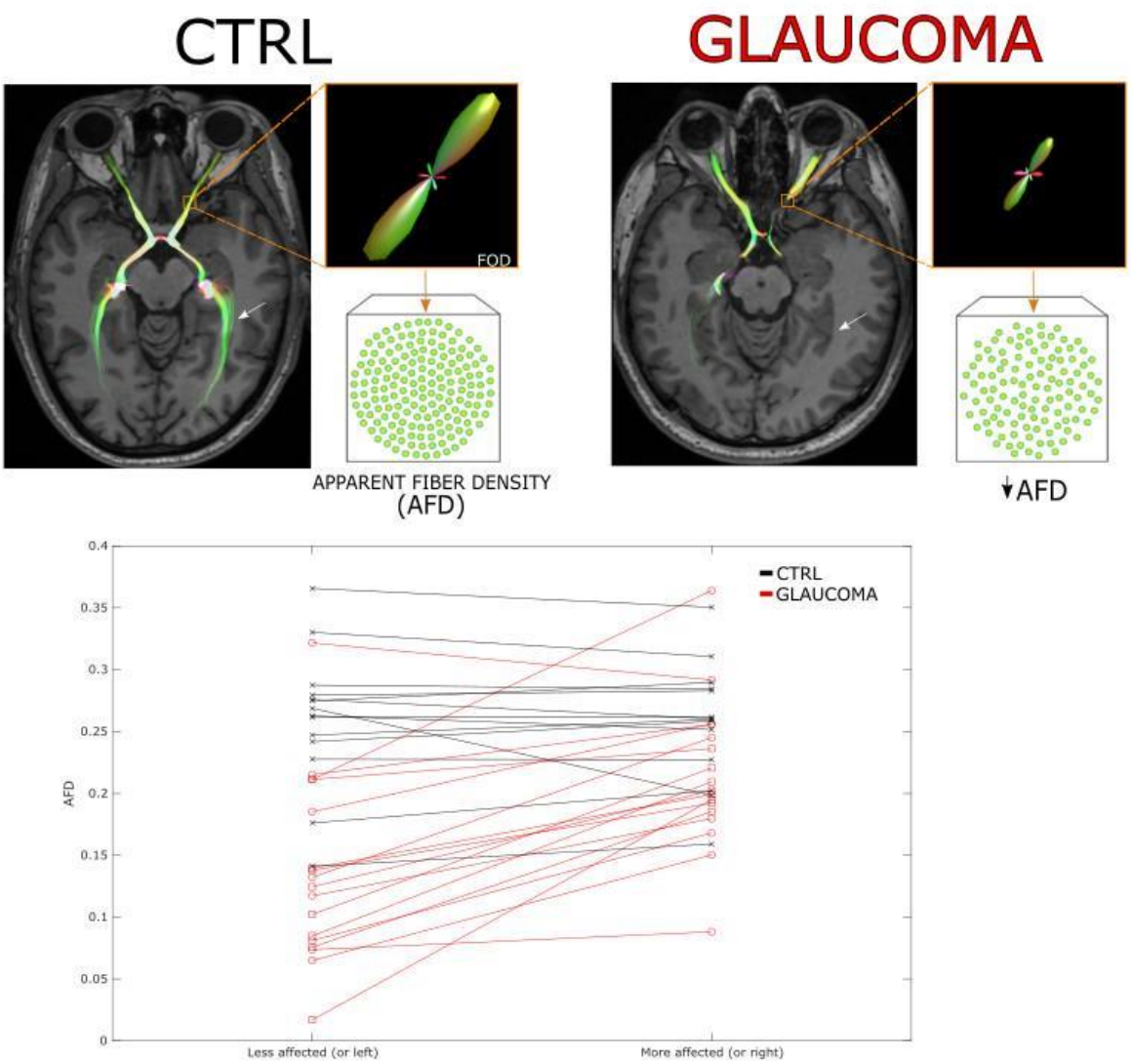
Abstract: Diffusion MRI (dMRI) serves as a valuable non-invasive method for exploring the microstructural properties of brain tissue. However, its reliability diminishes in regions with intricate configurations, such as fiber crossings, challenging histopathological inference in neurodegenerative disorders. Prior work performed in rodents has validated advanced dMRI for analyzing quantitative metrics related to axonal density and their sensitivity to axonal degeneration (Rojas-Vite 2019). This study aims to assess the potential of advanced dMRI in detecting axonal degeneration in vivo. Patients with asymmetric glaucoma provide an opportunity to assess the validity of dMRI to detect axonal degeneration in white matter. We scanned 25 control subjects and 25 patients with asymmetric glaucoma from the Instituto Mexicano de Oftalmología (IMO) using a 3T General Electric (GE) scanner. T1-weighted images were be obtained with resolution of 0.8x0.8x0.8 mm^3 Diffusion MRI were acquired with a multi-shot echo-planar imaging sequence with resolution of 1.7x1.7x1.7 mm^3 with 6 volumes with b=0 s/mm^2, and 16/32/64 volumes with unique directions for diffusion sensitization with b=500/1000/2000 s/mm^2. Constrained Spherical Deconvolution (CSD) was used to perform tractography to delineate the optic nerves. Apparent fiber density (AFD) was extracted from tractography-aided regions of interest of the optic nerves. Additionally, fractional anisotropy (FA) and mean diffusivity (MD), were obtained by fitting a diffusion tensor at each voxel. The average values of these diffusion metrics were compared between groups and between optic nerves for patients with asymmetric glaucoma (i.e., most affected vs least affected).AFD and tensor metrics of control subjects did not show asymmetries. The most affected optic nerves of patients with glaucoma showed a significant reduction of AFD and FA, and increased MD, as compared to their least affected nerves and were, in turn, statistically different from controls. Least affected nerves were also different from controls, albeit to a lesser degree (Figure).



Disclosures: D. Coutiño: None. **M. Garcia Guillen:** None. **J. Guerrero Zavala:** None. **L. Concha:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.13/C76

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Epidemiological Analysis of Mortality Predictors and Readmissions in Patients with Transverse Myelitis and/or Acute Disseminated Encephalomyelitis

Authors: S. GARG[1], D. KUMAR[1], A. SURABHI[2], A. RAO[3], A. AGRAWAL[3], A. GARYALI[4], D. KUMAR[5], R. PANJALA[6], *A. YOUNG[7];
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Abstract: Introduction Transverse Myelitis (TM) and Acute Disseminated Encephalomyelitis (ADEM) are neurological disorders associated with significant morbidity and mortality. Understanding the predictors of mortality and readmissions in these patients is crucial for improving clinical management and resource allocation.
Methods We conducted a retrospective cohort study utilizing data from 25,240 patients with TM and/or ADEM discharged between 2010 and 2020, excluding 2015, from a nationwide database. Baseline characteristics and predictors of mortality were analyzed using logistic regression models. Readmissions within 30 days were assessed across different calendar years.
Results The mortality rate among the cohort of patients with Transverse Myelitis (TM) and/or Acute Disseminated Encephalomyelitis (ADEM) was 2.56%. Analysis of hazard ratios revealed several significant predictors of mortality. Advanced age emerged as a strong predictor, with hazard ratios ranging from 2.382 (95% CI: 1.242-4.568) for the 45 to <65 years age group to 3.735 (95% CI: 1.716-8.131) for patients aged 85 years and older, compared to those under 10 years. Male sex did not significantly impact mortality risk (adjusted OR 0.924, 95% CI: 0.783-1.09). Patients with a longer length of stay (LOS) during the index admission, defined as seven days or more, exhibited a higher risk of mortality (adjusted OR 1.633, 95% CI: 1.371-1.944). Several comorbidities were identified as significant predictors of mortality. Notably, patients with congestive heart failure (adjusted OR 2.311, 95% CI: 1.87-2.857), cerebrovascular disease (adjusted OR 2.803, 95% CI: 2.281-3.445), moderate/severe liver disease (adjusted OR 3.468, 95% CI: 1.986-6.055), metastatic cancer (adjusted OR 2.201, 95% CI: 1.437-3.371), and other types of cancer (adjusted OR 2.256, 95% CI: 1.692-3.007) had significantly higher mortality risks compared to those without these conditions. Analysis of readmissions within 30 days of index admission revealed varying rates across different calendar years, ranging from 7.27% to 12.82%. This variability suggests potential temporal trends in post-discharge healthcare utilization among TM and ADEM patients, warranting further investigation.
Conclusion: In this large hospital-based discharge data analysis, we identified that advanced age, moderate to severe liver disease, congestive heart failure and cancer to be predictors of mortality.

Disclosures: S. Garg: None. **D. Kumar:** None. **A. Surabhi:** None. **A. Rao:** None. **A. Agrawal:** None. **A. Garyali:** None. **D. Kumar:** None. **R. Panjala:** None. **A. Young:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.14/C77

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS129407
NIH Grant P20GM109089
NIH Grant P30GM122734

Title: Thalamic neuroinflammation exerts distant effects on white matter health in post-acute sequelae of COVID-19

Authors: *A. VAKHTIN[1], H. LIN[2], A. BIRG[2], S. RYMAN[1];
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Abstract: One third of mild COVID-19 cases produce chronic symptoms, many of which are neurocognitive in nature. We previously described an indirect pathogenesis model for post-acute sequelae of COVID-19 (PASC) via chronic dysfunctional gut-brain interactions. Given its common gastrointestinal disturbances, COVID-19 can induce or exacerbate gut dysbiosis, facilitating systemic proliferation of microbial byproducts that can reach the brain and produce neurocognitive deficits. Indeed, we have previously demonstrated that gut-derived systemic levels of lipopolysaccharide - a potent activator of the immune system - are strongly related to thalamic microglial activation, as measured using diffusion weighted magnetic resonance spectroscopy (dMRS). In expanding the gut-brain model of PASC, here we examined whether the dMRS-based measure of thalamic neuroinflammation is related to health levels of white matter (WM) tracts. In addition, the associations between thalamic neuroinflammation and WM tract properties were contrasted between patients who have neurocognitive post-acute sequelae of COVID-19 (PASC+) and former COVID-19 patients who have recovered fully (PASC-). Microglial activation-related morphological changes in the left thalamic region were captured using dMRS as increased apparent diffusivity coefficient (ADC) of choline, which resides in microglia in concentrations that are higher than other cells in the brain. To obtain localized WM properties within 20 major WM tracts, conventional water-based diffusion tensor imaging (DTI) data were processed using Automated Fiber Quantification (AFQ). Increased neuroinflammation in the thalamic region was associated with aberrant WM DTI metrics (fractional anisotropy and mean/axial/radial diffusivities) in several major WM tracts ($p = 0.05$). While tracts with direct and proximal thalamic projections, such as the anterior thalamic radiation, were affected, WM metrics related to thalamic neuroinflammation were not necessarily adjacent to the thalamus. Rather, effects of regional thalamic neuroinflammation on WM properties were detected in distant sections of tracts as well. Further, the observed relationships were driven by the PASC+ group, suggesting that WM health in long COVID patients may be more vulnerable to neuroinflammatory damage relative to controls. Collectively, our findings raise the possibility that localized neuroinflammation may exert its detrimental effects via WM tracts remotely, affecting distant regions of the brain.

Disclosures: A. Vakhtin: None. **H. Lin:** None. **A. Birg:** None. **S. Ryman:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.15/C78

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Zika virus Infection stimulates inflammatory cytokine production in the human brain of endothelial cells monoculture

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Abstract: Zika virus (ZIKV), a neurotropic arbovirus from the *Flaviviridae* family is associated to neurological diseases including encephalitis, microcephaly, Guillain-Barre syndrome, and myelitis. However, the mechanisms underlying the neuropathogenesis of ZIKV infection are not yet fully understood, and the role of cytokines are still controversial. ZIKV infection induced the expression of pro-inflammatory and anti-inflammatory cytokines, causing inflammation and altered physiological functions in multiple organs. In this study, African ZIKV isolate (MR-766) infection to human brain endothelial cell (HBEC-5i) were used to investigate the mechanisms underlying neuroinflammation and neuropathogenesis. It was established monoculture of HBEC-5i with standard conditions and we selected A549 cells for the viral titer due for their susceptibility to ZIKV infection. The cytokines were measured by microbead-based Bio-Plex Pro Human Cytokine 27-plex Assay (BioRad, Hercules, CA, USA) by triplicate. The cytokines expressions were evaluated with 0.1, 1.0 and 10 MOI during through 30 min, 1, 3, 6, 12 and 24 h in the culture supernatant of ZIKV-infected cells. All experimental results are represented as mean values ± standard error of the mean and data results are expressed in terms of pg/ml. Statistically significant differences between the groups were determined using Student t test. Our study demonstrated that ZIKV infection promotes pro-inflammatory, anti-inflammatory cytokines and chemokines expression. ZIKV infection induced the maximum increase secretion for IL-1β (6.11 ± 0.16 vs control 2.84 ± 0.12; $p<0.0005$), IL-6 (938.36 ± 11.57 vs control 531.36 ± 9.79; $p<0.0005$), IL-9 (67.77 ± 3.26 vs control 53.85 ± 1.34; $p<0.05$), IFN-γ (17.92 ± 0.77 vs control 8.95 ± 0.36; $p<0.005$) and TNFα (26.09 ± 0.38 vs control 13.16 ± 0.62; $p<0.0001$) expression at 24 h with MOI 0.1. The ZIKV infection induces anti-inflammatory IL-5 expression (122.96 ± 4.81 vs control 61.94 ± 1.38) at 24 h with MOI 0.1. IP-10 (35.93 ± 2.19 vs. 18.43 ± 1.83; $p<0.003$), MCP-1 (174 ± 2.32 vs control 99.50 ± 3.18; $p<0.0005$); MIP-1a (10.39 ± 0.08 vs control 6.53 ± 0.13; $p<0.0005$), MIP-1b (19.16 ± 0.32 vs control 13.54 ± 0.50; $p<0.005$) at 24 h with MOI 0.1, meanwhile RANTES 27.61 ± 0.53 vs control 4.49 ± 0.20) at 1h MOI 0.1. Some

cytokines expression (IL-10, IL-12 and IL-13) were not present in detectable limits These results showed ZIKV infection induced exacerbated expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-9, IL-12, IFN-γ, and TNF-α), anti-inflammatory citokine (IL-5) and chemokines (IP-10, MCP-1a, MCP-1b and RANTES) in HBEC-5i

Disclosures: P. Campos-Bedolla: None. **P. Ruvalcaba Hernández:** None. **J.E. Marquez:** None. **R. Lira:** None. **J. Arellano-Galindo:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.16/C79

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: McKnight Scholar
Rita Allen Scholar
Klingenstein-Simons Fellow
DP2MH132943

Title: Single-cell analysis reveals the transcriptional characteristics and spatial distribution of glial cells in the epileptic microenvironment

Authors: *Q. LIN[1], C. KANG[1], L. CHEADLE[2,3];
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Abstract: Epilepsy is a common neurological disorder characterized by recurrent seizures often localized to the temporal lobe. The intricate cell communication pathways and molecular characteristics of the epileptiform microenvironment are yet to be fully understood. Research has shown that microglia in drug-resistant epilepsy (DRE) patients release canonical proinflammatory cytokines such as IL1α, IL1β, and TNFα, the release of which can induce neurotoxic effects by astrocytes. Furthermore, white matter pathology observed in epilepsy could reflect impairments in oligodendroglial function as well. Taken together, these observations suggest that dysfunctional signaling between glia and neurons may contribute to the pathogenesis of epilepsy. To test this hypothesis, we collaborated with a neurosurgery team to collect and immediately flash freeze brain tissue and blood from 6 DRE patients undergoing surgical resection. Three sample types were obtain: (1) Epileptiform tissue; (2) Neighboring but non-epileptiform tissue; and (3) non-epileptiform tissue that had been acutely stimulated with a bipolar electrode. We are currently applying a combination of single-nucleus RNA-sequencing and high-resolution spatial transcriptomics to map transcriptional changes across different features of the epileptiform microenvironment, employing data from an existing database as a non-epilepsy control sample. This dataset differs from those published before because it contains

non-epileptiform tissue alongside epileptiform tissue from a given patient, providing greater insights into the microenvironment. Our ultimate goals are to profile the transcriptional characteristics related to different cellular subpopulations and spatial distribution within the epileptic microenvironment in order to uncover the mechanisms of glial cell involvement in epilepsy. We aim to provide new insights that could lead to better approaches for the alleviation and treatment of epilepsy.

Disclosures: Q. Lin: None. **C. Kang:** None. **L. Cheadle:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.17/C80

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01- NS135162

Title: Myeloid CRISPR/Cas-9 repeat editing as a therapeutic approach for cerebellar neurodegeneration in Friedreich's ataxia.

Authors: *C. PERNACI[1,2], S. GILLETTE[1,2], A. JOHNSON[3], S. WEISER NOVAK[2,4], A. SIVAKUMAR[2], P. MISHRA[2], S. CHERQUI[2], N. G. COUFAL[1,2];
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Abstract: Friedreich's ataxia (FRDA) is the most prevalent inherited ataxia caused by an aberrant GAA trinucleotide repeat expansion inside the Frataxin (FXN) gene. This results in halted transcription and a significant reduction in FXN protein expression; a mitochondrial protein that plays a role in iron-sulfur cluster formation and iron homeostasis. Its absence causes progressive, ataxia, cognitive decline, and cardiomyopathy. Disease onset occurs between the ages of 5-15, and no cure is available. Identifying novel therapeutics and disease targets hinges on expanding the understanding of cell type contributions and the subcellular pathways of neurodegeneration in FRDA. As tools to tackle these questions, we have generated iPSCs from patients with FRDA, carriers and healthy controls, and differentiated them into microglia (iMG) and neurons to query the cell type-specific contribution to FRDA. A therapeutic approach for FRDA currently in characterization for future clinical trials, involves autologous bone marrow transplant after dual-guide CRISPR-mediated gene therapy to excise the GAA repeat expansion. Whether this approach ameliorates the neurodegenerative disease progression is not well understood. We therefore applied this genetic approach to generate GAA repeat expansion corrected iPSCs and subsequently iMG to explore the therapeutic potential in FRDA. We identified broad transcriptomic alterations and stark defects in mitochondria morphology, biogenesis, and mitochondrial superoxides accumulation, suggesting enhanced mitochondrial

oxidative stress in FRDA iMG. We next investigated microglial-neuronal interactions, querying whether FRDA iMG were sufficient to cause neuronal injury in healthy neurons using innovative *in vitro* and *in vivo* models. We co-cultured iMGs with healthy neurons and found that FRDA iMG interfere with neuronal survival *in vitro*, triggering cell death through a caspase-3 dependent mechanism. We subsequently extended these findings to a murine xenotransplantation model wherein iPSC-derived iMG progenitors are transplanted into neonatal humanized mice genetically depleted for endogenous murine microglia. We found that human FRDA microglia in the murine cerebellum are hyperinflammatory, accumulate in the Purkinje cell layer and reduce Purkinje cell survival. Compellingly, all the above alterations both *in vitro* and *in vivo*, were strongly attenuated by the GAA gene editing therapeutic approach. Altogether, these findings identify a critical role for microglia in the pathogenesis of neurodegeneration in FRDA and suggest the potential for a dual-guide gene editing strategy as a therapeutic approach in FRDA.

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Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.18/C81

Topic: C.01. Brain Wellness and Aging

Support: VA CSR&D grant 1 I01 CX002182-01
Department of Veterans Affairs, Office of Academic Affiliations (OAA) Fellowship program

Title: Mri analyses of csf flow vs. perivascular spaces in the assessment of glymphatic function in veterans with chronic multisymptom illness

Authors: *Y. ZHANG[1], M. MOORE[2,3], Y. RAHIMPOUR[2,3], P. J. BAYLEY[2,3], J. W. ASHFORD, Jr.[4,5], A. J. FURST[2,3];
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Abstract: Background: The “glymphatic system”, a network of cerebrospinal fluid (CSF) waste clearance pathways, has been hypothesized to be critical for brain health and function in many studies. The DTI-ALPS (diffusion tensor image analysis along the perivascular space) index can be calculated from diffusion MRI and has shown promise for measuring interstitial fluid dynamics along the perivascular space (PVS). Other reports suggested increased PVS burden may be an appropriate indicator for glymphatic dysfunction. This study compared both potential

indicators of glymphatic function and their relations with the severity of clinical conditions in veterans with Chronic Multisymptom Illness (CMI).
Methods: MRI data was acquired for 204 veterans (ages between 23-73 years old) who returned from the Persian Gulf War and Iraq/Afghanistan Wars. 203 veterans met the CDC criteria for CMI. Severities of sleep disturbances, pain, and fatigue symptoms were assessed based on self-report questionnaires. DTI-ALPS indices were calculated bilaterally along the paraventricular PVS. Quantification of the PVS burden included: volumes of PVS calculated from automatic computerized segmentation, and visual scoring of PVS grades, which grossly indicates numbers of enlarged PVS. Statistical analyses were performed with age, sex, and cerebral white matter volume as confounding factors.
Results: Older age was not associated with severities of sleep/pain/fatigue symptoms but was significantly associated with greater PVS burden and marginally associated with lower DTI-ALPS. Severities of sleep/pain/fatigue scores were significantly intercorrelated with each other; lower DTI-ALPS correlated with greater PVS burden, particularly with the number of enlarged cerebral type-II PVS. Imaging-clinical correlations showed that, after regressing out effects of confounders, lower DTI-ALPS was associated with greater sleep disturbances, worse pain levels in the past week, and greater degrees of prolonged fatigue; greater numbers (but not volume) of the enlarged type-II PVS were associated with greater sleep disturbances and worse pain levels in the past week. Comparisons between categorized groups showed that DTI-APLS was higher in the minimally symptomatic group versus the symptomatic group, whereas PVS burden could not specify the symptomatic group.
Conclusion: Both indices seem to capture different aspects of glymphatic function. DTI-ALPS is sensitive to glymphatic dysfunction with respect to multiple chronic symptoms such as sleep disturbances, pain severities and prolonged fatigue levels while PVS burden is primarily associated with age-related glymphatic dysfunctions.

Disclosures: Y. Zhang: None. **M. Moore:** None. **Y. Rahimpour:** None. **P.J. Bayley:** None. **J.W. Ashford:** None. **A.J. Furst:** None.

Poster

PSTR213: Neuroinflammation and Neurodegeneration: Studies in Humans and Human Tissues

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR213.19/C82

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DOD DA PR221066

Title: Neuroinflammation and Tight Junction Disruption in the Choroid Plexus are Associated with Posthemorrhagic Hydrocephalus in Preterm Infants and a Neonatal Mouse Model

Authors: *M. GARCIA BONILLA[1], R. SWARUP[2], O. LIMBRICK[2], C. BOYSEN[2], K. SHUMILOV BARTENEV[1], H. VOHRA[3], M. MICHENKOVA[1], J. P. MCALLISTER[1], D.

LIMBRICK[1];
[1]Neurosurg., Virginia Commonwealth Univ., Richmond, VA; [2]Neurosurg., Washington Univ. in St Louis, St Louis, MO; [3]Neurosurg., Virginia Commonwealth Univ., Midlothian, VA

Abstract: The pathophysiology of posthemorrhagic hydrocephalus (PHH), a major neurological disorder treated by pediatric neurosurgeons in preterm infants, is complex, multidimensional, and poorly understood. We hypothesized that neuroinflammation and alterations in the junctional biology of the choroid plexus (ChP) are associated with PHH. We aimed to analyze zona occludens-1 (ZO-1) and claudin 1, important tight junction proteins in the epithelial cells, claudin-5 in the endothelial cells, and quantify inflammatory cells in both human ChP (EGFA 25 ± 2 weeks) and CSF (EGA 34 ± 5 weeks) samples and a neonatal mouse model of PHH. Immunofluorescence was performed in human post-mortem samples; high-throughput proteomics and western blot in human CSF and extracellular vesicles. At 4 days of age (P4), mice received bilateral intraventricular injections of 5 ul lysed blood or saline to induce PHH or represent sham controls, respectively. Seven days post-induction (P11), MRI neuroimaging was performed, mice were euthanized, and brains were fixed in 4% paraformaldehyde in preparation for histology and immunohistochemistry. Ventricular volumes were quantified on T2-weighted MRI scans. Disruption of tight junctions (ZO-1 in epithelial cells and claudin-5 in endothelial cells) and increased number of inflammatory cells (T cells, neutrophils) was observed in human postmortem ChP. There was an increase in delocalized ZO1 junctional protein in the CSF, as well as several proteins involved in the tight junction complex (ANK1, ITGB2, CAPZB, ACTR3) within CSF extracellular vesicles in human PHH compared to controls. In mice, ventricular volume was increased ($p = 0.0025$) in the PHH group (3.8 ± 2.3 mm3) compared to sham controls (0.3 ± 0.1 mm3). Significant decreases in ZO-1 ($p = 0.0075$) and claudin-1 ($p = 0.0155$) were detected in the ChP epithelial cells in PHH. PHH was also associated with an increase in the number of GFAP+ astrocytes ($p = 0.0144$) and Iba1+ microglia/macrophages ($p = 0.0346$) in the stroma of the ChP. Microglia/macrophages displayed multiple autophagosomes and lysosomes by transmission electron microscopy, suggesting activation in PHH. These results demonstrate significant neuroinflammation and alterations in the junctional biology of ChP epithelial cells, the critically important blood-CSF barrier, in PHH. These results open the possibility of exploring novel immunomodulatory treatments to prevent the pathogenesis and development of PHH.

Disclosures: M. Garcia Bonilla: None. **R. Swarup:** None. **O. Limbrick:** None. **C. Boysen:** None. **K. Shumilov Bartenev:** None. **H. Vohra:** None. **M. Michenkova:** None. **J.P. McAllister:** None. **D. Limbrick:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.01/C83

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JP24K12795
JP23K06818
JP22K09804
JP21H04786
Shiseido Female Researcher Science Grant

Title: Oligodendrocyte targeted gene therapy for demyelination in experimental autoimmune encephalomyelitis

Authors: *X. GUO, K. NAMEKATA, Y. SHINOZAKI, C. HARADA, T. HARADA;
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Abstract: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) characterized by localized areas of demyelination. Experimental autoimmune encephalomyelitis (EAE) is a classic model widely used to explore pathogenic mechanisms of MS, generated by administering a myelin basic protein peptide that induces an autoimmune response directed to myelin. We recently developed a system that forces membrane localization of the intracellular domain of tropomyosin receptor kinase B (iTrkB) by farnesylation (F-iTrkB). Overexpression of F-iTrkB results in constitutive activation of downstream signaling pathways in the absence of TrkB's ligand, brain-derived neurotrophic factor (BDNF). Using AAV-mediated gene therapy in the eyes, we demonstrated that F-iTrkB expression (AAV-F-iTrkB) enhances neuroprotection in mouse models of glaucoma, stimulates robust axon regeneration after optic nerve injury and is effective in an optic tract transection model. In the present study, we examined the effects of oligodendrocyte targeted gene therapy of F-iTrkB for demyelination in EAE. We used AAV-PHP.eB, a highly BBB-permeable capsid variant of AAV9, and human myelin associated glycoprotein (*MAG*) promoter for reliable expression of F-iTrkB (AAV-PHP.eB-MAG-iTrkB) in oligodendrocytes *in vivo*. AAV-PHP.eB-MAG-GFP were used as a control. Both viruses were prepared according to a minimal purification method (Konno and Hirai, 2020). We found that about 80% of oligodendrocytes expressed GFP in the spinal cord of mice administrated with AAV-PHP.eB-MAG-GFP. Both viruses were then injected retro-orbitally to 5 week-old female mice followed by a myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide immunization after two weeks. Clinical scores of EAE mice were evaluated daily. We found that AAV-PHP.eB-MAG-iTrkB treatment significantly ameliorated the severity of EAE, which was confirmed by decreased demyelination and neuroinflammation in the spinal cord as revealed by histopathological analysis. However, the severity of EAE mice treated with AAV-PHP.eB-MAG-iTrkB after EAE induction remained comparable with that of AAV-PHP.eB-MAG-GFP treated EAE mice. Our results suggested that oligodendrocyte targeted TrkB therapy can protect mature oligodendrocytes in EAE mice.

Disclosures: X. Guo: None. **K. Namekata:** None. **Y. Shinozaki:** None. **C. Harada:** None. **T. Harada:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.02/C84

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DOD/USAMRAA W81XWH2210819
90108250 - F31NS132520
90105756 - Jackson Mace -Toffler Award

Title: Inhibition of MIF nuclease-mediated parthanatos mitigates neurodegenerative pathology in a mouse model of multiple sclerosis

Authors: *J. W. MACE[1], S. P. GADANI[2], D. GALLEGUILLOS[2], M. SMITH[2], B. KANG[3], M. GHARAGOZLOO[2], V. L. DAWSON[3], T. M. DAWSON[3], P. A. CALABRESI[2];
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Abstract: Nearly three million people worldwide are living with multiple sclerosis (MS), an autoimmune condition characterized by peripheral immune cell infiltration into the central nervous system (CNS) and reactive gliosis, demyelination, and neuroaxonal degeneration. Existing therapies target adaptive immune cells and treat relapsing MS, but are not effective in halting neurodegeneration in progressive MS. Numerous studies have identified prominent dysregulation of macrophage migration inhibitory factor (MIF), a multifunctional protein with cytokine and enzyme activity, in MS. Several years ago, our lab showed that MIF acts as the final nuclease executioner of a caspase-independent cell death pathway, parthanatos, that is triggered upon DNA damage and contributes to neuronal loss in Parkinson's disease and stroke. Because parthanatos-inducing conditions, such as high ROS concentrations, are pathologically prevalent in MS, we hypothesized that MIF nuclease contributes to neurodegeneration in this disease. Herein, our lab created a mouse line with a point mutation that selectively ablates MIF nuclease enzymatic activity and synthesized a compound that inhibits MIF nuclease. These experimental tools were used to assess the therapeutic efficacy of targeting MIF nuclease in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS. In this study, we revealed that EAE mice undergo parthanatic neurodegeneration. We determined that genetic ablation and pharmacologic inhibition of MIF nuclease in EAE mice led to decreased clinical disease severity (paralysis) over time. We also showed that genetic ablation and pharmacologic inhibition of MIF nuclease in EAE mice protected against retinal ganglion cell and lumbar spinal cord neuron loss. MIF nuclease ablation did not affect peripheral immune cell infiltration into the central nervous system of EAE mice, suggesting that parthanatic inhibition is therapeutically distinct from blocking the immune system. Altogether, if neurons degenerate by a MIF-mediated parthanatic process in the context of MS as indicated by our data, this research would establish a novel target that could be inhibited to directly treat the ongoing loss of grey matter in patients with this disease.

Disclosures: J.W. Mace: None. **S.P. Gadani:** None. **D. Galleguillos:** None. **M. Smith:** None. **B. Kang:** None. **M. Gharagozloo:** None. **V.L. Dawson:** None. **T.M. Dawson:** None. **P.A. Calabresi:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.03/C85

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Scleroseforeining
PMSMatTrain, The European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813263

Title: Synergistic Targeting of Innate Receptors TLR7 and NOD2 for Therapeutic Intervention in Multiple Sclerosis

Authors: M. DUBIK[1], J. MARCZYNSKA-GRZELAK[1], A. WLODARCZYK[1], M. SØRENSEN[1], R. DIEU[2], D. RUSIN[1], B. OJHA[1], E. SIGURÐARDÓTTIR SCHIÖTH[3], J. KRIEGER[1], T. OWENS[4], ***R. M. KHOROOSHI**[5];
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Abstract: Regulation of neuroinflammation is critical for maintaining CNS homeostasis and holds therapeutic promise in autoimmune diseases such as multiple sclerosis (MS). Previous studies have demonstrated the significance of selective innate signaling in triggering anti-inflammatory mechanisms, including the mobilization of suppressive cells and production of type I IFN, which play a protective role in MS-like disease, Experimental Autoimmune Encephalomyelitis (EAE). However, the individual intra-CNS administration of specific innate receptor ligands or agonists, such as TLR7 and NOD2, failed to trigger anti-inflammatory mechanisms in EAE. In this study, we investigated the potential synergistic effect of targeting both TLR7 and NOD2 simultaneously to prevent EAE progression. Our findings demonstrate that intrathecal administration of a combined NOD2 and TLR7 agonist led to a synergistic induction of type I IFN and effectively suppressed EAE in an IFN I-dependent manner. Suppression of EAE was correlated with decreased infiltration of immune cells, reduced demyelination, and downregulation of selective chemokine and cytokine gene expression in the spinal cord. These results underscore the therapeutic promise of concurrently targeting the TLR7 and NOD2 pathways in alleviating neuroinflammation associated with MS, paving the way for novel and more efficacious treatment strategies.

Disclosures: M. Dubik: None. **J. Marczynska-Grzelak:** None. **A. Wlodarczyk:** None. **M. Sørensen:** None. **R. Dieu:** None. **D. Rusin:** None. **B. Ojha:** None. **E. Sigurðardóttir Schiöth:** None. **J. Krieger:** None. **T. Owens:** None. **R.M. Khorooshi:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.04/C86

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Fate mapping mouse endogenous neuronal progenitor cells after exposure to EAE-upregulated cytokines

Authors: *J. SAYLOR[1], M. INOUE[2];
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Abstract: FATE MAPPING MOUSE ENDOGENOUS NEURONAL PROGENITOR CELLS AFTER EXPOSURE TO EAE-UPREGULATED CYTOKINES Joshua Saylor[1,2] and Makoto Inoue[1,2]*[1]University of Illinois at Urbana-Champaign Department of Comparative Biosciences, 2001 South Lincoln Avenue, Urbana, IL, 61802, USA.[2]University of Illinois at Urbana-Champaign Neuroscience Program, 405 North Matthews Avenue, Urbana, IL, 61801, USA.*Multiple Sclerosis (MS) is a chronic demyelinating disease that affects the central nervous system (CNS). Current disease-modifying therapies rely on modulation or suppression of the immune system, but do not reverse existing damage to the CNS. Stem cell therapy has long been proposed as a solution to this issue, but graft rejection and issues with differentiation of implanted cells are problems that stop the injection of cells from being a common treatment. An alternative source of stem cells is the endogenous populations in the CNS. Under CNS injury or inflammation, including MS, neural progenitor cells (NPCs) migrate to and proliferate at the sites of injury or inflammation. These cells have the potential to replace those lost from neurodegeneration. Focusing on endogenous NPCs avoids the risks associated with implantation and is a promising target for therapy. However, they do not behave as expected in disease conditions. In vitro NPCs can differentiate into neurons, oligodendrocytes, and astrocytes, but in vivo they are often restricted to astrocytes. Therefore, we investigated the in vivo regulatory factors of NPC differentiation into neurons and oligodendrocytes. Using a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), we found multiple cytokines were upregulated in the spinal cord of EAE mice, compared with naïve mice. Then, we isolated and cultured endogenous NPCs from EAE mice and treated them with upregulated cytokines (recombinant cytokines). We detected the alterations of NPC proliferation and differentiation and are seeking to better understand the how these cytokines directly affect the cells. Our study will lead to identifying the promising targets for MS therapy in order to promote the recovery cells lost to neurodegeneration. .

Disclosures: J. Saylor: None. **M. Inoue:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.05/C87

Topic: B.10. Multiple Sclerosis and Other Demyelinating Diseases

Support: Ph.D. fellowship to P.R from CAPES [process #88887.501568/2020-00].
G.T is the recipient of a fellowship from CNPq [process #303531/2020-7]
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G.T is the recipient of a fellowship from CNPq Nº 26/2021 [process# 401653/2022-6]

Title: <Apocynin impact on AOPPs-mediated TRPA1 activation in RR-EAE induction of neuroinflammation and neuropathic pain>

Authors: *P. RODRIGUES[1], F. T. VIERO[1], D. P. SPAT[1], J. M. FRARE[1], N. A. RUVIARO[1], D. S. M. ARAUJO[2], M. MARINI[2], L. LANDINI[2], F. DE LOGU[2], P. GEPPETTI[2], R. NASSINI[2], G. BOCHI[1], G. TREVISAN[1];
[1]Federal Univ. of Santa Maria, Santa Maria, Brazil; [2]Univ. of Florence, Florence, Italy

Abstract: <Relapsing-remitting multiple sclerosis (RRMS) elicits neuropathic pain in 28% of the patients. In the context of the relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE) model, nociception and neuroinflammation are observed, linked to transient receptor potential ankyrin 1 (TRPA1) and NADPH oxidase (Nox) activation. Acute treatment with apocynin (APO), a non-specific Nox inhibitor, mitigates nociception in RR-EAE. Nox triggers myeloperoxidase (MPO) activation, fostering advanced oxidative protein products (AOPPs) formation, thus stimulating TRP and inducing pain. Despite the absence of a clinical TRPA1 antagonist for MS-related neuropathic pain, Nox inhibitors, tested in phase II trials for diabetic neuropathy, prompt our investigation. We aimed to assess whether 15 days of APO repeated treatment reduces TRPA1-induced neuropathic-like symptoms and neuroinflammation in RR-EAE mice by modulating AOPPs levels. Using *Trpa1*$^{+/+}$ and *Trpa1*$^{-/-}$ female mice, we conducted intracellular calcium assays in dorsal root ganglion (DRG) neurons (protocol#9746010620/1194/2015-PR). *Trpa1*$^{+/+}$ mice were immunized with 200 µg of MOG_{35-55}, 45 µg of QuilA (subcutaneous), and two doses of 1 ng/µl pertussis toxin (intraperitoneal). We measured clinical score, mechanical and cold allodynia, grip test, rotarod test from days 7 to 35 post-induction and the nest building test on days 34 to 35 day after RR-EAE induction. We measured AOPPs levels, Nox/MPO activity, Mog, Iba-1, Gfap, Olig-1 or Olig-2 by PCR and/or immunofluorescence in the spinal cord. Administration of AOPPs in DRG cells of Trpa1$^{+/+}$ mice induced calcium influx, while genetic deletion of TRPA1 prevented it, suggesting AOPPs-induced TRPA1 activation. APO treatment (100 mg/kg oral gavage), initiated 20 days post-RR-EAE induction, ameliorated clinical score, allodynia, nociception, and grip strength, without affecting weight or locomotion. AOPPs and Nox/MPO activity increased in the spinal cord, reversed by APO treatment. APO mitigated demyelination, astrocyte and glial activation, and oligodendrocyte dysregulation. AOPPs-induced TRPA1 activation in mouse DRG suggests its

role in neuroinflammation and neuropathic pain in RR-EAE. Repeated APO treatment likely reduces AOPPs levels via MPO/Nox modulation, restoring myelin and reducing neuroinflammation, reflected in lowered RR-EAE clinical scores. These findings propose AOPPs-targeting compounds as potential therapeutic adjuncts for RRMS patients. AOPP-induced TRPA1 activation and its association with neuroinflammation and nociception provide insights into therapeutic strategies for managing RRMS symptoms.>

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Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.06/C88

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AT10980
NIH Grant AT10980-03S1

Title: HMB promotes the maturations of OPC to OL using PPARa, implications for MS

Authors: *S. PRIETO[1], K. PAHAN[2];
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Abstract: Oligodendrocytes are the myelinating cells in the CNS and multiple sclerosis (MS) is a demyelinating disorder that is characterized by progressive loss of myelin because immune cells induce the apoptosis of oligodendrocytes. Although oligodendroglial progenitor cells (OPCs) should be differentiated to oligodendrocytes, for multiple reasons, OPCs fail to differentiate to oligodendrocytes in MS. Therefore, increasing the maturation of OPCs to oligodendrocytes maybe of therapeutic benefit for MS. The β-hydroxy β-methylbutyrate (HMB) is a muscle-building supplement in human and this study underlines the importance of HMB in stimulating the maturation of OPCs to oligodendrocytes. OPC cells from C57BL/6 pups were isolated and purified. After HMB treatment, we studied the differentiation of the cultured OPCs by mRNA, double-label immunofluorescence and immunoblot analyses. As evident from mRNA analysis HMB treatment upregulated the expression of different maturation markers including PLP, MBP and MOG in cultured OPCs. Double-label immunofluorescence followed by immunoblot analyses confirmed the upregulation of OPC maturation by HMB. While investigating mechanisms, we found that HMB increased the maturation of OPCs isolated from peroxisome proliferator-activated receptor $\beta^{-/-}$ (PPAR$\beta^{-/-}$) mice, but not PPAR$\alpha^{-/-}$ mice. Furthermore, HMB treatment of OPCs led to the recruitment of PPARα, but neither PPARβ nor PPARγ, to the *PLP* gene promoter. These results suggest that HMB stimulates the maturation of

OPCs via PPARα and that HMB may have therapeutic prospect in remyelination. This opens the possibility to a novel application of HMB for MS patients.

Disclosures: S. Prieto: None. **K. Pahan:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.07/C89

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1 NS123532
NINDS R21 NS123546

Title: Involvement of the circadian system in the mouse cuprizone model

Authors: *T. NIEPOKNY[1], J. JOO[2], H. MENDRIES[3], H. COURTNEY[3], K. DOCKREY[3], P. BANGALORE PARTHASARATHY[4], S. RAO[5], R. DUTTA[6];
[1]Dept. of Neurosciences, Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [2]Cleveland Clin. Lerner Col. of Med., Cleveland, OH; [3]Dept. of Neurosciences, Lerner Res. Inst., Cleveland, OH; [4]CITI, Cleveland Clin., Cleveland, OH; [5]Dept. of Opthalmic Res., Cole Eye Inst., Cleveland, OH; [6]Lerner Res. Inst., Cleveland, OH

Abstract: Introduction: The central circadian pacemaker in mammals is located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Environmental light is the strongest external cue, or zeitgeber, which entrains the SCN pacemaker. A molecular circadian clock is found in nearly ever cell of the body and is implicated in neurodegenerative diseases. An association between the circadian clock and multiple sclerosis (MS) has been identified in individuals who perform shift work at a young age, and with certain polymorphisms in key circadian genes. Further, patients with MS often report sleep disruptions which negatively affect their quality of life. We hypothesize that disruptions in circadian rhythms negatively affect the disease course in MS. Methods: We utilize the cuprizone (CUP) model of demyelination in C57BL/6J mice and are focused on the involvement of the central SCN clock and local clocks in the corpus callosum (CC), the white matter tract most affected by CUP treatment. Mice were placed in a 14:10 light/dark cycle or in constant light (LL) to induce circadian disruption and were split into three treatment groups within each light condition: demyelination (6 weeks CUP), remyelination (6 weeks CUP + 4 weeks regular chow), or control (10 weeks regular chow). Under both light conditions, mice were sacrificed at the same external time (1400 hours) which corresponds to zeitgeber time 8 in the LD cycle. Brains were removed for RNA isolation (fresh-frozen) or immunofluorescence staining (perfusions). A subsect of animals within each cohort had cage activity monitored to assess CUP effects on rhythmic locomotor activity. The SCN (n=4/cohort) was collected from fresh-frozen tissue sections (600um) with a 1mm punch tool for

total RNA isolation and sent for bulk RNA-sequencing. Perfused brains (n=3-7/cohort) were sectioned at 30um for immunostaining of the SCN and CC. Results: Animals on CUP in an LD cycle had no differences in circadian period (p=0.092), and under LL conditions, animals became arrhythmic regardless of diet. QIAGEN Ingenuity Pathway Analysis of SCN sequencing data revealed gene changes in pathways related to cholesterol biosynthesis, immunogenic cell death signaling, and myelination signaling in comparisons of SCNs (CUP vs control and CUP-treated LD vs LL). Lastly, in the CC, CUP treatment paired with constant light further exacerbated the extent of demyelination (PLP coverage) compared to CUP treated animals in an LD cycle (p=0.036). Conclusions: Our preliminary data suggests that CUP affects the SCN pacemaker, and that environmentally induced circadian disruption exacerbates the demyelinating effects of CUP in the CC.

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Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.08/C90

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Hotchkiss Brain Institute MS Program
MS Canada endMS Doctoral Studentship Award
Hotchkiss Brain Institute International Graduate Recruitment Scholarship

Title: The role of the gut microbiota in the onset of progressive experimental allergic encephalomyelitis

Authors: *R. WANG[1], K. MCCOY[2], S. S. OUSMAN[1];
[1]Hotchkiss Brain Inst., Calgary, AB, Canada; [2]Snyder Inst. for Chronic Dis., Calgary, AB, Canada

Abstract: Multiple sclerosis (MS) is an autoimmune disease characterized by neuroinflammation, demyelination and neurodegeneration. Relapsing-remitting MS (RRMS) is the most common subtype, marked by periods of new or worsened symptoms with intervals of remission in between. Despite current disease modifying therapies, approximately 50% of RRMS patients will develop progressive MS within 10-15 years (secondary progressive MS - SPMS). In addition to RRMS, about 15% of MS patients are diagnosed with progressive MS from onset-called primary progressive MS (PPMS). SPMS and PPMS are associated with serious physical disability and poor quality of life and are unfortunately refractory to current approved therapies. Therefore, preventing or delaying the onset of progressive MS is critical for ameliorating disability in the disease. Our goal is to identify the mechanisms that underlie

progressive MS. Aging is currently the only factor that correlates with progressive MS. Interestingly, the gut microbiota, a mechanism of aging, is altered in people with MS relative to healthy, control individuals.
The objective of this project is to ascertain whether dysbiosis of the gut microbiome contributes to the development of progressive MS. To test this idea, we are using the 1C6 T cell receptor transgenic mouse that initially displays an acute MS-like (experimental allergic encephalomyelitis - EAE) phenotype that subsequently becomes progressive. Thus far, we have observed that 1C6 animals display two EAE phenotypes in both females and males - progressive (P-EAE) and non-progressive (NP-EAE) - and that male progressive animals display weaker grip strength. Through 16S rRNA sequencing, we found that the gut microbiota composition is significantly different between the two phenotypes during the transition and progressive phases. To determine if modulation of the gut microbiota impacts the development of P- or NP-EAE, 1C6 EAE animals were administered a cocktail of antibiotics after the acute phase. Antibiotic treatment markedly increased not only disease severity but the ratio of animals that developed P-EAE. Future experiments will determine if a) bacterial composition predict the onset of P-EAE, b) whether fecal microbiota transplantation of P- and NP-EAE bacteria will phenocopy the two EAE states in germ-free 1C6 mice, and c) if and how immune cell and central nervous system cell number and function are affected in P- and NP-EAE.

Disclosures: R. Wang: None. **K. McCoy:** None. **S.S. Ousman:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.09/C91

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Department of Biological Sciences, Kent State University
Graduate Student Senate, Kent State University

Title: Neuronal Mitochondrial Metabolite NAA Regulates Myelin Stability by Epigenetic Mechanisms

Authors: *N. AL RAMEL[1], N. K. SINGHAL[2], M. SHALIH MARAICAR[1], F. GUO[3], D. E. PLEASURE[4], J. MCDONOUGH[5];
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Abstract: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease of the central nervous system (CNS) resulting in progressive neurological disability. This disability results from demyelination or nerve damage disrupting the communication between the brain and the

body. It has been demonstrated that the neuronal mitochondrial metabolite N-acetylaspartate (NAA) is decreased in the MS brain, but the significance of reduced NAA to MS pathology is not clear. It is known that NAA mediates cross-talk between axons and oligodendrocytes. NAA is made in neurons and taken up by oligodendrocytes where it is catabolized by ASPA to acetate and L-aspartate. We have previously shown that NAA catabolism is involved in regulating metabolic and gene expression changes that are characteristic of myelinating oligodendrocytes. Deleting the N-acetyltransferase-8-like (NAT8L) enzyme that synthesizes NAA causes less compact myelin and sensorimotor disability in mice. We have hypothesized that NAA is involved in maintenance of myelin. In the present study, we used Nat8l$^{-/-}$ mouse model at the age of one year to test the effects of the lack of NAA on myelin stability during aging. Using fluorescent immunohistochemistry we evaluated myelin basic protein (MBP), and while we found no change in MBP levels in corpus callosum of the Nat8l KO, however qRT-PCR of mRNA isolated from NAT8l KO mice demonstrated that MOG and MAG expressions are decreased. These data suggests that NAA can enhance oligodendrocyte maturation. We also used CARS microscopy to quantitate myelin lipids. Our data show that there is less myelin in Nat8l $^{-/-}$ mice in corpus callosum compared to WT mice. In cell culture experiments we observed that NAA treatment was associated with increases in the expression of genes involved in myelin lipid synthesis and a decrease in electron transport chain complex expression. Seahorse respirometry shows that NAA inhibits mitochondrial respiration in oligodendrocytes and this inhibition is dependent on the activity of the KDM5 histone demethylase that demethylates H3K4me3. Treatment with CPI-455, an inhibitor of the KDM5 histone demethylases, blocked NAA effects on oligodendrocyte metabolism. Data were analyzed in GraphPad prism by ANOVA or two tailed t-test with P values less than 0.05 considered significant. Our data suggest that NAA catabolism in oligodendrocytes is necessary for maintaining proper epigenetic control in myelinating oligodendrocytes during aging in the CNS.

Disclosures: N. Al Ramel: None. **N.K. Singhal:** None. **M. Shalih Maraicar:** None. **F. Guo:** None. **D.E. Pleasure:** None. **J. McDonough:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.10/C92

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Training in the Molecular Basis of Autoimmunity and Autoinflammation 2T32AI132152-06

Title: Investigating the MS4A gene family as a key modulator of microglial function across neurodegenerative diseases

Authors: *J. FREEDMAN;
Univ. of Massachusetts Chan Med. Sch., Worcester, MA

Abstract: Neurodegenerative diseases, such as Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Multiple Sclerosis (MS), are debilitating, deadly conditions which lay an increasingly devastating burden on families and our health care systems alike. Despite decades of research, we have only limited treatment options for patients suffering from these conditions. While many aspects of these diseases are distinct, recent work has shown that dysregulation of microglia, the CNS-resident macrophage, may represent a uniting pathogenic mechanism across diseases. To this end, human genetics research has identified Membrane-Spanning-4A (*MS4A*) genes as a key modulator of microglial function in AD, and more recently, as a strongly upregulated gene in Multiple Sclerosis. Previous work in our lab has shown that *MS4A* knockout (*MS4A*-KO) mice have reduced disease severity in models of both AD and ALS. Furthermore, *MS4A*-KO is associated with reduced measures of microglial inflammation and dysfunction in these models. The present study is focused on elucidating the role of *MS4A* in Experimental Autoimmune Encephalomyelitis (EAE), a mouse model of Multiple Sclerosis. We have found that *MS4A* expression is upregulated in EAE. We also found that *MS4A*-KO mice have reduced severity of EAE, as measured by a reduction in clinical score and mitigated weight loss (n=10-15 per group), as well as by an AI-based mouse behavior analysis technique known as Motion Sequencing. We also found that *MS4A*-KO mice also have significantly reduced immunohistochemical markers of gliosis (including Iba1-positive and GFAP-positive cell area), microglial activation (including clec7a-positive Iba1 cells) within the brain. Additionally, we are in the process of assessing broader changes in immune cell populations via flow cytometry, as well as changes in microglial phagocytosis of synapse and myelin debris; preliminary results suggest that *MS4A*-KO mice have significant alterations in these processes. Overall, these data suggest that *MS4As* act within the brain to modulate microglial inflammatory state and function, providing key insight into the mechanism of MS4As in disease. This work also implicates *MS4As* as an intriguing target in the treatment of multiple neurodegenerative diseases, including MS, AD, and others.

Disclosures: J. Freedman: None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.11/C93

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH RO1 NS088566
Cure Alzheimer's Fund
Bright Focus Foundation

Lefler Center for Postdoctoral Fellowship
NIH RO1 RF1048790

Title: Plexus nexus: connecting the role of the choroid plexus in orchestrating an immune response in neuroinflammatory diseases

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Abstract: The brain was once considered immune-privileged, and neuro-immune functions were poorly understood. However, advances in neuro-immunology have inspired many new research directions to investigate mechanisms by which the brain tackles inflammation. Our lab investigates this question through the choroid plexus (ChP), a highly vascularized epithelial tissue responsible for producing cerebrospinal fluid (CSF) which also forms a critical brain barrier. Using lipopolysaccharides to experimentally trigger acute inflammation, we found that acute ChP responses to inflammation include weakened epithelial cell barriers and secretion of chemokines as well as macrophage sustaining factors. These events lead to rebuilding of barriers and resolution of inflammation. We next tested our hypothesis that neuroinflammation in context of neurodegenerative diseases may be associated with similar molecular mechanisms and functions. We examined human ChP tissues and mouse models of Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). AD is the most common type of dementia, and neuronal death is believed to result from accumulation of amyloid-β plaques. Postmortem ChP tissue from AD patients had macrophage accumulation. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that affects motor neurons in both the brain and spinal cord. Postmortem ChP tissue from ALS patients also showed barrier damage and macrophage accumulation (Saul et al., 2020). To recapitulate these findings in mice, AD pathology was studied using acute and chronic models of inflammation. For the acute model, adult WT mice received an intracerebroventricular injection of amyloid-β. The chronic model used adult 5xFAD mice that express five familial-linked-AD genes. To study ALS pathology, we used C9orf72 BAC mice, which contain the hexanucleotide repeat expansion mutation seen in ALS. Using immunohistochemistry and fluorescence, we stained and imaged cross-sections and whole-mounted ChP for various markers of immune responses. We found that in mouse models of AD and ALS, the ChP responded to neuroinflammation in three main ways: (1) the ChP increased immune cell presence through infiltration and recruitment of peripheral immune cells, (2) the ChP loosened its epithelial cell barriers to allow for immune cells to enter the CSF, and (3) vascular inflammation and blood platelet aggregates further facilitated immune cells trafficking from the periphery to bolster the immune response. Our findings uncover new aspects of ChP contributions to multi-faceted neuroinflammatory responses in the context of neurodegenerative disease.

Disclosures: H. Xu: None. **L. Byer:** None. **A. Dong:** None. **S. Gelb:** None. **L. Myllykangas:** None. **M. Lehtinen:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.12/C94

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH 1 T32 AI132152-06
RO1 NS118145

Title: Als citrullinome outlines disease progression

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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease that is characterized by progressive motor neuron loss, muscle wasting, paralysis, and death. 90% of cases are sporadic (sALS), while the remaining 10% are familial (fALS). A hallmark of ALS neuropathology is aberrant protein aggregation and inclusion body formation in neurons leading to eventual degeneration of motor neurons in the brain and spinal cord. Protein Citrullination (PC), a post-translational modification (PTM), and Peptidyl Arginine Deiminase 2 (PAD2) expression has been shown to play a fundamental role in ALS protein solubility and is altered dynamically in the spinal cord during disease progression. These citrullinated proteins are shown to accumulate in protein aggregates. Beyond the effects on protein solubility, our understanding of PC in ALS is incomplete. However, PC can alter many protein functions that potentially contributing to ALS pathogenesis. Here, we identified insoluble citrullinated proteins in mice and soluble citrullinated proteins in ALS mice and humans. The ALS citrullinome outlines disease progression distinctly from normal aging in mice. We found that glia proteins contribute the most to the ALS citrullinome, validating PC as a marker of reactive astrogliosis and neuroinflammation. Additionally, we found that PC severely compromises Heat Shock proteins by altering their structure and disrupting key functions. These results have improved our understanding of the effects of citrullination on proteins and uncover potential biomarkers of early-stage ALS. These studies have developed a new framework to investigate ALS, allowing more exploration into the underlining biology of citrullination to develop novel therapeutics.

Disclosures: W. Camille: None. **S. Rajoria:** None. **I. Yusuf:** None. **Z. Xu:** None. **P. Thompson:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.13/C95

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Research Council
Swedish Brain Foundation
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Institutional support RVO

Title: Genetic ablation of C3aR slows down ALS progression via modulation of astrocyte reactivity

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Abstract: Background: Amyotrophic lateral sclerosis (ALS) is an incurable disease characterized by progressive loss of motor neurons in the spinal cord and the brain. Neuroinflammation and reactive gliosis are prominent neuropathological hallmarks and the expression of C3 is increased in the spinal cord and motor cortex of ALS post-mortem tissue. C3-expressing astrocytes were proposed to play a role in the pathogenesis of ALS by exerting a toxic effect on motor neurons but genetic deletion of C3 did not affect disease progression in mice expressing ALS-causing mutation in superoxide dismutase 1 and pharmacological inhibition of C3 activation failed to affect ALS progression in a recent clinical trial (NCT04579666).
Methods: To investigate the role of C3aR in ALS, we crossed the *wobbler (Vps54$^{wr/wr}$)* model of sporadic ALS with mice deficient in C3aR. We recorded body weight, paw atrophy, motor performance and survival. Lumbrical muscles and cervical spinal cord were used to evaluate the effect of C3aR ablation at the tissue level.
Results: Genetic ablation of C3aR resulted in longer survival and slower loss of muscle strength. The *C3aR$^{-/-}$wobbler* mice had fewer degenerating neurons in the cervical spinal cord and better innervation of neuromuscular junctions. Our single-cell RNA sequencing data showed that in the *wobbler* spinal cord, C3aR is predominatly expressed in microglia and C3aR ablation led to altered gene expression profile of interferon responsive microglia. Our findings of fewer activated Clec7a-positive microglia and fewer C3-positive reactive astrocytes in the *C3aR$^{-/-}$ wobbler* spinal cord together with reduced expression and colocalization of C1q with degenerating neurons point to microglia-derived C1q as an important mechanistic link between microglial C3aR and astrocyte reactivity. Robust microglial immunoreactivity of C3aR in human postmortem ALS spinal cord supports the clinical relevance of our experimental findings.
Conclusions: Our study points to C3aR as an important disease modifier and potential therapeutic target in ALS.

Disclosures: M. Pekna: None. **Y. Li:** None. **U. Wilhelmsson:** None. **P. Abaffy:** None. **C. Penningh:** None. **S. Chytilova:** None. **Y. de Pablo:** None. **M. Kubista:** None. **L. Valihrach:** None. **M. Pekny:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.14/Web Only

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Therapeutic Effect of Quercetin on Paraquat-induced Parkinsonism in Mice

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Abstract: Background: Parkinson's disease (PD) is a neurodegenerative disease with three (3) classical motor symptoms — tremors, bradykinesia, and muscle rigidity. These symptoms are collectively referred to as Parkinsonism. Paraquat (PQ) is a potent yet non-selective herbicide which has been banned in over 50 countries of the world mainly because of its severe neurotoxic effect. Studies reveal that PQ can induce PD.Aim: This research was aimed at exploring the therapeutic potential of quercetin (QCT) against motor and non-motor damage as well as neuronal loss and oxidative stress induced by PQ poisoning.Methodology: Swiss Albino mice (male, 22.0±2.0 g, n=20) were procured, housed, and fed according to internationally-recognized ethical standards. Following a weeklong acclimatisation, the mice were randomly assigned (n=5) into — group 1 (10 mL/kg distilled water p.o.), group 2 (10 mL/kg distilled water p.o. + 10 mg/kg PQ i.p.), group 3 (100 mg/kg quercetin p.o. + 10 mg/kg PQ i.p.), group 4 (15 mg/kg carbido-levodopa p.o. + 10 mg/kg PQ i.p.). Treatment was done for twenty-one (21) consecutive days but PQ was only administered on even days approximately one hour after treatment. Behavioural tests for motor and non-motor function were carried out on day 22. A day later, selected mice were euthanized, whole brains were harvested, and cardiac blood was collected in preparation for biochemical assays. Selected mice brains were appropriately fixed using formalin solutions in preparation for immunohistochemistry of the hippocampus, dentate gyrus, prefrontal cortex and substantia nigra. Data was analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison post hoc test using the GraphPad Prism software version 7. The results are presented as mean±standard deviation (SD) at a significance level of 0.05. Results: The mice of the QCT group demonstrated a longer latency of fall in the rotarod test and a higher frequency of line crossing in the open field test as well as a higher percentage of alternation and discrimination ratio in the Y-maze and novel object recognition test, respectively. Oxidative stress was also significantly ($p<0.05$) attenuated in the QCT group as the level of antioxidants was increased while the level of pro-oxidants was decreased. There was also significant ($p<0.05$) abrogation of PQ-induced neuronal loss in the various brain regions. Conclusion: Quercetin possesses therapeutic benefit in ameliorating Parkinson-like symptoms and even slowing down the progression of the disease. It is therefore important to incorporate quercetin supplementation into our daily life.

Disclosures: A.T. Eduviere: None. **L.O. Otomewo:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.15/C96

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Evaluation of the Gut-Microbiome as a Modulator of Parkinson's Disease in a Genetic Mouse Model

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Abstract: Investigation of the relationship between prodromal symptoms and onset of Parkinson's Disease have illustrated that neurodegeneration may accompany microbial dysbiosis (GMD) up to 20 years prior to clinical diagnosis. Moreover, the risk of onset, disease severity and survival rate may be exacerbated by dietary habits impacting the gut-microbiome (GM). Yet, despite evidence of the gut-immune axis's (GIA) influence over neuroinflammatory effects, the link between GMD and neurodegeneration at preclinical stages remains unclear. In my predoctoral project, we evaluated the role that the GM may have in mediating neurodegeneration of dopamine neurons in the mid-brain through dietary High-Fat challenge of DAT-cre/TFAM KO mice (MitoPark Mice). MitoPark mice are a Cre/LoxP specific mitochondrial maintenance gene knock out in dopaminergic neurons leading to progressive-onset of PD-like symptoms as soon as 15 weeks. Coincidentally, previous studies have demonstrated a dietary link to enhanced neurodegeneration and *SCNA* expression in MitoPark mice. To evaluate the influence of the GM, a pilot-study used MitoPark mice and their littermate controls to single-house and assign to a dietary condition - High-Fat diet or Regular chow - at 6-weeks of age. Stool, open-field, and blood samples were collected at 6, 10, 15, 20, and 30 weeks of age. Terminal collection began with a cohort of mice being sacrificed at 30 weeks of age, and the subsequent cohorts at earlier stages. We expected High-Fat Diet to influence the disease severity of MitoPark mice. Analysis of ambulatory distance traveled (cm) within 30 minutes revealed that while MitoPark mice developed the PD-like phenotype by 15 weeks, MitoPark fed a High-Fat Diet seemed to avoid significant decline up to 20 weeks, when compared to their littermate controls on either diet. A closer look into a pilot-16s analysis of stool-derived microbiome revealed that a high-fat diet impacted the composition of the gut-microbiome as early as 8 weeks of age, to include a loss of Lactic Acid producing bacteria associated with neuroprotection, and an enrichment of families associated with proinflammatory conditions. To better understand the significance of these early findings, I propose to expand on my pilot-studies with multiplex staining of the mid-brain to evaluate DAN counts, microglia activation and recruitment of adaptive immune cells. Further, we will evaluate plasma and lymph tissue to characterize GIA and neuroimmune profile associated with alterations of the gut. Together, these findings will establish new peripheral

biomarkers that may lead to novel methods for diagnosis and manipulation of PD at preclinical stages.

Disclosures: A.L. Rodriguez: None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.16/C97

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RS-2022-00141392

Title: Effective attenuation of hypersensitivity by downregulating GCH1 in neuropathic pain

Authors: *H. CHANG[1], J. YU[2], M. PARK[1], K. LEE[3,4], H.-N. WOO[4,5], J. KIM[3,4], C. KOH[1], H. LEE[4,6], H. JUNG[1];
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Abstract: Introduction: BH4 (tetrahydrobiopterin) is involved in the synthesis of various neurotransmitters affecting homeostatic balance. Although tightly regulated under normal conditions, its intracellular concentration is impaired after nerve injury. GTP cyclohydrolase I (GCH1) has been identified as a critical enzyme responsible for regulation of BH4 levels and is dramatically upregulated in the dorsal root ganglion (DRG) in the course of neuropathic pain. Despite advances in the development of new drugs that address the persistent effects of neuropathic pain, traditional analgesics often lacked specificity and failed to target the underlying causes of neuropathic pain, providing only partial relief. In this study, we investigate to develop and evaluate an RNA interference (RNAi)-based AAV designed to specifically downregulate GCH1 mRNA from multiple species and subsequently downregulate BH4 levels, ultimately mitigating neuropathic symptoms in neuropathic pain model. **Methods**: We first designed u-siGCH1 and selected most effective multispecies-compatible u-siGCH1 through RT-qPCR and western blot. We then constructed AAV expressing u-siGCH1 (AAV-u-shGCH1) and verified its ability downregulating GCH1. Adult male Sprague-Dawley rats (200g-220g) were used and grouped into normal (NM), spared nerve injury (SNI), AAV-u-shCON, and AAV-u-shGCH1. Spared nerve injury model was applied and the virus was injected on the post-operative day (POD) 16. Behavior tests were conducted before surgery, post-injection day (PID) 3, 7, 14, 21 and 28. Rats were sacrificed on PID 28 and DRG tissues and blood were harvested for immunohistochemical analyses and for examining BH4 concentration, respectively. **Results:** In

vitro assessments showed that the selected u-siGCH1 was remarkably effective in downregulating both GCH1 mRNA and protein levels in human (HeLa), monkey (Vero), and rat (C6) cells. In neuropathic pain model, it was found that AAV-u-shGCH1 group showed a gradual increase in mechanical withdrawal threshold until PID 28, while the AAV-shCON group remained hypersensitivity. Compared to SNI and AAV-shCON group, AAV-shGCH1 group immunohistochemical analysis indicated an alleviation in the expression of GCH1 in the DRG. Not only u-siGCH1, but also AAV-shGCH1 effectively downregulated BH4 levels in HeLa cells. **Conclusion**: This study demonstrated that this novel therapeutic approach can provide a long-term strategy to alleviate neuropathic pain through a single treatment, and suggested the potential of gene therapy in the field of neuropathic pain.

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Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.17/C98

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RS-2022-00141392

Title: Pain alleviation using a novel soft neural electrode based on liquid metal in a rat model of neuropathic pain

Authors: *T. KIM[1], C. KOH[1], W. MUN[1], Y. KWON[2], H. JUNG[1];
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Abstract: Objectives: Neuropathic pain is treated with DBS using metal-based electrodes. However, too rigid electrodes can damage adjacent soft brain tissue, limit precise stimulation of local areas, and cause immune responses and inflammation, which can interfere with long-term stable stimulation and recording processes. Liquid metal-based electrodes can stimulate and record nerve signals in the brain with minimal damage to the brain. To overcome the conventional limitation of electrodes, we presented facile Gallium-Indium eutectic (EGAIn) based liquid metal electrodes to detect neural activities and deliver micro-electrical stimulation in the deep brain region to see pain modulation effects in the rat model of neuropathic pain. **Methods:**The tip of the electrodes was coated with platinum black for higher electrochemical properties and biocompatibility. Two liquid probes were implanted in ACC for stimulation and VPL for recording using liquid metal electrodes made of EGAln using polyimide tube. Neural activities in the VPL of a neuropathic pain rat model were recorded without electrical stimulation. Next, the charge-balanced biphasic electrical stimulation (130 Hz, 100 μA) was

delivered to the ACC for one hour using the same rat. Von Frey test was performed with continuous stimulation of the VPL using liquid metal electrodes implanted in the rat model of neuropathic pain.
Results: The rat after ACC-treated condition showed a significant reduction of after discharge in the VPL, of which firing rate and mean amplitude were decreased from 23.85 to 9.3 Hz (*$p<0.05$), respectively. When using a biphasic current of 80 µA with a frequency of 50 Hz, the modulation of pain sensitivity was notably effective, as indicated by a higher withdrawal threshold (10.51 g).
Conclusion: This study provides a new concept for preparing a liquid metal electrode with high output performance and mechanical properties. In vivo experiment using in the rat model of neuropathic pain, liquid metal electrodes presented a multifunctional nature of EGAIn in stimulating and recording neural tissues for neuro-modulation. However, there is a limitation that the process of implanting liquid metal electrodes into the brain must be differentiated from solid metal electrodes. Collectively, the proposed liquid metal electrode can be an alternative option for long-term, stable recording and stimulation to treat various neurological disorders.

Disclosures: T. Kim: None. **C. Koh:** None. **W. mun:** None. **Y. Kwon:** None. **H. Jung:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 1R01NS118198
NIH Grant R01NS100924
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Title: A Novel Common Cascade for Neurodegeneration in Alzheimer's Disease and Multiple Sclerosis: Toxic Misplacement of a Mitochondrial Anchor into Neuronal Dendrites triggered by Interaction between Amyloid Beta, Cytokines and NMDA-R

Authors: D. MATHUR[1], *C.-L. ZHANG[2], K. DU[3], R. R. RAJAII[4], J. E. PLOSKI[5], S.-Y. CHIU[6];
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Abstract: A Novel Common Cascade for Neurodegeneration in Alzheimer's Disease and Multiple Sclerosis: Toxic Misplacement of a Mitochondrial Anchor into Neuronal

Dendrites triggered by Interaction between Amyloid Beta, Cytokines and NMDA-R

Authors
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Disclosures
Mathur: None. Zhang: None. Du: None. Rajaii: None. Ploski: None. Chiu: None.

Abstract
Alzheimer's Disease (AD) and Multiple Sclerosis (MS) are two neurodegenerative diseases with prominent comorbidity. This AD/MS comorbidity suggests a common neurodegeneration mechanism and therapeutic targets. A key toxic player in MS is Cytokine IL-1β, while it is Amyloid β42 (Aβ42) in AD. In addition, N-methyl-D-aspartate (NMDA) excitotoxicity is common to both diseases. Here we show that these three players in MS and AD, namely IL-1β, Aβ42 and NMDA-Receptor (NMDAR), converge on a common target, the mitochondrial anchor Syntaphilin (SNPH), to cause dendritic degeneration. We have previously shown that SNPH, normally present only in axons, is misplaced to dendrites in diseases, causing excitotoxicity, dendritic fragmentation and ensuing neuronal death (Joshi et al., 2019, 2022). Here we used hippocampal neuronal cultures to show that this toxic SNPH intrusion into MAP2 positive dendrites is triggered by IL-1β (C) and Aβ42 (D) from both diseases (Figure below). Intriguingly, NMDAR, a common player to both diseases, exerts opposing roles in dendritic SNPH intrusion depending on the NMDAR subtype. The GluN2B is pro-death and triggers toxic SNPH intrusion, while the GluN2A is pro-survival and prevents SNPH intrusion. Lenti-viral overexpression of GluN2B, but not GluN2A, triggers massive SNPH intrusion. Critically, Aβ42 and IL-1β interact with NMDAR. For Aβ42, pre-inhibition of GluN2B completely prevents Aβ42 from triggering SNPH intrusion, suggesting that Aβ42 acts via GluN2B. For IL-1β, pre-inhibition of GluN2B still allows IL-1β to trigger SNPH intrusion, but this intrusion can be prevented by activation of GluN2A. Collectively, dendritic intrusion by the mitochondrial anchor SNPH triggered by Aβ42 in AD and IL-1β in MS is a common neurotoxic mechanism underlying the comorbidity between the two diseases. Targeting SNPH is therefore a novel, common therapy for both AD and MS. One exciting therapy is to design viral vectors to selectively overexpress GluN2A to block toxic intrusion of SNPH to prevent dendritic fragmentation and synaptic dysfunction in both AD and MS.

Disclosures: D. Mathur: None. **C. Zhang:** None. **K. Du:** None. **R.R. Rajaii:** None. **J.E. Ploski:** None. **S. Chiu:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.19/C99

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01AI136999

Title: Infiltrated T cells elicit neuronal damage in the brains in murine cryptococcus-associated IRIS

Authors: *J. ZHOU[1,2], S. ANWAR[2,3], M. INOUE[1,2];
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Abstract: *Cryptococcus*-associated immune reconstitution inflammatory syndrome (C-IRIS) is a condition that frequently occurs in immunocompromised patients receiving reconstitution therapy. The immune system becomes overreactive, and patients with C-IRIS exhibit many central nervous system complications, including headache, fever, cranial neuropathy, and visual disturbance, potentially complicating the progression and recovery processes. Pulmonary disease has also been reported in patients with C-IRIS, where symptom presentation can include herniation and pulmonary nodules. However, little is understood about its etiology and pathogenesis, making clinical diagnosis and treatment highly inefficient. Previously, we have developed a mouse model of C-IRIS using immunocompromised mice, with intranasal infection of Cn serotype A H99 (CnH99) and intravenous transfer of $CD4^+$ T cells after CnH99 infection. This mouse model showed weight loss, high mortality, systemic upregulation of pro-inflammatory cytokines, elevated levels of $CD4^+$ T cells in the lungs, infiltration of $CD4^+$ T cells into the brain, and cerebral edema. Here, utilizing our established mouse model of unmasking C-IRIS, we investigate how pulmonary dysfunctions are mediated, and demonstrate that pulmonary dysfunctions associated with the C-IRIS condition in mice could be attributed to the infiltration of $CD4^+$ T cells into the brain via the CCL8-CCR5 axis. Infiltrated T cells express higher levels of ephrin B3 and semaphorin 6B, axon guidance molecules during development, and elicit neuronal damage in the nucleus tractus solitarius, a region located in the hindbrain and known for processing information related to respiration. Our findings provide unique insight into the mechanism behind pulmonary dysfunctions in C-IRIS and nominate potential therapeutic targets for treatment.

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Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

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Program #/Poster #: PSTR214.20/C100

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RF1 NS085070

Title: The impact of altered PINK1 enzymatic activity on neuronal resilience and glial inflammatory signaling

Authors: *G. FIORINO[1], X. HOU[2], F. C. FIESEL[1], W. SPRINGER[3];
[2]Neurosci., [3]Neurosci. Res., [1]Mayo Clin., Jacksonville, FL

Abstract: Background: Parkinson's disease (PD) is characterized by loss of nigrostriatal dopaminergic (DA) neurons. Numerous studies have highlighted that mitochondrial dysfunction and neuroinflammation serve as both a cause and consequence of DA neuron damage. Loss-of-function (LOF) mutation in the mitochondrial quality control enzymes PINK1 and PRKN are the main genetic causes of early-onset PD. There remains a gap-in-knowledge surrounding the impact of PINK1-PRKN LOF to non-neuronal CNS cell types. In this study, we will elucidate the mechanisms through which loss of PINK1-PRKN signaling impacts non-neuronal cell type function and secretome, thereby contributing to neuroinflammation. There is also increasing evidence that enhancing mitochondrial autophagy (mitophagy) activation is neuroprotective. Our lab has discovered a PINK1 gain-of-function (GOF) variant that endogenously enhances kinase activity and mitophagy rates and will serve as a genetic proof of concept to broadly determine the therapeutic potential of mitophagy activation in PD. We hypothesize that PINK1 GOF will provide neuroprotective benefits and reduce neuroinflammation, while PINK1 LOF will have opposite results. **Methods and Results:** We used an ELISA-based assay to detect PINK1-PRKN pathway activation in iPSC-derived midbrain DA neurons and glia under basal and acute stress conditions. We found that PINK1-PRKN signaling is active in all CNS cell types but to different extent. Using a Cytokine Array Kit, we then measured cytokine and chemokine levels present in conditioned media from PINK1 LOF and isogenic wild-type (isoWT) control glia. Interestingly, we report that PINK1 LOF leads to significant changes in basal levels of certain cytokines and chemokines in glia. To investigate the therapeutic potential of PINK1 GOF, we generated PINK1 GOF and isoWT NGN2-inducible i3Neurons and found that PINK1 GOF increases mitochondrial respiration, neurite outgrowth, and viability while decreasing ROS production compared to isoWT. **Conclusions:** These results indicate that a pro-inflammatory response may be directly elicited in glia by loss of PINK1, highlighting the need to further investigate the cell-type specific importance of PINK1-PRKN signaling in non-neuronal CNS cell types that may contribute to neuroinflammation in PD. Lastly, we found that the PINK1 GOF variant enhances neuronal resilience, which will be further investigated in the context of preventing neuroinflammation.

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Poster

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NHMRC Project Grant 1156744
Brain Foundation Grant

Title: Targeting STING, TOLLIP and the unfolded protein response to limit secondary injury post-TBI

Authors: *A. L. FRYER[1], A. ABDULLAH[2], J. M. TAYLOR[3], P. J. CRACK[4];
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Abstract: Traumatic brain injury (TBI) is a major cause of death and disability worldwide with limited pharmacological interventions available to slow the inflammation and neurodegeneration that ensues post-injury. Our lab has demonstrated the cGAS-STING pathway to be a key driver of neuroinflammation-mediated neurodegeneration in TBI and other neuropathologies[1]. Known to be a regulator of type-I interferon production, there is increasing evidence for additional roles for STING in mediating cell death and ER-stress[2]. Toll interacting protein (TOLLIP), is an endogenous negative regulator of TLR signaling with roles in misfolded protein trafficking. Recently, TOLLIP has been identified to regulate STING activity by stabilising STING at its resting state on the ER[3]. This study aims to evaluate the role of TOLLIP in regulating STING and ER-stress activity in the CNS post-TBI. 10-12-week-old male C57Bl/6 mice were exposed to brain injury using the controlled-cortical impact model (CCI). 30-minutes post-injury, mice were intravenously administered a single 750nmol dose of the STING inhibitor, C-176, or a saline (vehicle). Analysis was conducted 2 and 24-hours post-TBI (n=7-9 mice for each treatment group). Western blot analysis revealed a significant reduction in the expression of TOLLIP and total STING in the cortex 24h-post TBI and not 2h-post TBI in both C-176 (0.65±0.08) and vehicle-treated mice (0.65±0.10) when compared to sham (1.55±0.15). C-176-treated mice alone exhibited significantly increased phosphorylation of the unfolded protein response (UPR) regulator IRE1-α (6.14±1.24) compared to both sham (1.00±0.12) and vehicle-treated TBI mice (1.37±0.28). Striatal mRNA expression levels of its downstream mediator *Xbp1* were also elevated at 24h-post TBI when compared to sham mice (C-176=1.24±0.11; sham=1.03±0.08). Together these findings suggest TOLLIP and its stabilising activity on resting-state STING is lost-post TBI with the pharmacological inhibition of STING increasing activation of the IRE1-XBP1 branch of the unfolded protein response. This study provides novel mechanistic insight into the regulation and activity of STING post-TBI in mice.
1. Abdullah, A. *et al.* STING-mediated type-I interferons contribute to the neuroinflammatory process and detrimental effects following traumatic brain injury. *J. Neuroinflamm* **15**, 17 (2018). 2.Yang, K., Tang, Z., Xing, C. & Yan, N. STING signaling in the brain: Molecular threats, signaling activities, and therapeutic challenges. *Neuron* **112**, 539-557 (2024). 3. Pokatayev, V. *et al.* Homeostatic regulation of STING protein at the resting state by stabilizer TOLLIP. *Nat Immunol* **21**, 158-167 (2020).

Disclosures: A.L. Fryer: None. **A. Abdullah:** None. **J.M. Taylor:** None. **P.J. Crack:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.22/C102

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: U01 CA266981

Title: Involvement of the NLRP3 Inflammasome in Neuropathology in the Aged Brain Following Traumatic Brain Injury

Authors: *I. COLELLO[1], D. SUN[3], J. GREEN[2], S. ZHANG[1], Y. XU[1];
[2]Anat. and Neurobio., [1]Virginia Commonwealth Univ., Richmond, VA; [3]Dept Anat. and Neurobio., Med. Col. Of Virginia, Richmond, VA

Abstract: Traumatic brain injury (TBI) is a significant health issue. Approximately 2.5 million people sustain a TBI each year in the United States, with the elderly population at the age of 75 and older among the highest incidence group. Currently there is no effective treatment for TBI. Moreover there is limited research examining the effects of TBI on the aged brain. Following TBI, the neuroinflammatory response acts as a secondary injury leading to further brain damage. Inflammasomes are important inflammatory, multiprotein complexes that regulate the innate immune response, production of inflammatory cytokines such as interleukin-1 beta (IL-1β), activation of caspase-1 and induction of cell death. Among inflammasome family members, leucine-rich repeat and pyrin containing protein 3 (NLRP3) is the most extensively studied in neurological diseases including TBI. In this study, we examined TBI-induced changes to the NLRP3 inflammasome and its downstream effectors, and assessed the therapeutic effects of a novel NLRP3 inhibitor (YM-III-109) on brain tissue damage and the inflammatory response following a moderate focal brain injury. A group of 20-month-old Sprague Dawley male rats were subjected to a moderate cortical impact injury (CCI). Following TBI, rats were treated with 4 doses of YM-III-109 (20mg/kg, i.p., at 30 minutes, 6, 24 and 30 hours post-injury). Animals were sacrificed at 2 days post-injury and brain tissues were harvested. For neuropathological assessment, cortical lesion volume, the extent of injury-induced degenerative neurons and the number of activated microglia/macrophages were quantified. Additionally, protein expression levels of inflammatory mediators including pro-inflammatory cytokines TNF-α and IL-1β were measured via ELISA. Furthermore, protein expression levels of inflammatory markers including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), high mobility group box 1 (HMGB1), and caspase -1 along with NLRP3 and its adaptor protein apoptotic speck-containing protein (ASC) were measured via Western blotting. We found that focal brain injury induces significant activations of NLRP3 inflammasome and downstream effectors in both the ipsilateral cortex and hippocampus. Post-injury treatment with our novel NLRP3 inhibitor YM-III-109 can mitigate the injury-induced inflammatory responses and neurodegeneration. Further studies assessing the therapeutic potential for YM-III-109 are ongoing. Our findings provide further insight on the NLRP3 inflammatory response following TBI in the aged brain and the prospect of NLRP3 as a potential therapeutic target.

Disclosures: I. Colello: None. **D. Sun:** None. **J. Green:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.23/C103

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Discovery of IGFBPL1-fusion Proteins as Multifunctional Therapeutics for Retinal Degenerative Disorders

Authors: *J. M. LEVENSON[1], B. MA[1], Y. WANG[1], J. SLOCUM[2], M. BRAUN[2], S. SUMMERS[2], A. JACKSON[2], D. HICKSON[2], K. MICHAUD[1], L. R. TEAL[1], A. KAZANTSEV[2], J. W. BRYSON[1], E. FURFINE[2], W. YELLE[1];
[1]FireCyte Therapeut., Beverly, MA; [2]Mosaic Biosci., Boulder, CO

Abstract: Glaucoma is the second leading cause of blindness worldwide. Current standard of care centers around control of intraocular pressure, however no treatment prevents progression to blindness. Recent studies have linked inflammatory processes to glaucoma in patients and preclinical rodent models of disease. This indicates glaucoma is in a class of neurodegenerative conditions that include a neuroinflammatory component. More recently it has been shown that therapeutic administration of the protein insulin growth factor-1 binding protein-like 1 (IGFBPL1) to preclinical models of glaucomatous neurodegeneration and retinal wound healing preserve visual function and accelerate resolution of lesions, respectively. To better understand the mechanism by which IGFBPL1 exhibited both anti-inflammatory and neuroprotective pharmacology, we screened the cell surface proteome for binding to IGFBPL1. This screen revealed IGFBPL1 interacted with 2 distinct receptors: Sortilin 1 (SORT1) and the netrin-1 receptor / deleted in colorectal cancer (DCC). To enable robust optimization of IGFBPL1, over 100k different truncated proteins were screened for stability and solubility to precisely map functional domain boundaries. Using this knowledge, we screened over 200 candidates for binding to SORT1 and DCC, mapping SAR to presence of specific functional domains. Proprietary FireCyte fusion proteins exhibit robust binding to SORT1 and DCC and robust binding to neurons and microglia. These IGFBPL1-fusion proteins exhibit potent anti-inflammatory activity in hIPSC-derived microglia. In addition, IGFBPL1-fusion proteins are directly neuroprotective in monocultures of hIPSC-derived glutamatergic neurons. Direct comparisons to Latozinemab, a clinical-stage anti-SORT1 monoclonal antibody in development for ALS-FTD, demonstrate superiority of FireCyte IGFBPL1-fusion proteins in every measure tested. Collectively, these observations support the further development of IGFBPL1-derived therapeutics for retinal degenerative disorders. Our results are one of the first to reduce to practice the potent pharmacology of a multifunctional, multi-targeting therapeutic for neurodegenerative disease.

Disclosures: J.M. Levenson: A. Employment/Salary (full or part-time):; FireCyte Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics. **B. Ma:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics. E. Ownership Interest (stock,

stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics. **Y. Wang:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ireCyte Therapeutics. **J. Slocum:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. **M. Braun:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. **S. Summers:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. **A. Jackson:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. **D. Hickson:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. **K. Michaud:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics. **L.R. Teal:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics. **A. Kazantsev:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics. **J.W. Bryson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics. F. Consulting Fees (e.g., advisory boards); FireCyte Therapeutics. **E. Furfine:** A. Employment/Salary (full or part-time):; Mosaic Biosciences. 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.; FireCyte Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics. F. Consulting Fees (e.g., advisory boards); FireCyte Therapeutics. **W. Yelle:** A. Employment/Salary (full or part-time):; FireCyte Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FireCyte Therapeutics.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.24/C104

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Treatment with leuprolide acetate, a gnrh agonist, enhaces cognitive function in rats with hepatic encephalopathy

Authors: *B. L. GUTIERREZ-ESPARZA[1], M. L. GONZALEZ-TORRES[2], A. QUINTANAR-STEPHANO[1], J. L. QUINTANAR[1];
[1]Fisiología y Farmacología, [2]Psicología, Univ. Autónoma de Aguascalientes, Aguascalientes, Mexico

Abstract: Hepatic encephalopathy (HE) is a neuropsychiatric complication of acute liver failure or chronic liver injury. Liver failure disrupts ammonia detoxification, leading to its accumulation in the bloodstream. This increased ammonia can breach the blood-brain barrier, resulting in brain damage. The hippocampus becomes a crucial target during events of elevated ammonia levels, causing episodic and spatial memory impairment, a decrease in learning ability, and altered navigation function. Leuprolide acetate (LA), a GnRH agonist, has been implicated in neuroprotection and neuroregeneration in several regions of the central nervous system, including the hippocampus. In this study, we aim to evaluate the effects of LA treatment on the hippocampus of rats with HE induced by portocaval anastomosis (PCA) through cognitive tests, histology analysis, and expression of neuronal recovery marker proteins, such as neurofilament (NF200) and neurabin II, and the astrocyte marker glial fibrillary acidic protein (GFAP). Wistar rats weighing 250-300g were divided into three groups: Sham, portocaval anastomosis with saline solution (PCA+SS), and portocaval anastomosis treated with LA (PCA+LA). To evaluate learning and spatial memory, elevated T-maze (ETM) and Y-maze tests (YMT) were respectively used. Results showed that in both ETM and YMT, rats treated with LA had significantly better performance than rats without treatment. Furthermore, coronal brain slices were obtained and stained with hematoxylin-eosin. The histological analysis of the hippocampus revealed an increase in neuron density, nuclear area, and thickness of the dentate gyrus region in the PCA+LA group compared to PCA+SS. Moreover, the expression of neurabin II and NF200 in LA-treated rats showed an increase compared to the untreated group. Additionally, it was demonstrated that there was an increase in the expression of GFAP in the PCA+SS group compared to the control and PCA+LA group. In conclusion, LA promotes the improvement of cognitive function, recovery in hippocampal neurons, and a reduction in astrogliosis in a hepatic encephalopathy model. These findings indicate that leuprolide acetate could be a potential therapeutic intervention for attenuating hippocampal damage during HE.

Disclosures: B.L. Gutierrez-Esparza: None. **M.L. Gonzalez-Torres:** None. **A. Quintanar-Stephano:** None. **J.L. Quintanar:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.25/C105

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIA ViCTER RO1ES033462-01

Title: Regulator of G-protein signaling 10 ameliorates NLPR3-mediated inflammation and metabolic dysfunction.

Authors: *D. J. GRESHAM[1], J. CHUNG[1], J. BYUN[1], N. DIEP[1], M. C. HAVRDA[2], J. LEE[1]; [1]Physiol. and Pharmacol., Univ. of Georgia, Athens, GA; [2]Mol. and Systems Biol., Geisel Sch. of Med. at Dartmouth, Lebanon, NH

Abstract: Systemic inflammation and metabolic dysfunction are highly implicated in the pathogenesis of neurodegenerative diseases. The NLRP3 inflammasome is a multimeric protein complex that responds to cellular stress signals and is induced by many factors, including environmental toxicants and amyloid aggregates. Chronic inflammasome activation has been shown to cause insulin resistance, which is associated with the development of neurodegenerative diseases. Increased inflammasome activity has been observed in both Alzheimer's and Parkinson's disease patients, making the NLRP3 inflammasome a target for disease intervention. Previously, regulator of G-protein signaling 10 (RGS10) was identified as a key regulator of activation and homeostasis in microglia and peripheral macrophages. This study aimed to determine whether RGS10 plays a role in limiting NLRP3 inflammasome activity. Here, we conducted both in-vivo and in-vitro studies to elucidate immune homeostasis and explore the potential interaction between NLRP3 and RGS10. Previously, we observed impaired glucose tolerance, insulin resistance, and increased proinflammatory cytokine production in $RGS10^{-/-}$ knockout mice. Our in-vitro data show that $RGS10^{-/-}$ knockout bone marrow-derived macrophages produce increased levels of cleaved caspase-1 (p20) and mature IL-1b (p17) in response to LPS in combination with ATP or nigericin, suggesting that RGS10 mitigates NLRP3 inflammasome activity. Our in-vivo high-fat diet study showed that improved glucose tolerance and insulin sensitivity in $NLRP3^{-/-}$ female mice were no longer seen in $NLRP3^{-/-}RGS10^{-/-}$ double knockout (dKO) female mice. Both male and female dKO mice showed bodyweight increases comparable to $RGS10^{-/-}$ mice, though some sex differences were observed in their overall metabolic performance. Our data suggest that RGS10 is required for metabolic improvements resulting from knockout of NLRP3, indicating the role of RGS10 as an upstream regulator of NLRP3 inflammasome activity.

Disclosures: D.J. Gresham: None. **J. Chung:** None. **J. Byun:** None. **N. Diep:** None. **M.C. Havrda:** None. **J. Lee:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.26/C106

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of a novel exercise metabolite on blood-brain barrier disruption

Authors: P. SHRIVASTAVA[1], ***I. J. BIOSE**[1,2];
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Abstract: Neurological and cardiometabolic conditions result in blood-brain barrier (BBB) dysfunction due to inflammation. Increased circulating gut bacterial toxins and lipopolysaccharide (LPS), a component of gram-negative bacteria cell wall, are linked to these observations. Our goal is to test, in mouse brain endothelial (bEND.3) cells, whether a recently characterized exercise metabolite (EM1) can mitigate (100 ng or 1 µg of) LPS-induced BBB tight-junction protein disruption as well as ameliorate pro-inflammatory signaling. Our results demonstrate that EM1 significantly increases ($p<0.01$) ZO-1 and Occludin protein levels without LPS insult. Further, EM1 significantly upregulates ($p<0.01$) ZO-1 expression against LPS-induced downregulation but EM1 did not change Occludin and Claudin-5 expressions. Among the tested doses, 0.3 and 5 µM of EM1 show the most beneficial effect on cell viability in the presence of LPS. For reproducibility, we incorporated two repeats for each experimental paradigm. Also, our data are expressed as mean ± standard deviation. Results are underway to show the effect of EM1 on BBB permeability using transepithelial electrical resistance (TEER) and transwell assays. Also, we are presently investigating whether EM1 has anti-inflammatory and antioxidant properties. We expect to demonstrate the mechanism by which EM1 attenuates BBB dysfunction in bEND.3 cells.

Disclosures: P. Shrivastava: None. **I.J. Biose:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.27/C107

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS122973
NIH Grant R01NS089640

Title: Fabp7 expression modulates astrocyte response in lps-induced endotoxemia

Authors: *M. BRESQUE TOLEDO[1], D. ESTEVE[2], M. PEHAR[3], M. R. VARGAS[2];
[1]Neurol., UW-Madison, Madison, WI; [2]Neurol., Univ. of Wisconsin, Madison, Madison, WI;
[3]Medicine, Div. of Geriatrics, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Fatty acid binding proteins (FABPs) have recently drawn attention due to their key role in regulating lipid metabolism, energy homeostasis, gene expression, and inflammation. FABPs are a family of small proteins classically identified as lipid chaperones that reversibly bind fatty acids (FA) and participate in FA subcellular traffic. FABP7, one of the members of this family, also known as brain lipid binding protein, is highly expressed in astrocytes. Recent reports suggested a pathogenic role of FABP7 upregulation in astrocytes during neurodegenerative processes, including amyotrophic lateral sclerosis and Alzheimer's disease. In addition, FABP7 over-expression in astrocytes induces a pro-inflammatory response detrimental to co-cultured neurons. Here we explored how FABP7 expression modulates the inflammatory response in astrocytes and its consequences on neighboring cells. We first evaluated the effect of Fabp7 downregulation in the response of cultured astrocytes to inflammatory stimuli. Next, we analyzed the impact of Fabp7 silencing in activated astrocytes in co-cultured neurons and microglia. Lastly, using mice injected with a viral vector expressing a scramble shRNA (control) or a Fabp7-shRNA, we analyzed cortical neuroinflammation in response to LPS systemic administration. Our results showed that silencing FABP7 expression in activated astrocytes decreases the expression of a luciferase reporter expressed under the control of NF-kB-response elements. Accordingly, decreased FABP7 expression reduced nuclear translocation of the p65 subunit of NF-kB in response to inflammatory stimuli. Consequently, silencing FABP7 decreases the toxicity of stimulated astrocytes toward co-cultured motor neurons. Similar results were obtained after silencing FABP7 in astrocytes differentiated from human induced pluripotent stem cells (i-astrocytes). In addition, silencing Fabp7 in astrocytes enhances the phagocytic activity of co-cultured microglia. Finally, mice with astrocyte-specific Fabp7 silencing displayed reduced cortex gliosis after LPS administration. Moreover, whole transcriptome RNA sequencing analysis from the cortex of LPS-treated mice showed a differential inflammatory transcriptional profile following Fabp7 silencing, with an attenuated NF-kB-mediated response. Together, our results highlight the potential of FABP7 as a target to modulate neuroinflammatory responses in the central nervous system.

Disclosures: M. Bresque Toledo: None. **D. Esteve:** None. **M. Pehar:** None. **M.R. Vargas:** None.

Poster

PSTR214: The Contributions of Neuroinflammation to Neurodegeneration: Animals Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR214.28/C108

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Zucker Diabetty Fatty Rat as a model of diabetes: alterations in Hipoccampus, Prefrontal Cortex and evidence of memory deficit

Authors: *R. VAZQUEZ-ROQUE[1,2], J. MORALES-MEDINA[3], S. TREVIÑO[4];
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Abstract: Diabetes Type 2 (DT2) is a chronic metabolic disease of multifactorial aetiology, characterized by a hyperglycemic state as a result of alterations in insulin secretion, production or signaling. Cognitive declines as well as Alzheimer's and other dementias have been associated with Diabetes. In fact, an increased incidence of cognitive decline, as well as cortical and subcortical atrophy, has been reported in association with it. Due to the fact that DT2 affects ~6.3% of global population, and it's the 3rd cause of death in Mexico, its relevance is undisputable for the health burden it carries. In this manner, the objective of this work is the evaluation of the effect of the diabetic condition in the behaviour and brain of the Zucked Diabetic rat. Short- and Long-term recognition memory were analyzed by the Novel Object Recognition Test (NORT), while motor activity was evaluated by total distance, total time and velocity during the habituation step of NORT. Additionally, morphology and cell density of the Hippocampus (CA1 and Dentate Gyrus) and Prefrontal Cortex were evaluated. Our results demostrated a decrease in motor activity (as measured by total distance), as well as a deficit in long term recognition memory. This deficit is consistent with findings of a decrease in neuron density and atrophies in neuron morphology in the regions mentioned, as compared with controls. This findings are consistent with reports in human patients, however, more research is needed to elucidate the mechanisms behind these changes, and how they are reflected in the rat strain.

Disclosures: R. Vazquez-Roque: None. **J. Morales-Medina:** None. **S. Treviño:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.01/C109

Topic: C.08. Ischemia

Support: NIH grant NS121227

Title: The novel MCU blocker Ru265, delivered after transient global ischemia, attenuates neuronal injury and mitochondrial dysfunction both in vitro (hippocampal slice) and in vivo (rat cardiac arrest).

Authors: Y. V. MEDVEDEVA[1], E. SHARMAN[2], H. YIN[1], A. BAZRAFKAN[2], H. GHAZNAVI[2], E. WANG[2], S. KHATAMI[2], R. LOTFY[2], M. RAFI[2], C. VU[2], N. ELLAHIB[2], P. BORHANIKHABAZ[2], K. SUAYNGAM[1], J. WILSON[3], Y. AKBARI[1], ***J. WEISS**[4];

[1]Neurol., [2]Univ. of California, Irvine, Irvine, CA; [3]Chem. and Chem. Biol., Univ. of California, Santa Barbara, Santa Barbara, CA; [4]Univ. of California Irvine, Irvine, CA

Abstract: Despite high morbidity and mortality after brain ischemia, treatments are limited in part due to inadequate understanding of injury mechanisms. After transient ischemia, many neurons initially recover but die hours or days later. Our recent studies used a mouse brain slice oxygen glucose deprivation (OGD)/reperfusion model to compare events occurring after ischemia in particularly vulnerable CA1 and more resistant CA3 hippocampal neurons. We found substantial Zn^{2+} accumulation in mitochondria of CA1 (but not CA3) neurons 1 hour after ischemia, occurring largely via the mitochondrial Ca^{2+} uniporter (MCU). We further found that preventing this Zn^{2+} accumulation (with Zn^{2+} chelation or MCU blockade) attenuated mitochondrial and neuronal dysfunction 4 h later. Our current studies tested the recently developed specific MCU inhibitor Ru265, which has potential for usability in humans. Ru265 was applied after termination of the ischemic episode, a period amenable to therapeutic interventions, both in slice and in an in vivo cardiac arrest model. Acute brain slices were subjected to short (8 min) OGD /reperfusion. After 1-4 h we assessed mitochondrial state (by evaluating mitochondrial potential, $\Delta\Psi_m$, using Rhodamine123) and the ability of neurons to maintain Ca^{2+} homeostasis (by measuring $[Ca^{2+}]_i$; using brain slices from transgenic mice expressing a protein based ratiometric Ca^{2+} indicator, GCaMP6f/tdTomato in forebrain pyramidal neurons). We found that both $\Delta\Psi_m$ and $[Ca^{2+}]_i$ largely recover by 1 h after OGD, but after a longer delay (2 h) mitochondria start to show progressive depolarization and $[Ca^{2+}]_i$ increases. Application of Ru265 after OGD termination for 30 min substantially attenuated both of these delayed changes. Parallel studies employed an in vivo rat asphyxial cardiac arrest model of transient global ischemia. We found that 8 min asphyxia induces considerable neuronal injury (evaluated with vanadium acid fuchsin staining) and mitochondrial swelling in CA1 24 h later, that is accompanied by impaired brain function, assessed by behavioral neurological deficit score (NDS) testing. These effects were substantially attenuated by delayed (upon reperfusion) intravenous infusion of Ru265, leading to significantly improved NDS scores versus placebo at 4 h and 20 hours. We also noted improvements in hemodynamic function, including more stable heart rate and blood pressure after reperfusion. MCU inhibition with RU265 may provide a new approach for neuroprotective treatment after ischemia. As this treatment can be applied after restoration of blood flow it may present a promising therapeutic approach.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.02/C110

Topic: C.08. Ischemia

Support: CONAHCYT Grant A1-S-21433

Title: Morphological and oxidative changes in the striatum, cortex, and hippocampus of rats subjected to ischemia and reperfusion

Authors: J. RIVERO SAN JUAN[1], **S. BAUTISTA PÉREZ**[1], C. A. SILVA-ISLAS[2], D. BARRERA-OVIEDO[1], *P. MALDONADO[3];
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Abstract: A transient ischemic attack (TIA) is the most common type of stroke, occurring by the occlusion of a cerebral artery. Reperfusion is vital for restoring blood flow to ischemic tissue and prevent cell death; however, it also causes secondary damage by oxidative stress, inflammation, and even blood-brain barrier disruption. The aim is to evaluate the effect of ischemia and reperfusion on structural and oxidative changes in striatum, cortex, and hippocampus.Male Wistar rats (280-310 g and aged 8 weeks) were divided in 7 groups: sham, ischemia (I)15min, I30min, I60min, I60min and reperfusion (R)15 min, I60minR30min, and I60minR60min.No morphological changes (Nissl and H&E) and low oxidative damage (8-OHdG and 3-NT) were observed in hippocampus. Cortex shows slight changes in cell morphology and increases in oxidative damage with reperfusion. Striatum exhibited the most significant morphological changes and oxidative damage with reperfusion. None of the studied regions presented FJ-positive cells, suggesting the absence of neurodegeneration.Ischemia does not induce morphological an oxidative alteration in hippocampus, cortex, and striatum. However, reperfusion caused the major structural and oxidative changes in striatum and cortex, possibly due to its proximity to the ischemic core.This work was supported by project A1-S-21433, CONACYT (PDM).JRS received a scholarship from project A1-S-21433, CONACYT.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.03/C111

Topic: C.08. Ischemia

Support: RO1003146

Title: Investigating the role of mitochondrial quality control mechanisms in the induction of microglial polarization following oxygen and glucose deprivation.

Authors: *F. J. TORRES TORRES[1], G. M. FOGO[2], K. J. EMAUS[3];
[1]Neurosci., [2]Univ. of Michigan, Ann Arbor, MI; [3]Emergency Med., Univ. of Michigan, ANN ARBOR, MI

Abstract: Ischemic injury is the leading global cause of death and long-term disability. During ischemia, deprivation of key metabolites such as oxygen and glucose contribute to cell death from mitochondrial dysfunction. Microglia, the resident immune cells of the brain, initiate immune responses in response to injury in a process termed polarization. The classically defined 'pro-inflammatory' polarized microglia harbor perturbed mitochondrial structure and quality control mechanisms that result in accumulation of fragmented mitochondria. Prolonged mitochondrial dysfunction from ischemia reperfusion may commit microglia towards a chronic pro-inflammatory phenotype and the development of neurodevelopmental diseases following stroke. However, the specific contribution of mitochondrial dysfunction to microglial polarization mechanisms have yet to be defined. Parkin is a ubiquitin ligase that signals for clearance of dysfunctional mitochondria through PINK1/Parkin mitophagy. Conversely, Parkin-mediated ubiquitination may initiate IKK-complex formation on fragmented mitochondria, a potentially critical step in the NFKB-polarization pathway. Parkin-null ($Parkin^{-/-}$) primary microglia cell lines were utilized along with autophagic inhibitors to separate the contribution of Parkin-dependent ubiquitination in mitophagy and the IKK-complex formation. Primary microglia were exposed to 6hrs oxygen and glucose deprivation (OGD/R), comparing markers of activation following 2hrs and 18hrs of reperfusion. Immunofluorescent labeling was used to measure mitochondrial morphology, phosphorylated-NFKB internalization to the nucleus, and colocalization of IKK-gamma/NEMO with fragmented mitochondria. WT and $Parkin^{-/-}$ present vastly different mitochondrial morphology profiles, with greater accumulation of punctate mitochondria in the $Parkin^{-/-}$ group. IKK-complex recruitment to mitochondria, pNFKB internalization, and expression levels of NLRP3 and pNFKB were diminished in the $Parkin^{-/-}$ group. Ongoing studies will integrate the genetically-encoded fluorescent probe MitoQC, to visualize changes in mitophagic flux in WT and $Parkin^{-/-}$ primary microglia following OGD/R.

Disclosures: F.J. Torres Torres: None. **G.M. Fogo:** None. **K.J. Emaus:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR215.04/C112

Topic: C.08. Ischemia

Support: Nebraska DHHS LB692
NIH/NIGMS P20GM130447 COBRE/CoNDA Pilot Award

Title: Trem1-mediated neuroinflammation contributes to global ischemia pathology

Authors: *R. URQUHART, H. KIM, G. JADHAV, J.-Y. HWANG;
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Abstract: Global ischemic stroke, occurring most often following cardiac arrest, is a state in which cerebral blood flow is globally impaired. Loss of blood flow and oxygenation throughout the brain leads to delayed and selective death of hippocampal CA1 pyramidal neurons which leads to impaired learning and memory. With any stroke, the first priority is restoration of blood flow, but the mechanisms driving the long term effects of global ischemia are not fully understood. Neuroinflammation is receiving increased attention for its potential contributions to neurodegenerative conditions, particularly in stroke. In this study, we sought to explore how dysregulation of genes might promote the neurodegeneration and cognitive deficit associated with global ischemia. Towards this end, rats were subjected to global ischemia via 4-vessel occlusion (4VO), then RNA-seq was performed to assess altered mRNA patterns in post-ischemic hippocampal CA1. Canonical pathway analysis using the Ingenuity Pathway Analysis (IPA) software revealed upregulated expression of genes related to 'neuroinflammation' and 'TREM1 signaling'. The triggering receptor expressed on myeloid cells-1 (TREM1) is a myeloid-derived surface receptor involved in immunity and inflammation. It has known roles in conditions such as myocardial ischemia and sepsis. However, the role of TREM1 in global ischemia pathology remains unknown. Our results using RT-qPCR and Western blot analyses further show elevated TREM1 expression within 48HR of ischemia, with downstream inflammatory cytokines and transcription factors also showing differential expression. These findings validate RNAseq and IPA and confirm TREM1 activation and signaling are impacted by global ischemia. To establish a causal relationship between TREM1 and global ischemia pathology, the effects of TREM1 inhibitory peptide LR12 were assessed. LR12 administration reduced neurodegeneration of hippocampal CA1, minimized blood brain barrier breakdown and loss of tight junction proteins, and attenuated the inflammatory profile observed following global ischemia. Current experiments will address the effects of LR12 on global ischemia-induced cognitive deficits. Overall, this research establishes the role of TREM1 mediated neuroinflammation in global ischemia pathology and identifies TREM1 as a potential therapeutic target for attenuating global ischemia-induced neurodegeneration and cognitive deficits.

Disclosures: R. Urquhart: None. **H. Kim:** None. **G. Jadhav:** None. **J. Hwang:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Topic: C.08. Ischemia

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National Institute of Aging (RF1AG078352)
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National Cancer Institute to ALE (K08 CA100526)

Title: Rab7a activation promotes degradation of select tight junction proteins at the blood-brain barrier after ischemic stroke

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1. **Abstract:** The stability of tight junctions (TJs) between endothelial cells (ECs) is essential to maintain blood- brain barrier (BBB) function in the healthy brain. Following ischemic stroke, TJ strand dismantlement due to protein degradation leads to BBB dysfunction, yet the mechanisms driving this process are poorly understood. Here, we show that endothelial-specific ablation of Rab7a, a small GTPase that regulates endolysosomal protein degradation, reduces stroke-induced TJ strand disassembly resulting in decreased paracellular BBB permeability and improved neuronal outcomes. Two pro-inflammatory cytokines, TNFα and IL1β, but not glucose and oxygen deprivation, induce Rab7a activation via Ccz1 in brain ECs in vitro, leading to increased TJ protein degradation and impaired paracellular barrier function. Silencing Rab7a in brain ECs in vitro reduces cytokine-driven endothelial barrier dysfunction by suppressing degradation of a key BBB TJ protein, Claudin-5. Thus, Rab7a activation by inflammatory cytokines promotes degradation of select TJ proteins leading to BBB dysfunction after ischemic stroke.

Disclosures: D. Jamoul: None. **A. Cottarelli:** None. **D. Agalliu:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR215.06/C114

Topic: C.08. Ischemia

Support: NIH Grant R01NS116143
NIH Grant 1R21NS134813

Title: Stroke leads to changes in nuclear homeostasis that increase the risk of neurodegeneration in iPSC derived cortical neurons

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Neurosci., Univ. of Rhode Island, Kingston, RI; [5]Cell and Mol. Biol., Univ. of Rhode Island, Kingston, RI; [6]Cell and Mol. Biol., Univ. of Rhode Island Interdisciplinary Neurosci. Program, Kingston, RI

Abstract: Ischemia is a life-threatening condition resulting from medical emergencies like stroke. Lack of oxygen and glucose during stroke leads to acute neuronal death and long-lasting damage to affected brain regions. Having a stroke also greatly increases an individual's risk of developing Alzheimer's Disease later in life. During stroke neurons experience acute ischemia, which impacts the actin cytoskeleton through aggregation of actin and cofilin, formation of actin stress fibers, and neurofilament destabilization. RNA-binding proteins (RBPs) then begin to accumulate in the cytoplasm, similar to pathology seen in neurodegenerative disorders. However, the molecular link between ischemia-induced cellular changes and neurodegeneration is unclear. Our previous research has established a link between actin homeostasis and the functional stability of the nuclear pore complex (NPC). Thus, we hypothesize that rearrangement of the actin cytoskeleton after stroke may have long-term impacts on the NPC, leading to the mislocalization and aggregation of nuclear proteins with downstream effects on neuronal survival. To test this, we expose iPSC-derived cortical neurons to acute oxygen and glucose deprivation (OGD) followed by a recovery period under normoxic conditions. We examine changes to nuclear structures, the cytoskeleton, gene regulatory proteins, and viability. Our results show that acute ischemia induces long-lasting changes that affect the abundance of regulatory proteins and the ability to respond to age-related stressors, leading to cell death. Our research yields novel insights into early changes occurring after a stroke that may compound the effects of normal aging to increase the risk of neurodegeneration.

Disclosures: E. Potts: None. **B. Chatragadda:** None. **A. Collins:** None. **M. Gregoire:** None. **C. Fallini:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.07/C115

Topic: C.08. Ischemia

Support: CalciGenix, LLC

Title: Impact of a transient ischemic attack on neuronal excitability

Authors: *B. NATWORA, M. MASSMAN, I. MORLEY, J. R. MOYER, JR.;
Univ. of Wisconsin-Milwaukee Grad. Training Program In Neurosci., Milwaukee, WI

Abstract: Current reports rank stroke as the fifth leading cause of death in the United States, and the source of life-altering cognitive impairments in survivors. Approximately 87% of reported stroke cases are the result of an ischemic stroke, characterized by the occlusion of an artery that supplies oxygen-rich blood to the brain. Consequently, local networks exhibit a change in

excitability and homeostatic function, which can influence delayed neuronal death. However, inconsistent electrophysiological findings justify the need for additional research to uncover the pathophysiology of this neurovascular disease. Given that susceptibility is highest among aging women and greatly influenced by a prior transient ischemic attack (TIA), the present study investigated the impact of a TIA on female and male neuronal membrane properties, intrinsic excitability, and structural integrity in a region that is vital for memory formation. Acute coronal brain slices containing the dorsal hippocampus were prepared from adult female and male Fisher 344 rats and placed in oxygenated artificial cerebral spinal fluid (aCSF). Half the slices from each subject underwent 5 min of oxygen-glucose deprivation (OGD) to model a TIA, and the other half remained in oxygenated aCSF as a control. Cell death was assayed by trypan blue exclusion, which labels dead or dying neurons, to verify the efficacy of the OGD protocol. Neuronal damage was drastically increased, as indicated by a greater number of trypan blue-labeled neurons in OGD-treated slices compared to control-treated slices. To evaluate the impact of OGD on the physiological and morphological properties of CA1 pyramidal neurons, biocytin-filled patch electrodes were used to obtain whole-cell recordings from visually identified CA1 pyramidal neurons from control and OGD-treated slices. No sex differences were observed in the intrinsic excitability of CA1 neurons under control conditions. However, we observed an increase in neuronal output of CA1 neurons from female rats following OGD compared to neurons from control slices. In contrast, the intrinsic excitability of male CA1 neurons was similar between conditions. A difference in neuronal membrane properties between female and male rats was revealed by sag amplitude and action potential threshold following OGD. These observed changes imply a modulatory role of sex on the intrinsic excitability of hippocampal CA1 pyramidal neurons during a transient ischemic attack. These data provide insights into unique mechanisms that may underly the survival of CA1 hippocampal neurons during a TIA and offers avenues to enhance the restoration of both neuronal and cognitive functioning.

Disclosures: B. Natwora: None. **M. Massman:** None. **I. Morley:** None. **J.R. Moyer, Jr.:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR215.08/C116

Topic: C.08. Ischemia

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I01 BX005127
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IK6BX005690

Title: Stroke Triggers Dynamic m^6A Reprogramming of Cerebral Circular RNAs

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Abstract: We previously showed that stroke alters circular RNA (circRNA) expression profiles. Many circRNAs undergo epitranscriptomic modifications, particularly methylation of adenosine to form N^6-methyladenosine (m^6A). This modification significantly influences the circRNA metabolism and functionality. Hence, we currently evaluated if transient focal ischemia in adult C57BL/6J mice alters the m^6A methylation of circRNAs. Changes in m^6A were profiled in the peri-infarct cortex following immunoprecipitation coupled with microarrays. Correlation and gene ontology analyses were performed to understand the association of m^6A changes with circRNA regulation and functional implications after stroke. Many circRNAs showed differential regulation (up or down) after stroke, and this change was highest at 24h of reperfusion. Notably, most circRNAs differentially regulated after stroke also exhibited temporal changes in m^6A modification patterns. The majority of circRNAs that showed post-stroke differential m^6A modifications were derived from protein-coding genes. Hyper- than hypomethylation of circRNAs was most prevalent after stroke. Gene ontology analysis of the host genes suggested that m^6A-modified circRNAs might regulate functions such as synapse-related processes, indicating that m^6A epitranscriptomic modification in circRNAs could potentially influence post-stroke synaptic pathophysiology. Funded by NIH and VA.

Disclosures: S.L. Mehta: None. **H. Namous:** None. **R. Vemuganti:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.09/Web Only

Topic: C.08. Ischemia

Title: Analyzing the expression of Prohibitin during focal cerebral ischemia and reperfusion in a rat model

Authors: R. GÓMEZ RIVERA[1], O. PULIDO MAZA[2], N. SERRANO[3], A. SÁNCHEZ GARCÍA[1], F. CÁZARES-RAGA[4], J. NADER[5], F. HERNÁNDEZ HERNÁNDEZ[6], ***A. ORTIZ-PLATA**[7];
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Abstract: Introduction: Cerebral ischemia (CI) is characterized by a sudden interruption of blood circulation, which triggers molecular mechanisms that produce changes in the expression

of genes and proteins. Prohibitins (Phb) are pleiotropic proteins commonly located in the mitochondrial inner membrane (MIM) that comprise two subunits (Phb1 and Phb2). Previously, through a proteomic strategy, the expression of Phb1 in focal cerebral ischemia (FCI) was identified. **Objective:** In this work we investigated the expression of Phb1 and Phb2 in the parietal cortex during FCI. **Methods**: FCI (middle cerebral artery occlusion) in male Wistar rats was induced for 15 minutes and 1 h without and with 24 h of blood reperfusion (R). Sham rats (without middle cerebral artery occlusion) were used as a control; n=3 for each group. After the experimental procedure, the rats were perfused in 4% paraformaldehyde-PBS; brain tissue was dehydrated, paraffin embedded and 4 μm sections were made. Phb-1 and 2 expressions were assessed by Immunohistochemistry. **Results and discussion**: In brain Phb expression has been reported in MIM. In our work, comparing to the control, Phb1 decreased cytoplasmic expression at 15 min of FCI, rising later in R conditions as well as at 60 min of FCI and 60 min/R. Unlike control, no nuclear expression of Phb1 was observed at 15 min of FCI, without and with R, as well as at 60 min of FCI; however, it was striking that after 60 min of FCI with 24 h/R, the immunodetection of Phb1 was also nuclear. Phb2 expression in the control group was scarce and no changes during FCI were found. With these results we show that in the cerebral cortex during FCI, Phb1 expression decreases since the first minutes and its expression increases as time passes. Here we found that Phb1 show cytoplasmic and nuclear localization, and not only mitochondrial as had already been reported. It has been reported that Phb1 and Phb2 expressions are interdependent; here we observed low and scarce expression of Phb2 compared with Phb1. Previous research in animal models and neoplastic cells, a relationship between the increase in Phb expression and cell survival, has been found. In cerebrovascular disease, its probable interaction with the release of cytochrome C from mitochondrial cristae has been described, which leads to an increase in the expression of antiapoptotic proteins and a reduction in the area of cerebral infarction. In the nucleus, Phb can interact with p53 participating in the regulation of transcriptional activation and the cell cycle. Thus Phb may have a protective role. We need more studies to understand its interaction with other proteins, which will allow us to understand its role in FCI.

Disclosures: R. Gómez Rivera: None. **O. Pulido Maza:** None. **N. Serrano:** None. **A. Sánchez García:** None. **F. Cázares-Raga:** None. **J. Nader:** None. **F. Hernández Hernández:** None. **A. Ortiz-Plata:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.10/C117

Topic: C.08. Ischemia

Title: Changes in the histoarchitecture of the sensorimotor cortex in white rats' brain during prolonged incomplete ischemia and severe head injury: a comparative study

Authors: *V. AKULININ[1], S. STEPANOV[1], A. SHORONOVA[1], L. MAKAREVA[1], M. KORZHUK[2], D. AVDEEV[1];
[1]Omsk State Med. Univ., Omsk, Russian Federation; [2]NMRC of Oncology nam. N.N.Petrov, Saint Petersburg, Russian Federation

Abstract: Head injury and prolonged brain ischemia are the two most common causes of encephalopathy. Comparative study of structural and functional changes in the cells of cerebral cortex helps to elucidate mechanisms and patterns of the neural networks reorganization under different types of stress. Severe Head Brain injury (SHI) was modeled in Wistar rats using falling weights. Prolonged incomplete ischemia was imitated by ligation of the common carotid arteries (LCCA). Neurons and glial cells of the sensorimotor cortex (SMC) were studied using light microscopy (hematoxylin-eosin and thionine staining, immunohistochemical reactions for NSE, MAP-2, GFAP, IBA1) at 1, 3, 7, 14, and 30 days post injury. Total numerical density of neurons (TND), numerical densities of normochromic, hyperchromic n-shrinking, and shrinking (NDN, NDHN, NDSN) neurons, hypochromic (NDHN) neurons and shadow cells (NDSC), astrocytes and microglia cells per 1 mm^2 field of view were determined, and the neuroglial index (NGI) was calculated. Statistical hypotheses were tested using non-parametric methods in R environment. After LCCA, compared to CHI, statistically significant differences were observed in the indicators of NDN and NGI. Lower values were found for NDHN, NDSN, NDHN, NDSC, NDA, and NDM. The most pronounced differences for neurons were observed in the acute period (1 and 3 days post-injury). For example, differences in NDN after 1 day were 26.3%, NDSN - 38.3%, NDHN - 42.9%, NDSC - 47.1%. The maximum differences for NDHN were found at 14 and 30 days - 38.1 and 44.4% respectively. NDA showed the maximum difference between groups at 1 and 3 days - 26.6 and 29.3%, while NDM - at 14 (23.1%) and 30 days (23.36%). The comparison revealed morphometric differences indicating the presence of specific implementation of reactive mechanisms, damage, and recovery throughout the observation period. As a result, post-ischemic and post-traumatic reorganization of the neuronal network of the SMC in white rats occurred with different levels of functioning pyramidal neurons. Thirty days after LCCA, 23.2% of neurons in layer V were disrupted, while after CHI - 18.5%. At the end of the experiment, prolonged chronic ischemia due to LCCA led to a greater deficit of structural-functional elements in the pyramidal layer of the cortex. Following the injury, active processes of restoration of damaged neurons with significant involvement of glial cells were observed.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR215.11/C118

Topic: C.08. Ischemia

Title: Lipocalin-2 modulates neuronal cell functions through altering inflammatory, migratory, and angiogenic pathways following ischemic injury

Authors: *U. V. WESLEY[1], D. E. NELSON[2], R. J. DEMPSEY[3];
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Abstract: Background: Neuronal cell migration and survival critically impact the post stroke brain angiogenesis, tissue remodeling/repair, and recovery of neurological functions. However, burst of inflammatory stimuli disrupt these neuronal cell functions following cerebral ischemia and reperfusion (I/R) injury. Lipocalin-2 (LCN-2) regulates inflammatory responses. In humans, serum levels of LCN-2 progressively increase following cerebral ischemia. We examined the role of LCN-2 in modulating biological functions such as survival and migration of neuronal cells under ischemic conditions. We further examined the specific molecular pathways affected by LCN-2. **Methods:** Neuronal cells SH-SY5Y were exposed to *in vitro* ischemia by oxygen glucose deprivation. The specific effects of recombinant LCN-2 were examined. Live/dead cell viability assay was performed to evaluate neuronal cell survival. Tetra-methyl-rhodamine ethyl ester (TMRE) staining was used to examine mitochondrial potential. The migratory potential was assessed by Boyden chamber assay. Inflammatory, angiogenic, and migratory pathway specific transcriptomic profiles were identified using PCR mRNA profiler arrays, and selected genes were validated by real-time PCR. Immunofluorescence staining was carried out to examine protein expression. **Results:** Exposure of neuronal cells to *in vitro* ischemia/reperfusion (I/R) injury resulted in significant upregulation of LCN-2 levels. This supports our previous observation that I/R in rat brain significantly increases LCN-2, particularly in neuronal cells. Both exogenous administration of LCN-2, as well as increased LCN-2 associated with I/R lead to decreased neuronal cell viability and migratory potential. LCN-2 also enhanced mitochondrial dysfunction. These effects were associated with an increase in inflammatory nuclear factor kappa B (*NF-κB*) pathway genes, and a decrease in angiogenic factors, EDN1, FLT1, NRP1, pro-survival Akt1, and pro-migratory Diaph1 mRNA levels. **Conclusion:** Increased LCN-2 promotes mitochondrial dysfunction and apoptosis of neuronal cells, and inhibits their migratory potential likely through modulating inflammatory, migratory, and angiogenic pathway genes.

Disclosures: U.V. Wesley: None. **D.E. Nelson:** None. **R.J. Dempsey:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Topic: C.08. Ischemia

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Title: The role of trpm7 in the immunological manifestations in ischemic stroke

Authors: K. BREWER[1], Z.-G. XIONG[2], *T. LENG[1];
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Abstract: An ischemic stroke elicits many pathophysiological changes in the brain, leading to sustained injury and delayed recovery. Substantial amounts of research have been conducted on the mechanisms of neuronal injury and neuroprotection; however, clinical trials have not yielded promising results. Excessive inflammation has been suggested to cause worse stroke outcomes. However, much remains unknown on the immunological responses of the brain and their regulations during a stroke. The role of transient receptor potential melastatin 7 (TRPM7), a ubiquitous non-selective cation channel, in oxidative stress-induced neuronal injury and metastasis of cancer cells has been investigated. In addition, activation of TRPM7 has been implicated in the proinflammatory activity of neutrophils. However, the detailed function of TRPM7 in regulating brain innate immunity and its involvement in ischemic brain injury are largely unknown. This study aimed to elucidate the role of TRPM7 in regulating brain microglia pro-inflammatory activity during an ischemic stroke, and its impact on ischemic brain injury. An in-vivo model of middle cerebral artery occlusion (MCAO) in mice was used to examine the effect of TRPM7 inhibition on short-term stroke injury and recovery. Motor activity before and after MCAO was evaluated by Open field tests, and infarct volume was quantified using TTC staining 24h after MCAO. Mice that received the TRPM7 inhibitor performed better on the open field test and the infarct size was smaller than mice that received the vehicle. In-vitro analysis showed that inhibition of TRPM7 reduced LPS-stimulated microglia proinflammatory functionality. Further investigation is ongoing to explore the molecular mechanisms underlying the regulation of microglia function by TRPM7 and its impact on ischemic brain injury.

Disclosures: K. Brewer: None. **Z. Xiong:** None. **T. Leng:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

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Japan Brain Foundation

Title: Temporal profile of Ezh2 in gerbil dentate gyrus in acute phase of forebrain ischemia

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Abstract: Introduction: It was established that neurogenesis occurs even in the adult brain of two limited regions, including the subventricular zone of lateral ventricle and the subgranular zone of dentate gyrus (DG) in hippocampus. Neurogenesis increases after brain insults, such as ischemia, trauma, epilepsy etc. Although this phenomenon is interesting, the regulatory system and the role of neurogenesis have not been elucidated. **Purpose:**This study focuses on the regulatory role of polycomb group complex (PcG) on DG neurogenesis after transient ischemia, which is known to regulate gene silencing through histone methylation. At this moment, we show the temporal profile of PcG proteins and the number of newborn neurons in the acute phase of ischemia. **Methods:** Six-week-old male gerbils (body weight: 48-54 g) were objected to transient bilateral carotid arteries occlusion for 5 min. For SDS-PAGE analysis, animals were decapitated 1, 2, 3, 4, and 5 days after ischemia (n = 4, each). For histological analysis of newborn cells, animals were decapitated 1, 2, 3, 4, 5, 6, and 7 days after ischemia (n = 3, each). Coronal sections of brains were made and were stained with anti-Ki67 antibody to determine the number of proliferative cells in the DG. ANOVA followed by Tukey. Data represent mean ± SD. **Results:**In the DG, the number of Ki67-positive cells gradually increased until 7 days after ischemia (sham: 9.7±3.1; 1 d: 9.2±0.7; 2 d: 11.3 ±3.5; 3 d: 16.4±4.1; 4 d: 24.7±4.6; 5 d: 27.9±5.0; 6 d: 50.0± 2.7; 7 d: 53.3±7.5). At 4 or more days after ischemia, the significant difference of Ki67-positive cells between sham and ischemia groups were found ($p < 0.05$ in sham vs 4 d; $p < 0.01$ in sham vs 5 to 7 d). On the other hand, SDS-PAGE analysis revealed that Ezh2, one of the components of PcG, significantly increased at 3 days after ischemia, and decreased at 4 and 5 days after ischemia (Ezh2/actin: 1.33±0.10 in sham, 1.25±0.02 in 1 d, 1.80±0.49 in 2 d, 3.11±0.69 in 3 d, 2.27±0.69 in 4 d, 2.33±0.14 in 5 d; $p < 0.001$ in sham vs 3 d; $p < 0.05$ in sham vs 4 d and 5 d). **Conclusions:**Up to now, we obtained data showing that neurogenesis in DG increased after transient ischemia 4 days after ischemia and even more in later time span, and that Ezh2 significantly increased before the increment of neurogenesis with its peak at 3 days after ischemia. We'll investigate the relationship between Ezh2 and neurogenesis in DG after forebrain ischemia in the future studies.

Disclosures: Y. Sehara: None. **Y. Hashimotodani:** None. **K. Shimazaki:** None. **K. Kawai:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.14/C121

Topic: C.08. Ischemia

Support: National Institute of Health (RO1 NS130763 and R35 NS132184)
U.S. Department of Veterans Affairs (I01 BX005127 and I01 BX006062)

Title: N^6-methyladenosine (m^6A) reader YTHDF1 regulates sex-specific immune responses after stroke

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Abstract: N^6-methyladenosine (m^6A) is a neuronal-enriched, reversible post-transcriptional modification that regulates RNA metabolism. The m^6A-modified RNAs recruit various m^6A-binding proteins that act as readers. Differential m^6A methylation patterns are implicated in ischemic brain damage, yet the precise role of m^6A readers in propagating post-stroke m^6A signaling remains unclear. We presently evaluated the functional significance of the brain-enriched m^6A reader YTHDF1, in post-stroke pathophysiology. Focal cerebral ischemia significantly increased YTHDF1 mRNA and protein expression in adult mice of both sexes. $YTHDF1^{-/-}$ male, but not female, mice subjected to transient middle cerebral artery occlusion (MCAO) showed worsened motor function recovery and increased infarction compared to sex-matched $YTHDF1^{+/+}$ mice. $YTHDF1^{-/-}$ male, but not female, mice subjected to transient MCAO also showed significantly perturbed expression of genes related to inflammation, and increased infiltration of peripheral immune cells into the peri-infarct cortex, compared with sex-matched $YTHDF1^{+/+}$ mice. Thus, this study demonstrates a sexual dimorphism of YTHDF1 and thus m^6A methylation in regulating post-ischemic inflammation and pathophysiology.

Disclosures: V. Arruri: None. **A. Chokkalla:** None. **S.L. Mehta:** None. **R. Vemuganti:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR215.15/C122

Topic: C.08. Ischemia

Support: NSTC grant, 112-2320-B-A49 -008 -MY3
NSTC grant, 112-2320-B-A49 -048 -

Title: Investigating the involvement of protein kinase in direct current stimulation-induced synaptic plasticity

Authors: *H.-C. LIN[1], C.-W. LEE[2], H. CHI[3], C.-Y. WU[2], W.-J. LEE[2], T. CHUNG[2], J. LI[2];
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Abstract: Transcranial direct current stimulation (tDCS) is an emergent non-invasive neuromodulation technique which applied on several psychiatric disorders. Previous studies have indicated that the direction of tDCS can produce different effects. The anodal stimulation exhibits an excitatory or long-term potentiation (LTP) effect, while the cathodal stimulation exhibits an inhibitory or long-term depression (LTD) effect. The intracellular Ca2+ concentration has been considered a key factor in tDCS-modulated synaptic plasticity. Moreover, the PKC family, which is sensitive to Ca2, represents an important role in LTP. The protein kinase M zeta (PKMζ), an autonomously active atypical protein kinase C zeta (PKCζ), is involved in Ca2+ dependent protease active during LTP. The PKMζ also necessary and sufficient for the maintenance of LTP thought prevents endocytosis of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor subunit GluA2. However, whether PKMζ is involved in tDCS modulating synaptic plasticity still unclear. Hence, we applied the anodal DCS in brain slice to investigate the role of PKMζ in DCS modulated-synaptic plasticity. The results indicated that the synaptic response was potentiated by anodal DCS application. The expression of PKMζ was increased; whereas the expression of PKCζ was not change after anodal DCS application. The synaptic potentiation, which induced by anodal DCS, was blocked by zeta inhibitory peptide. Lastly, GluA2 was involved in anodal DCS modulated-synaptic response. Taken together, the potentiated synaptic response by anodal DCS was via PKMζ-related mechanism.

Disclosures: H. Lin: None. **C. Lee:** None. **H. Chi:** None. **C. Wu:** None. **W. Lee:** None. **T. chung:** None. **J. Li:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.16/C123

Topic: C.08. Ischemia

Support: National Research Foundation of Korea (NRF-2021R1I1A1A01046548)
National Research Foundation of Korea (NRF-RS-2023-00278593)
The Catholic Medical Center Research Foundation program year of 2022

Title: Ultrastructural analysis of scar-forming PDGFR-β^+ fibroblasts and infiltrating macrophages after photothrombotic stroke

Authors: *J.-W. HWANG[1,2], D.-G. KIM[1,2], H.-L. KIM[3], Y. OH[3], A. CHO[1], M.-Y. LEE[1,2], T.-R. T. RIEW[1,2];
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Abstract: The morphological characteristics of fibroblasts in fibrotic scar formation after CNS injury remain elusive. We previously identified PDGFR-β as a common marker for leptomeningeal and perivascular fibroblasts in healthy rats, with leptomeningeal fibroblasts being activated and perivascular fibroblasts in a resting state, based on their molecular and ultrastructural characteristics. In this study, we induced focal cortical injury by photothrombotic stroke and investigated the spatiotemporal dynamics of PDGFR-β^+ leptomeningeal and perivascular fibroblasts. Multi-label immunohistochemistry revealed that perivascular as well as leptomeningeal fibroblasts co-expressed intermediate filament protein, extracellular matrix, and proliferation markers in the acute and chronic phases, implying their activation states. Immuno-electron microscopy showed that both fibroblasts had euchromatic nuclei with dilated endoplasmic reticulum, corroborating immunohistochemical findings. At 14 days post-stroke, fibroblast processes elongated to form a web-like network in the extravascular space, intermingling and closely contacting macrophages. 3D electron microscopy reconstruction of PDGFR-β^+ fibroblasts showed sheet-like extensions and highly-branched patterns. Notably, the cytoplasm at branching points exhibited dilated endoplasmic reticulum and close contact with macrophages. In addition, lysosome-like electron-dense organelles were observed in fibroblasts 14 days post-injury, confirmed by correlative light and electron microscopy. Finally, quantitative analysis revealed a loss of activation-related characteristics in late fibroblasts compared to early fibroblasts. Collectively, our data provide new insights into key cellular players in the tissue repair response by spatiotemporally investigating the dynamic morphological characteristics of post-stroke fibroblasts.

Disclosures: J. Hwang: None. **D. Kim:** None. **H. Kim:** None. **Y. Oh:** None. **A. Cho:** None. **M. Lee:** None. **T.T. Riew:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.17/C124

Topic: C.08. Ischemia

Support: CIHR

Title: Identifying underlying functional pathways and regulatory networks of peripheral recanalization responses in a murine model of ischemic stroke

Authors: *T. BUI[1,2], Y. MA[2], S. ABBASI-HABASHI[1,3], P. KENT[2], G. C. JICKLING[4,3], I. R. WINSHIP[3,2];
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Abstract: Ischemic stroke is one of the leading causes of death worldwide that leaves substantial long-term disabilities. Age and sex have interactive effects on ischemic pathophysiology as the

risks and mortality increase with age and older women are more affected by stroke than their male counterparts. Current treatments for ischemic stroke focus on recanalization via mechanical thrombectomy and rt-PA delivery are limited, as even when recanalization is successful, many patients experience poor clinical outcomes. This is defined as futile recanalization, in which patients have impaired cognitive and motor functions post-treatment. In vivo animal studies support the role of neutrophil activation and stalls in the microvascular as a potential contributor to futile recanalization. However, patterns of gene expression associated with increased neutrophil stalls are not known. Using the murine middle cerebral occlusion (MCAO) model that mimics a vessel occlusion with recanalization, we performed a comprehensive microarray analysis of blood samples post-MCAO and recanalization in male, female, young adult, and aged mice. Blood samples collected from sham, 1-hour MCAO, and MCAO with 3-hour recovery post-recanalization (N=44), were examined. Total RNA was extracted from blood samples and analyzed using Affymetrix Clariom™ S Assay. Downstream bioinformatics analysis was then performed in RStudio and Bioconductor. We identified 19 new genetic markers of recanalization injury. Our analyses revealed neutrophils and neutrophil signaling pathways to be the main differential driver of transcriptional changes in female and, to a lesser degree, aged animals. Using GeneOntology, miTarBase, and CHEA3, we constructed an interactive neutrophil signalling network between differentially expressed genes, their regulatory miRNAs, and transcription factors. We demonstrated in this network that after recanalization, female and aged animals have distinct miRNAs, transcription factors, and expressed genes that collectively contribute to an upregulation of neutrophil activities in peripheral blood. Our results suggest that early signs of neutrophil stalls (possibly futile recanalization) can be observed in peripheral blood as early as 3-hour post-recanalization.

Disclosures: T. Bui: None. **Y. Ma:** None. **S. Abbasi-Habashi:** None. **P. Kent:** None. **G.C. Jickling:** None. **I.R. Winship:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.18/C125

Topic: C.08. Ischemia

Support: National Research Foundation (NRF) (2021R1A2C2010920)

Title: Hmgb1 contribute to netosis induction and thrombus formation in the photothrombosis stroke model

Authors: *J.-K. LEE[1], S.-A. OH[2], S. SEOL[3];
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Abstract: In various brain diseases, neutrophils infiltrate damaged tissue soon after injury, worsening inflammation. In the permanent MCAO model, neutrophil extracellular traps (NETs) were detected both in brain tissue and within blood vessels, playing a critical role in aggravating damage by vascular injury. Danger-associated molecular pattern (DAMP) molecules have been reported to induce NETosis following cerebral ischemia. This study investigated the role of High mobility group box 1 (HMGB1), a well-known DAMP molecule, in NETosis induction,particularly in neutrophil-platelets interactions. We employed the photothrombotic stroke (PTS) model, where thrombi consists primarily of aggregated platelets and neutrophils, lacking fibrin. TTC staining revealed rapid and progressive infarct formation in the PTS model. Notably, progressive NETosis, especially intravascular NETosis, was prominent, as evidenced by increased levels of citrullinated histone $H3^{+}$ (CitH3, a marker of NETosis) and NE-DNA complex in neutrophils isolated after PTS (demonstrated by immunoblotting and immunofluorescence staining). Functional inhibition of HMGB1 by intranasal administration of a MGB1 A box significantly reduced infarct volume and improved neurological deficits in the PTS model. Importantly, neuroprotection was achieved by both pre-administration and administration 2 h after stroke, suggesting that suppression of intravascular NETosis is crucial for mitigating ischemic brain damage in this model. Platelet activation in PTS model was confirmed by increased expression of CD41 and GPIbβ (platelet activation markers) and surface HMGB1. These activated platelets can induce neutrophil activation and intravascular NETosis, with platelet-derived HMGB1 plays a key role. Notably, TLR4 and RAGE signaling pathways appear to be involved in platelet-derived HMGB1-mediated neutrophil activation. Our findings demonstrate that HMGB1 contribute to NETosis induction not only during initial thrombus formation but also in subsequent damage progression in the PTS animal model. Therefore, NETosis may serve as a valuable prognostic maker in acute ischemic stroke. Targeting NETosis by modulating HMGB1 offers a promising multipotent therapeutic strategy to mitigate ischemic brain damage.

Disclosures: J. Lee: None. **S. Oh:** None. **S. Seol:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.19/C126

Topic: C.08. Ischemia

Support: Oklahoma Center for the Advancement of Science and Technology to QG
Alzheimer's Association to QG
National Institutes of Health to QG
Department of Biochemistry and Physiology, OUHSC to QG and AB

Title: An unconventional extrinsic cytoprotective mechanism with novel therapeutic applications in ischemic brain injury and stroke

Authors: J. XIE[1], ***J. BELOUSOVA**[2], A. BELOUSOV[2], Q. GUO[2];
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Abstract: Apoptosis antagonizing transcription factor (AATF) is a novel and highly potent neuroprotective agent. AATF lacks a classical N-terminal signal peptide and is normally found in intracellular compartments. However, we noted that a significant amount of AATF was secreted extracellularly by cortical neurons following exposure to hypoxic insults, suggesting the existence of an unconventional extrinsic pathway of neuroprotection mediated by secreted AATF (sAATF). In neurons, receptor-interacting protein kinase 3 (RIPK3) recruits and phosphorylates the executioner mixed lineage kinase domain-like protein (MLKL) to signal necroptosis. We found that sAATF binds to cell surface TLR-4 (Toll-like Receptor-4) and blocks RIPK3/MLKL-dependent necroptosis in models of ischemic/reperfusion (I/R), suggesting that sAATF is an endogenous ligand and blocker of TLR4-4-mediated pro-inflammation and pro-cell death signaling. The extracellular release of AATF is negatively regulated by an intracellular process involving binding of AATF by Par-4 (prostate apoptosis response- 4), a 38 kDa leucine zipper protein that promotes neuronal cell death. In neurons from both Par-4 overexpressing transgenic mice and Par-4 knockout (Par-$4^{-/-}$) mice, we found that Par-4 binds to AATF intracellularly via the leucine zipper domain, and AATF/Par-4 complex formation blocks the extracellular secretion of AATF. A region corresponding to the amino acid sequence between AATF179 and AATF279 was found to be responsible for interacting with TLR-4. Surprisingly, a small core peptide identified from this region of AATF, termed as SAP-12 (secreted AATF peptide of 12 amino acids), confers a much greater neuroprotective potency and broader effective dose range than the full length sAATF. Recently, the potent cytoprotective effects of sAATF/SAP-12 were also observed in C166 vascular endothelial cells following exposure to hypoxia. These studies set the groundwork for producing novel therapeutics for neuronal and vascular endothelial cell deaths in ischemic brain injury, including the optimization of the pharmacokinetic properties and target specificity of SAP-12 through amino acid modification and incorporation, as well as conjugation of moieties that improve efficacy and stability.

Disclosures: J. Xie: None. **J. Belousova:** None. **A. Belousov:** None. **Q. Guo:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR215.20/C127

Topic: C.08. Ischemia

Support: MIBTP2020: BB/T00746X/1
Medical and Life Sciences Research Fund

Title: Measuring purine salvage for hypoxic-ischemic brain tissue metabolic support using a newly developed liquid chromatography-mass spectrometry method.

Authors: *P. M. MICHOR, B. G. FRENGUELLI, Y. TIMOFEEVA, J. BOLTZE, A. BOTTRILL;
Univ. of Warwick, Coventry, United Kingdom

Abstract: The Purine Salvage Pathway (PSP) is a critical pathway for the synthesis of ATP and other adenine nucleotides in the mammalian brain. It utilizes ATP metabolites to regenerate AMP, ADP, and ATP levels. However, cerebral ischemia causes rapid depletion of cellular ATP, and ATP metabolites are lost from the brain into circulation, hindering PSP function. Supplementation of PSP with ribose and adenine (RibAde) could lead to increased ATP production and better recovery after stroke. However, the exact mechanism of action of this treatment and its effect on the pathway is not known. We developed a novel liquid chromatography - tandem mass spectrometry (LC-MS/MS) method utilizing hydrophilic interaction liquid chromatography with an aqueous buffer containing 20mM ammonium acetate, 45.7mM ammonium hydroxide and 5% acetonitrile, and acetonitrile as an organic buffer. This was combined with a triple quadrupole mass spectrometry with electrospray ionization and selected reaction monitoring. An internal standard chlorphenylalanine was used to correct for sample preparation variations and to compensate for instrument fluctuations. This LC-MS method is able of separating, detecting and quantifying ATP, ADP, AMP, IMP, adenosine, inosine, adenine, hypoxanthine, and xanthine, with good sensitivity, linearity, precision, and accuracy. We also optimized a compatible metabolite extraction method from brain samples using acetonitrile, methanol, and formic acid in water solution, allowing us to track the metabolic states of the brain slices. We then carried out a time course incubation experiment to compare the effects of RibAde on ATP and PSP metabolites ex vivo. While brain slices incubated in artificial cerebrospinal fluid with RibAde did not show a significant increase in ATP levels, the overall sum of ATP and its metabolites was significantly increased in RibAde, as compared to the control group [two-way ANOVA $F(5, 90) = 4.747$, $p = 0.0007$], for all incubation times between 1h and 5h [two-way ANOVA $F(1, 90) = 22.40$, $p < 0.0001$], suggesting a higher turnaround of molecules in the RibAde group. Furthermore, this method is able of distinguishing between the endogenous metabolites, and the metabolites arising from the exogenously added, stable isotope-labeled RibAde. We used this approach to show that stable isotope-labeled RibAde gets directly incorporated into ATP molecules. This newly developed LC-MS method is capable of separating and quantifying ATP and other molecules involved in the PSP and can be used to analyze brain metabolite extracts. Ribose and adenine combine in tissue to produce ATP and could be used to metabolically protect the penumbra in ischemic stroke.

Disclosures: P.M. Michor: None. **B.G. Frenguelli:** None. **Y. Timofeeva:** None. **J. Boltze:** None. **A. Bottrill:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR215.21/C128

Topic: C.08. Ischemia

Support: NIH Grant R01MH086638

Title: Human capillary identification used to simulate spreading depression in control and ischemic neocortical microcircuits

Authors: *A. J. H. NEWTON[1], C. KELLEY[2], Y.-C. WANG[3], S. ZINK[4], M. M. DISTASIO[5], R. A. MCDOUGAL[6,7,8], W. W. LYTTON[1,9,10];
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Abstract: Spreading depolarization (SD) is a wave of neuronal depolarization that propagates through gray matter at 2-7mm/min: a brief period of hyperexcitability at the wavefront is followed by spreading depression, neuronal silence from depolarization block; as well as by disruption of ion homeostasis. Blood vessels play a key role in SD as the source of the energy needed for ion pumps. Multiple mechanisms underlie SD initiation and propagation which we explored using simulation.

NEURON/RxD/NetPyNE were used: point neurons with Hodgkin-Huxley style ion channels augmented with homeostatic mechanisms, including Na^+/K^+-ATPase, NKCC1, KCC2, and dynamic volume changes. We simulated the intracellular and extracellular concentrations of Na^+, K^+, Cl^-, and O_2. The contribution of astrocytes was modeled as the O2-dependent clearance of K^+. The evolutional-optimization Python framework Optuna was used to find parameters in simulating 13,000 neurons representing ~1 mm^3 of mouse cortex (layers 2-6). Connectivity was based on prior cortical modeling after scaling excitatory and inhibitory synaptic weights and the distribution of external drive to account for differences between conductance-based neuronal models used here and the original Integrate-and-fire models.

Oxygen sources in the model were determined using histologic images showing capillary locations from human tissue: a 2.0 x 2.3 cm cross-section of the V1 cortex was immunostained for CD34. This identified 918 capillaries with mean density of 199.6/cm^2 and capillary cross-sectional area of 16.7±11.9μm^2. For simulation, we used a biased random walk to expand the 2D cross-section of capillaries to a 3-dimensional distribution of oxygen sources. SD was reliably triggered in this model by a bolus of extracellular K^+ applied to cortical layer 4. Our model showed neurons closer to capillaries were more able to maintain homeostasis and physiological firing. We also found that neuronal depolarization occurred in all cortical layers, with pathological activity spreading both through extracellular K^+ diffusion and through network connectivity.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

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Program #/Poster #: PSTR215.22/C129

Topic: C.08. Ischemia

Support: JSPS KAKENHI Grant 17H02117
JSPS KAKENHI Grant 23H03241

Title: Expression of angiotensin converting enzyme-2 (ACE2) modified by ischemic stroke and neurorehabilitation

Authors: *H. MAEJIMA[1], T. INOUE[3,2], M. OKAMURA[2], Y. TAKAMATSU[4,1], Y. OKA[1]; [1]Dept. of Rehabil. Science, Fac. of Hlth. Sci., [2]Grad. Sch. of Hlth. Sci., Hokkaido Univ., Sapporo, Japan; [3]Brain Res. Inst., Niigata Univ., Niigata, Japan; [4]Chubu Univ., Kasugai / Aich, Japan

Abstract: Angiotensin-converting enzyme 1 (ACE1) converts angiotensin I (Ang I) into Ang II which associates with AT1 receptor and induces vasoconstriction, inflammation and cellular apoptosis. Meanwhile, angiotensin-converting enzyme 2 (ACE2) has an enzymatic role to convert angiotensin II into angiotensin-(1-7), Ang (1-7), or convert Ang I into Ang (1-9) which also yields Ang (1-7). Ang (1-7) binds to AT2 and Mas receptors, inducing vasodilation, anti-inflammation, and tissue survival. Therefore, ACE2 has been suggested to beneficially contribute to the neuronal protection after stroke. Meanwhile, ACE2 is well known as a receptor of SARS-CoV-2 in COVID-19 infection, suggesting that ACE2 expression could be double-edged sword. In our previous study, we showed that therapeutic exercise combined with low-level inhibition of $GABA_A$ receptor potentiated the recovery after ischemic stroke. Based on the finding of the previous study, we here elucidated the effect of stroke and combined neurorehabilitation after stroke on ACE2 expression in the brain regions including motor cortex and hippocampus. Male Sprague-Dawley rats were divided into 5 groups: sham (SHAM), control (CON), exercise (EX), bicuculline (BIC), and bicuculline plus exercise (BICEX) groups. All rats, except those in the SHAM group, underwent middle cerebral artery occlusion (MCAO) surgery to induce an ischemic stroke. $GABA_A$ receptor antagonist, bicuculline (0.25 mg/kg, i.p.), was administered to the BIC and BICEX groups every day. The EX and BICEX groups exercised on a treadmill (11 m/min for 30 min) every day. Following the daily intervention for two weeks, ipsilateral motor cortex and hippocampus were excised and used for quantitative PCR to measure mRNA expression of ACE2. In the motor cortex, ACE2 expression was significantly enhanced by MCAO. In addition, a two-way ANOVA for post-MCAO intervention showed a significant main effect of exercise, no significant effect of bicuculline administration and no significant two-factor interaction, indicating that exercise decreased ACE2 expression enhanced after MCAO. Thus, ACE2 expression was enhanced after ischemic stroke and the increase was inhibited by exercise. Meanwhile, there was no significant modification of ACE2 expression in the hippocampus. This study showed region-specific modifications of ACE2 expression in the brain

after ischemic stroke, as if the increased ACE2 potentiated neuronal protection after stroke. it was suggested that the decrease of ACE2 expression caused by exercise could be attributed to a kind of compensation for the neuronal protection by exercise effect.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.23/C130

Topic: C.08. Ischemia

Title: Excitotoxic neuronal injury requires superoxide entry via Swell1-containing volume-regulated anion channels

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Abstract: Excitotoxicity is the most immediate cause of neuronal death in cerebral ischemia, and likewise contributes to neuronal death in several other disease states. The excitotoxic cell death pathway involves production of nitric oxide (NO) and superoxide (O_2^-), which combine to form the highly reactive peroxynitrite radical ($ONOO^{-\cdot}$). NO, being non-polar, easily crosses neuronal cell membranes, but how the polar superoxide anion enters neurons has not been established. Here we show instead that O_2^- enters neurons via volume-regulated organic anion channels (VRACs), and that blocking VRACs presents excitotoxic oxidative stress and neuronal death. In primary neuronal cultures, oxidative stress induced either by NMDA receptor stimulation or exposure to authentic superoxide was blocked by pharmacological inhibition of LRRC8A (i.e. Swell1), an obligatory subunit of volume-sensitive organic anion channels (VRACs). This oxidative stress was also attenuated by superoxide dismutase but not by the catalase, which degrades hydrogen peroxide, or L-NAME, which blocks NO production. VRAC inhibition also prevented NMDA-induced neuronal death in cell cultures. *In vivo*, the genetic knockdown of neuronal LRCC8A using LRRC8A-floxed mice attenuated neuronal oxidative stress induced by either NMDA injection or transient ischemia. VRACs comprise of an essential “A” subunit and one or more of 3 other subunits (B-D). We show in Hela cells that expression of the C subunit is necessary to permit superoxide entry, suggesting that VRAC channels containing specifically A and C subunits mediate superoxide entry. These findings identify a role for volume-regulated organic anion channels in neuronal oxidative signaling and stress, and support increasing evidence suggesting that VRAC channels have subunit specific properties.

Disclosures: K. Harris: None. **S. Won:** None. **N. Mai:** None. **D. Ogut:** None. **R.A. Swanson:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.24/C131

Topic: C.09. Stroke

Support: RFAG042189
NIH Fellowship 1F30NS131053

Title: Pre-treatment with Tamoxifen, a selective estrogen receptor modulator, reduces microglial mitochondrial stress under oxygen glucose deprivation

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Abstract: Introduction: Tamoxifen (TAM), a selective estrogen receptor modulator, is a first-line treatment for hormone receptor-positive breast cancer. Women who were treated with tamoxifen had an increased risk for ischemic stroke (82%). Since stroke is associated with increased neuroinflammation, we investigated the effects of TAM on microglia profiles and mitochondrial function. **Methods:** Primary microglia were isolated for RNA-seq from 5-7 mo. Sprague Dawley rats, were treated via subcutaneous pellet for 5 weeks with TAM, estradiol, or vehicle. Cultured HMC3 microglia were treated with TAM (1uM), 17ß-estradiol (E2, 10nM), or vehicle (DMSO) for 24 hours. At 18 hours, cells were separated into one of two culture conditions: either normoxia (21% O2, 25mM glucose) or oxygen and glucose deprivation (OGD) for 6 hours. At 24 hours post-treatment, mitochondrial function was assessed by Seahorse XFe96 Analyzer. **Results:** Hypothesis-generating RNA-seq showed TAM downregulated (unadjusted p=0.02, fc=0.62) and estradiol upregulated (unadjusted p=0.03, fc=1.71) *Slc25a2,* a mitochondrial efflux transporter of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase which is associated with increased reactive oxygen species. Following RNA-seq data, Mito Stress Test showed that TAM and E2 reduced basal respiration under normoxia, but only TAM reduced basal respiration (p =0.0149) under OGD conditions. **Conclusions:** Nitric oxide plays an important role in neuroinflammation and regulating mitochondrial dynamics. Our data suggest TAM may indirectly reduce NO levels in microglia via reduced efflux of NO synthase inhibitor. Our findings suggest that although TAM may increase risk of ischemic stroke, it may have a protective effect via reduced neuroinflammation and reduced mitochondrial stress.

Disclosures: M.E. Zardeneta: None. **F. Sohrabji:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.25/C132

Topic: C.09. Stroke

Support: Jax scholar 2019

Title: Genetically diverse cerebral organoids unveil potential therapeutic targets for stroke

Authors: *D. CORTES[1], K. CHARLAND[2], M. PERA[1];
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Abstract: Stroke is one of the leading causes of death. Although stroke is a multifactorial disease, the genetic differences between patients contribute significantly to variation in risk, survival, and recovery. Furthermore, the genetic and mechanistic differences that distinguish between susceptibility (death or severe sequelae) and resilience (recovery) in stroke patients are not well understood and this limits our ability to identify targets that can be translated into new therapeutics. Thus, there is a critical need for an efficient approach to elucidate the genetic mechanisms that drive resilience to stroke so that novel therapeutic targets can be identified and exploited. Here, we use a stem cell (SC)-based approach to rapidly analyze phenotypical differences among genetically diverse populations. Previously we developed a protocol to differentiate neurons and cerebral organoids from genetically diverse mouse and human SCs, making readouts between mouse and human more comparable. For this study, we developed an *in vitro* stroke-like paradigm which we used to test for phenotypical differences among genetically diverse neurons using SCs from the founders of the collaborative cross mice, and human SCs from the iPSCORE collection. After measuring oxidative stress, DNA fragmentation, LDH release, neuronal survival, mitochondrial, electrophysiological, and caspase activity; we found a varied response to the insult associated with the genetic diversity. We conducted RNA-seq analysis and found that Mgarp and Ptn, genes important for cell survival and mitochondrial activity were differentially expressed between the most susceptible and resilient strains. Transcriptional regulatory network analysis revealed that differences in resilience are linked to *Vdr*, *Tfap2c*, and *Irf7* transcriptional factors (TFs).To validate the effect of these TFs using our *in vitro* stroke model, we incubated human cerebral organoids with commercially available molecules that activate these TFs. We found that combination of these molecules can increase the survival of neurons after the insult. After generating CRISPRa human SCs to activate the endogenous TFs, we were able to corroborate the pro-survival effects of these TFs in cerebral organoids.Single nuclei RNA-seq revealed susceptibility to the stroke model amongst the different neuronal types within the cerebral organoids based on genetic diversity.The present model demonstrates how complex genetic factors modify differential responses to stroke. The transcriptional adaptation associated with a favorable response to stroke can lead to much needed new therapeutics.

Disclosures: D. Cortes: None. **K. Charland:** None. **M. Pera:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.26/C133

Topic: C.08. Ischemia

Support: NIH Grant NS112805

Title: Acid sensing ion channels and mitochondrial function

Authors: C. FORD[1], L. BAUER[1], S. SANGHVI[1], L. VARGHESE[1], M. SIMAITIS[1], H. SINGH[2], *C. ASKWITH[3];
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Abstract: The acid-sensing ion channel 1a (ASIC1a) contributes to normal brain function and promotes neuronal death in mouse models of stroke, traumatic injury, and multiple sclerosis. The most established model of ASIC1a-dependent toxicity involves acidosis-dependent activation of ASIC1a on the plasma membrane and induction of signaling cascades that promote cell death. Investigators have also reported that ASIC1a can regulate mitochondrial function to affect cell viability. To investigate ASIC1a-mediated control of mitochondria, we tested the consequences of ASIC1a expression in cultured neurons, cell lines, and brain tissue. We found that ASIC1a impacts hydrogen peroxide-mediated cell death and cellular metabolic activity. In subcellular fractions containing mitochondria, we identified ASIC1a-dependent changes in mitochondrial susceptibility to calcium overload and production of reactive oxygen species. Preliminary data suggest that these effects are dependent on the N-terminal intracellular region of the channel. Together, these data indicate that ASIC1a can regulate mitochondrial activity and suggest ASIC1a controls neuron sensitivity to ischemic insults in a manner independent of extracellular acidosis.

Disclosures: C. Ford: None. **L. Bauer:** None. **S. Sanghvi:** None. **L. Varghese:** None. **M. Simaitis:** None. **H. Singh:** None. **C. Askwith:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR215.27/C134

Topic: C.09. Stroke

Support: NIH Grant 1R01NS117606-01
NSF Grant 1916894
UTHealth New Faculty Start-up

Title: Endothelial-specific modulation of mir-34a attenuates blood-brain barrier permeability and improves outcomes after ischemic stroke in mice

Authors: *X. REN[1], H. HU[2], S. TABASSUM[3], S. WU[3], C.-H. LEE[4], J. LEE[5], C. PAYNE[6], A. GUSDON[6], H. CHOI[6], J. W. SIMPKINS[7];
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Abstract: The preservation of blood-brain barrier (BBB) integrity by cerebrovascular endothelial cells (CECs) is pivotal during ischemic episodes, wherein CEC damage can exacerbate stroke severity. MicroRNA-34a (miR-34a) has emerged as a significant modulator in this context, with its dysregulation contributing to heightened BBB permeability and worsened stroke outcomes. Our prior investigations demonstrated promising results in a global miR-34a knockout (KO) mouse model, prompting further exploration into the specific impact of miR-34a within endothelial cells on stroke pathology. By using Cre-lox technology by crossing miR-34a$^{fl/fl}$ floxed mutant mice and endothelial specific Cre (Tek-Cre) mice, we have produced endothelial-specific miR-34a knockout mice (miR-34a$^{fl/fl}$Cre^{+}). By using real-time PCR, we confirmed that miR-34a from isolated primary CECs were absent in miR-34a$^{fl/fl}$Cre^{+} mice and remained unchanged levels in brain tissue or microglia. We found specific deletion of miR-34a in endothelial cells did not alter cerebral blood flow or cerebrovascular anatomy. We subjected miR-34a$^{fl/fl}$ Cre^{+} mice and wild-type control mice to transient middle cerebral artery occlusion (tMCAO) model and evaluate outcomes after stroke. Interestingly, our findings delineate that miR-34a$^{fl/fl}$ Cre^{+} (KO) mice displayed reduced BBB permeability, ameliorated neurological deficits, and reduced infarct volume post-tMCAO, irrespective of sex, compared to WT counterparts. These findings highlight the therapeutic potential of endothelial-specific miR-34a manipulation in mitigating stroke severity. Furthermore, the study suggests a novel avenue for investigating miR-34a's involvement in endothelial dysfunction across various neurological disorders, paving the way for potential translational applications in stroke management and beyond.

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Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.28/C135

Topic: C.10. Brain Injury and Trauma

Support: National Research Foundation of Korea (NRF-2021R1I1A1A01046548)
National Research Foundation of Korea (NRF-RS-2023-00278593)
The Catholic Medical Center Research Foundation program year of 2022

Title: Morphological heterogeneity of CNS border-associated macrophages after photothrombotic stroke

Authors: *D.-G. KIM[1,2], J.-W. HWANG[1,2], A. CHO[1], M.-Y. LEE[1,2], T.-R. T. RIEW[1,2]; [1]Dept. of Anat., The Catholic Univ. of Korea, Seoul, Korea, Republic of; [2]Department of Biomedicine and Health Sciences, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of

Abstract: CNS border-associated macrophages (BAMs) constitute a small population of macrophages located in the meninges, choroid plexus, and perivascular spaces (PVS). Their unique anatomical position between the periphery and the brain parenchyma has sparked significant interest in exploring their roles in both normal CNS function and disease. In this study, we aimed to elucidate the anatomical and ultrastructural characteristics of BAMs in the context of ischemic stroke, which triggers rapid proliferation and recruitment of various myeloid cell types. At 7 days after the ischemia, the number of BAMs, identified by expression of CD206, Lyve1, and Iba1 increased compared to contralateral cortex. They were primarily distributed along the meninges and PVS within the lesion core, while their presence within the extravascular parenchyma was limited. Notably, a subset of BAMs did not express Lyve1, both in the meninges and PVS. The heterogeneity of BAMs based on their morphology and marker expression was revealed by triple-label immunohistochemistry using CD206, Lyve1, and platelet-derived growth factor receptor beta (PDGFR-β), a previously identified marker for leptomeningeal and perivascular fibroblasts. Meningeal BAMs intermingled within PDGFR-β(+) fibroblasts exhibited amoeboid morphology and expressed both CD206 and Lyve1. In contrast, BAMs located in the subpial space, characterized by a thin cell body and slender processes with partial contact with PDGFR-β (+) fibroblasts did not express Lyve1. BAMs in the PVS, surrounded by PDGFR-β(+) perivascular fibroblasts lacked Lyve1 immunoreactivity, and displayed elongated, stretched morphology with slender processes. BAMs exposed to the extravascular parenchyma exhibited variable morphologies and expressed both CD206 and Lyve1. These findings were further supported by the immuno-electron microscopy and correlative light- and electron- microscopy analyses.CD206(+) macrophages were found both abluminal and adluminal to the perivascular PDGFR-β(+) fibroblasts, whereas Lyve1(+) macrophages were only detected abluminal to the PDGFR-β(+) fibroblasts. Collectively, our findings suggest distinct morphological characteristics and spatial dynamics of BAMs in response to ischemic stroke.

Disclosures: D. Kim: None. **J. Hwang:** None. **A. Cho:** None. **M. Lee:** None. **T.T. Riew:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.29/C136

Topic: C.08. Ischemia

Support: NIH Grant NS121426
NSF GRFP

Title: Rapid purinergic contributions to modulation of dopaminergic dysregulation in severe ischemia

Authors: *M. WEESE-MYERS, K. CALDWELL, C. WITT, A. ROSS;
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Abstract: The CA1 region of the hippocampus is particularly susceptible to ischemic stroke due to its heavy innervation with glutamatergic and dopaminergic projections. Both neurotransmitters cause excitoxicity and oxidative stress during ischemia, leading to localized cell damage and propagation of spreading depolarization, causing global damage. Guanosine (GN) is a rapidly-signaling neuroprotective agent active in the CA1 during ischemia and is well-known for its role in dampening glutamate release and facilitating its reuptake. However, any rapid effects guanosine may exert on dopamine (DA) signaling in ischemia has gone uninvestigated. Previous studies have suggested that purinergic regulation of dopamine occurs through adenosine pathways. In this study, we examine the impacts of guanosine administration on dopamine signaling and accumulation of oxidative stress during ischemia in real time. We also investigate the respective contribution of guanosine to dopamine regulation through the adenosine receptors A1 and A2A. Transient DA was monitored in the stratum lacunosum moleculare of the CA1 in juvenile rat hippocampal slices for 45 minutes utilizing fast scan cyclic voltammetry (FSCV). The amount released, the duration in the extracellular space, and the frequency of events were analyzed. Baseline DA release patterns were established in normoxic, complete global oxygen-glucose deprivation (OGD), and focal OGD conditions. The effects of GN administration and its contributions through adenosine receptors on DA release in severe ischemia was also examined. Changes in cell morphology and mitochondrial stress were determined through immunohistochemical imaging. The effects of GN administration on the expression of NET and DAT transporters was examined with RT-qPCR. We establish significant differences in average dopamine event concentration and release frequency between global and focal ischemic models, with near-complete abolition of dopamine release in global OGD. Further, GN administration at concentrations representative of post-ischemic accumulation restores dopamine signaling and minimizes infarction. Here, we demonstrate for the first time at guanosine acts as an immediate neuroprotective agent on dopaminergic neurons following initiation of severe ischemia.

Disclosures: M. Weese-Myers: None. **K. Caldwell:** None. **C. Witt:** None. **A. Ross:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.30/Web Only

Topic: C.08. Ischemia

Support: NIA AG033720-11

Title: Dystrophic Microglia in White Matter Ischemic Injury

Authors: I. HARMON, **H. V. NGUYEN**, *S. BALTAN;
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Abstract: Major neurological disability from ischemic stroke is due to white matter injury (WMI), which can vary by age and sex. WMI, which varies by age and sex, contributes significantly to the neurological deficits seen in stroke patients. In ischemic gray matter, microglia have a dual role of exacerbating injury or promoting neuroprotection. However, the role of microglia in WMI is inconclusive and remains to be investigated. WM functional recovery is sex- and age-specific, for young female axons achieve greater functional recovery than young male axons, yet this difference is not observed within aging populations. Here, we defined the microglial dynamics in WMI by characterizing the morphological characteristics in response to ischemic injury as a function of age and sex. We used isolated mouse optic nerves (MONs), a pure white matter tract, from 3-month-old and 24-month-old male and female Cx3Cr1-GFP mice to capture the microglia morphological changes during an *ex vivo* WM ischemic model. Live cell images (z-stack of 10µm total thickness) were captured for 5 minutes every 20 minutes during baseline, 1 hour of oxygen-glucose deprivation (OGD), and 1 hour of recovery. Images were processed and scored for 8 morphological characteristics using ImageJ (NIH) with the fractal analysis (FracLac) plugins. Microglial characteristics were separated by sex and age, analyzed using principal component analysis, and grouped by Euclidean dendrogram and cluster mapping in R. We identified three distinct clusters which we classified as activated, unresponsive, and dystrophic based on morphological changes during OGD. Of the young male (YM) microglia, 10% were activated, 41% were unresponsive, and 49% were dystrophic. For aged male (AM) microglia, 25% were activated, 36% were unresponsive, and 39% were dystrophic. YM-activated and dystrophic microglia had greater structural variance between time points than AM microglia. YM unresponsive cells slightly increased in size while the aged male unresponsive cells slightly decreased in size. Interestingly, for both YM and AM, the activated microglia processes had a greater reduction in processes complexity over time than the dystrophic microglia. Our comparative analysis of young and aged female microglial dynamics is ongoing.We highlighted an unbiased method to define microglia behavioral subtypes during acute OGD in WM. Our findings indicate microglia vary in activation and resiliency during OGD which warrants further investigation in their contribution to WMI.

Disclosures: I. Harmon: None. **H.V. Nguyen:** None. **S. Baltan:** None.

Poster

PSTR215: Stroke and Ischemia: Molecular and Cellular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR215.31/C137

Topic: C.08. Ischemia

Support: AHA 24POST1192484

Title: Casein kinase 2 exerts post-ischemic protection of white matter against ischemia

Authors: *H. NGUYEN, I. HARMON, M. PIKE, S. BALTAN;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The advancement in stroke care and awareness has increased the number of survivors with long-term neurological deficits and disabilities. White matter injury (WMI) contributes to the majority of clinical deficits seen in stroke patients. However, a scientific gap remains in targeting WM functional recovery as a major outcome. Here, we utilize the selective focal WM injury (sfWMI) model to investigate a novel therapeutic approach for ischemic stroke. We showed that Casein Kinase 2 (CK2) upregulation after ischemia activates Cdk5 and AKT/GSK3β pathways causing mitochondrial dysfunction and WMI. Consequently, CX-4945, an FDA-approved, selective, and specific CK2 inhibitor that crosses the blood-brain barrier, confers post-ischemic protection by conserving mitochondria. Furthermore, prior reports showed that ischemia activates NADPH oxidase (NOX) in neurons to increase oxidative stress leading to mitochondrial damage. Therefore, we hypothesized that CK2 mediates WMI injury by activating NOX leading to mitochondrial dysfunction, and thus post-ischemic inhibition of CK2 with CX-4945 confers protection of WM against ischemia.Using 8-week-old C57BL/6 male mice, three injections each of 200nL of L-NIO (130µM) to create focal ischemic strokes or saline for sham were deposited at earlier identified coordinates. Six hours after WMI, CX-4945 (75mg/kg) or saline was administered for treatment and control group respectively for 5 days, twice daily. Behavioral deficits were assessed using cylinder test, and pasta-eating test at baseline, days 1, 7, 21, and 28 post-injuries. T2-weighted imaging and diffusion tensor imaging (DTI) were taken on days 2 and 10 post-injury to assess longitudinal WM changes. The loss of WM integrity was characterized by axon and myelin damage, astrocyte and microglia activation, mitochondrial dysfunction and upregulation of NOX activity correlated with impaired bilateral paw use in cylinder tests and dexterity in pasta-eating tests in male and female mice. The deficits corresponded with edema formation and changes in fractional anisotropy measured in magnetic resonance (MR) images. Application of CX-4945 preserved WM integrity histologically, alleviated behavioral deficits, attenuated NOX activity, and improved WM structure in MRI.We demonstrate that sfWMI causes significant damage to axon integrity which correlates with WM damage seen in MRI, behavioral deficits, and upregulation of NOX activity. Moreover, CX-4945 exerts post-ischemic protection of axons, oligodendrocytes, and myelin, effectively alleviating behavioral deficits, and decreasing NOX activity with improved WM integrity observed with MRI images.

Disclosures: H. Nguyen: None. **I. Harmon:** None. **M. PIke:** None. **S. Baltan:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.01/C138

Topic: C.10. Brain Injury and Trauma

Support: USU grant: MDO-70-12756
MTBI2 Graduate Fellowship Award

Title: How severity of traumatic brain injury impacts behavior?

Authors: *C. BOUSLOG[1], X. XU[2], F. W. LISCHKA[3], V. TSYTSAREV[2], C. A. BROWNE[4], D. L. DICKSTEIN[5], Z. GALDZICKI[6];
[1]Dept. of Anat., Physiol. and Genet., Neurosci. Grad. Program, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [2]Dept. of Anat., Physiol. and Genet., Uniformed Services Univ. of the Hlth. Sci., The Henry M Jackson Fndn. for the Advancement of Military Hlth. Inc., Bethesda, MD; [3]Dept. of Anat., Physiol. and Genet., Uniformed Services Univ. of the Hlth. Sci., Henry M. Jackson Fndn. for the Advancement of Military, Bethesda, MD; [4]Dept. Pharmacol. & Mol. Therapeut., Henry M Jackson Fndn., Uniformed Services Univ. of the Hlth. Sci., Neurosci. Grad. Program, Bethesda, MD; [5]Dept. Pathology, The Henry M Jackson Fndn. for the Advancement of Military Hlth. Inc., Uniformed Services Univ. of Hlth. Sci., Neurosci. Grad. Program, Bethesda, MD; [6]Dept. of Anat., Physiol. and Genet., Neurosci. Grad. Program, Uniformed Services Univ. of the Hlth. Sci., Sch. of Med., Bethesda, MD

Abstract: Traumatic brain injuries (TBIs) are a global health emergency with an estimate of over 55 million people affected worldwide. These injuries can lead to progressive memory/cognitive dysfunction, emotional dysregulation, sleep impairments and gait instability. Using an open head controlled-cortical impact (CCI) model of TBI in male and female C57BL/6J mice, the effect of varying TBI intensity on behavior was examined. Animals were randomly assigned to the Sham control group, or one of three CCI groups, differing in impact depth, Z = 1, 1.5 or 2.0 mm. All surgeries were conducted under isoflurane anesthesia. Mice were evaluated on a beam walking assay on day seven post injury. The primary outcome measure was number of slips. On day eight post injury mice were exposed to a five-minute fear conditioning paradigm, in which mice received two tone-shock pairings (30 s tone (3 kHz, 80 dB) co-terminating with 0.4 mA (2 s duration) shock). One day later, mice were tested for fear memory recall (percent time spent freezing) in response to the context (shock chamber, no shock, no tone), or cue (novel chamber, no shock, tone). Data was analyzed using Kruskal Wallis ANOVA and Dunn's multiple comparisons. Balance was impacted by CCI ($H_4 = 22.50$, $P<0.0001$), with more slips observed in injured mice relative to Sham controls; CCI Z = 1 mm (P = 0.0230), CCI Z = 1.5 (P = 0.001) and CCI Z = 2.0 mm (P = 0.0054). Recall of contextual fear memory was significantly altered following injury ($H_4 = 33.93$, $P<0.0001$). Injured mice in the CCI Z = 1.5 group exhibited robust reductions in % time freezing (P = 0.0015) and those in the CCI Z = 2.0 group displayed increased time spent freezing (P = 0.0257). No changes were noted in cued fear memory recall. As anticipated, moderate (Z = 1 and 1.5 mm) and severe (Z = 2 mm) injuries produced gross motor impairment. Interestingly, moderate injured resulted in memory

impairment, decreased recall of the tone-shock association. In contrast, mice in the severely injured group exhibited enhanced contextual fear memory recall. This particular phenotype is pertinent to understanding the underlying neurobiology of individuals that exhibit post concussive symptoms and comorbid post-traumatic stress disorder. Future studies will evaluate the correlation between injury severity and behavioral phenotypes with glymphatic flow (vascular pulsatility) and TBI associated abnormalities in neurovasculature. **Disclaimer:** The views expressed in this scientific presentation are those of the author(s) and do not reflect the official policy or position of the U.S. government or the Department of Defense.

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Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.02/C139

Topic: C.10. Brain Injury and Trauma

Title: Characterization of a mouse model of infant traumatic brain injury

Authors: *N. SAVORY[1], R. DESANTI[2], T. BRISCOE[3], J. W. HUH[4], R. RAGHUPATHI[5]; [1]Anat. and Neurobio., Drexel Univ. Col. of Med., Phila, PA; [2]Anat. and Neurobio., Drexel Univ. Col. of Med., Philadelphia, PA; [3]Drexel Univ. Col. of Med., Anatomy and Neurobiology, PA; [4]Anesthesiol. and Critical Care, Children's Hosp. of Philadelphia, WYNNEWOOD, PA; [5]Anat. and Neurobio., Drexel Univ., Philadelphia, PA

Abstract: Traumatic brain injury (TBI) is a leading cause of mortality and morbidity in children below the age of 4 and can lead to psychosocial and cognitive deficits well into adulthood. The progression of pathophysiologic alterations and behavioral deficits may differ between adult and pediatric TBI patients and, therefore, therapies that are effective in adults may be ineffective or danger in children. Multiple animal models of pediatric TBI have been characterized, though only a few correlate to infant TBI, the highest risk age group. A mouse model of infant TBI presents certain advantages with respect to examining mechanisms underlying acute and chronic functional deficits that follow TBI in this group. Infant (11-day-old) male and female C57BL/6 mice (n=26) underwent closed head injury with a silicone-tipped indenter on the exposed intact skull above the left parietal cortex (5m/s to a distance of 2.5mm from the skull zero point); sham-uninjured mice (n=27) were surgically prepared but did not receive an impact. At 3 days post-injury, a subset of sham-uninjured and brain-injured mice were euthanized, and brains were assessed for gross morphologic alterations (Nissl-myelin staining), axonal injury (amyloid precursor protein), reactive astrocytes (glial fibrillary acidic protein), and microglia activation (Iba1/CD68). Histological assessment showed robust glial activation and traumatic axonal injury in multiple white matter tracts as well as the thalamus in the injured hemisphere. Behavioral assessments conducted at 4-5 weeks post-injury (adolescent age) revealed significant

impairments in spatial learning and an increase in risk-taking behaviors ($p<0.05$ compared to sham-uninjured mice); depressive-like behaviors were not evident in brain-injured mice. Axonal injury and microglia activation were not apparent at 6 weeks, although reactive astrocytes were present. At both time points, no gross morphologic alterations were observed as a result of the impact. These data provide evidence of a working model of TBI in the infant mouse that will allow us to use genetic tools to investigate the cellular and circuit mechanisms underlying long-term deficits caused by pediatric TBI.

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Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.03/C140

Topic: C.10. Brain Injury and Trauma

Support: Coordinación de Investigación en Salud Fundación IMSS (NL) R-2019-785-060

Title: Early life stress affects behavioral flexibility after a traumatic brain injury in adult rats, but prevents the increase in IL-1b expression in the prefrontal cortex

Authors: *M. A. ROQUE[1,2], A. HERNANDEZ FLORES[1], E. ARREOLA[3], C. O. BONDI[4], N. LAJUD[5];
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Abstract: Early life stress (ELS) increases vulnerability to traumatic brain injury (TBI) and affects behavioral flexibility. Previous findings showed that ELS does not affect behavioral flexibility after TBI in the parietal cortex; however, it is still necessary to study the effects of ELS post-TBI in the prefrontal cortex. We used the Attentional Set-Shifting Task (AST) in adult male Sprague-Dawley rats, using maternal separation (MS) as an ELS model conducted from postnatal day one (PND1) to PND21 for 3 hours per day. On PND75, they received a controlled cortical impact in the prefrontal cortex (2.4 mm, 4 m/s, TBI) or remained as sham controls (SHAM). We evaluated behavioral flexibility 14 days after the injury. AST task involved a series of discriminative task of increasing difficulty, such as simple and compound discrimination, stimulus reversals, and intra- and extradimentional shifts. The results showed that TBI increased the number of trials required to reach criterion in the first reversal ($p<0.05$); however, only MS +

TBI rats showed an increase in the number of trials to criterion in the second ($p<0.05$) and third ($p<0.05$) reversals. TBI increased ($p<0.01$) prefrontal cortex interleukin 1 beta (IL-1β) expression in control but not MS rats. In conclusion, our data indicated that MS impairs cognitive flexibility after a mild frontal TBI in adults; however, it did not increase IL-1β expression in the site adjacent to the injury. These results shed some light into the complex interrelation between early life stress and adaptation to a secondary challenge in adulthood.

Disclosures: M.A. Roque: None. **A. Hernandez Flores:** None. **E. Arreola:** None. **C.O. Bondi:** None. **N. Lajud:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.04/C141

Topic: C.10. Brain Injury and Trauma

Support: FIMSS-CIS IMSS R-2019-785-060
Ana Fernanda Salinas-García was supported by CONACYT (CVU 805864)
Jonathan Zamudio-Flores was supported by CONACYT (CVU 1187161)

Title: Early life stress increase depressive type behavior after a traumatic brain injury in adult rats

Authors: *A. SALINAS GARCÍA[1], M. A. ROQUE[2], J. ZAMUDIO FLORES[1], A. E. KLINE[3], N. LAJUD[4];
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Abstract: Early life stress (ELS) affects object location memory and increases depressive-like behavior as well as hypothalamus-pituitary-adrenal axis activity. Previous studies have demonstrated that ELS impacts spatial learning after adult mild traumatic brain injury (mTBI). However, it remains unclear whether TBI increases the effects of ELS on object location memory and emotionality. The aim of the study was to assess cognitive performance, emotionality, and HPA axis reactivity in adult male rats that were subjected to ELS prior to a mTBI.. Specifically, maternal separation for 180 min per day (MS180) during the first 21 post-natal days (PND21) was used as an ELS model, while controls (CONT) remained undisturbed. At PND110 the rats were subjected to a mild controlled cortical impact (2.6 mm) or sham injury. Cognition was evaluated in the object location test 14 days after injury and emotionality was assessed with open field and forced swimming tests. On post injury day 22 glucose

concentrations were quantified after 12-h of fasting and the rats were injected with intravenous glucose. Glucose levels were evaluated 30, 60 and 120 min after administration. The data indicate that MS180+Sham and CONT+TBI, but not MS180+TBI rats show cognitive impairments in the object location test ($p< 0.05$). Additionally, only MS180 + TBI rats showed a passive coping strategy in the forced swimming test ($p< 0.05$). All groups showed increased glucose levels 30 min after administration; however, this increase was still present in MS180+Sham and CONT+TBI rats at 120 min. In conclusion, the combination of ELS and TBI does not exacerbate the effects on object location memory and anxiety-like behavior associated with ELS, but it does increase depressive-type behavior in the forced swim test.

Disclosures: A. Salinas García: None. **M.A. Roque:** None. **J. Zamudio Flores:** None. **A.E. Kline:** None. **N. Lajud:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.05/C142

Topic: C.10. Brain Injury and Trauma

Support: CONACyT- FOSISS No. FIS/IMSS/PROT/1769

Title: Early life stress increases depressive- like behavior and affects object location memory after a pediatric mild traumatic brain injury, but does not exacerbate stress reactivity

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Abstract: Early life stress (ELS) affects cognitive performance in the Morris water maze and increases hippocampal neuroinflammation after a pediatric mild traumatic brain injury (mTBI) without increasing baseline hypothalamic- pituitary- adrenal axis (HPA) activity. However, whether the same paradigm affects emotionality and stress reactivity remains unknown. To evaluate this, control (CONT) or maternally separated (3 hours/day from postnatal day 1-21, MS180) rats were subjected to a pediatric mTBI or sham injury at weaning and allowed to recover for 10 days. We evaluated object location memory and emotionality. Further, rats were implanted with a jugular vein catheter and handled for one week before evaluating their HPA axis response after an acute cold- swim stressor. Our results indicate that, in adolescents, only MS180 + TBI rats show a passive coping strategy on the forced swimming test and cognitive

impairments in the object location test. No effects were observed when evaluating anxiety- like behavior in the open field. Furthermore, MS180 + TBI rats showed an increase in hypothalamic corticotropin releasing factor (CRH) expression, but a decrease in corticosterone response to a cold- swim stress. These changes were associated to increased IL-1β expression in the ipsilateral hippocampus. Our findings strengthen the idea that early life stress increases the vulnerability to a mild TBI, and suggest that previous chronic stress exposure could have important implications for recovery after and abusive head trauma.

Disclosures: N. Lajud: None. **A. Díaz:** None. **R. Ruiz:** None. **E. Meléndez:** None. **A.E. Kline:** None. **M.A. Roque:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.06/C143

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01AA028218
NIH Grant R01NS110898

Title: Limbic neuropeptides may mediate sex differences in affective behavior following repetitive mild traumatic brain injury in adolescent rats

Authors: *C. R. MARTIN, J. R. BARSON, R. RAGHUPATHI;
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Abstract: Mild traumatic brain injury (mTBI) is common among adolescents and often occurs repetitively because of their participation in contact sports. Growing evidence suggests that girls are more likely to sustain sports related concussions and are more likely to report deficits in affect such as depression and lack of motivation for pleasurable activities, which often persist for years following injury. Using adolescent-age rats, this study focused on depression-like and motivated behaviors and changes in the expression of limbic neuropeptides implicated in these behaviors in the acute and chronic phase after repetitive mTBI. Adolescent male and female Long-Evans rats were anesthetized and subjected to 3 mild closed-skull impacts over 7 days (brain-injured males $n = 21$, females $n = 27$) or were anesthetized without injury (sham-injured males $n = 20$, females $n = 26$). In the first subset of animals at 5 weeks, but not the subset at 2 weeks following injury, injured females but not males displayed increased immobility in the forced swim test compared to sham controls ($p < 0.05$). In another subset of animals, injured females drank less 20% ethanol in the 2-bottle choice intermittent access paradigm, and they displayed less active lever pressing for a saccharin reward in operant chambers than sham females. In the nucleus accumbens (NAc) shell, injured females displayed decreased dynorphin (-38%) and enkephalin (-33%, injury effect $p < 0.05$) mRNA expression 5 weeks following injury as assessed by quantitative real-time PCR. In the posterior paraventricular nucleus of the

thalamus (pPVT, which sends dense projections to the NAc), mRNA expression of pituitary adenylate cyclase-activating polypeptide (PACAP), was increased in injured females but not males at 2 (+215%, $p < 0.05$) and 5 weeks (+162%, $p < 0.05$) compared to shams. These data suggest that depression-like and motivated behavior emerge in the chronic phase following repetitive mTBI in adolescent females. Moreover, PACAP in the pPVT may contribute to these injury-induced behavior differences by regulating neuropeptide expression NAc shell in a sex-dependent manner.

Disclosures: C.R. Martin: None. **J.R. Barson:** None. **R. Raghupathi:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.07/C144

Topic: C.10. Brain Injury and Trauma

Support: Jonathan Zamudio-Flores was supported by CONACyT (CVU 1187161)
Ana Fernanda Salinas-García was supported by CONACyT (CVU 805864)

Title: Environmental enrichment-induced cognitive recovery after a moderate pediatric traumatic brain injury is related to gut microbiome and neuroinflammation

Authors: *J. ZAMUDIO FLORES[1], A. SALINAS GARCÍA[1], D. CERQUEDA-GARCÍA[2], S. GUERRERO FLORES[3], N. SELEM-MOJICA[3], E. MELÉNDEZ[4], A. E. KLINE[5], N. LAJUD[6]; [1]Ctr. de Investigación Biomédica de Michoacán, Inst. Mexicano del Seguro Social, Morelia, Mexico; [2]Inst. de Ecología, A. C., Coatepec, Mexico; [3]Ctr. de Ciencias Matemáticas, Univ. Nacional Autónoma de México, Morelia, Mexico; [4]BASIC Biol., UMSNH: Univ. Michoacana de San Nicolas de Hidalgo, Morelia, Mexico; [5]Phys Med. & Rehab, Psych, Safar Ctr. Resuscitation Res., Univ. of Pittsburgh, Pittsburgh, PA; [6]Ctr. de Investigación Biomédica de Michoacán, IMSS - Inst. Mexicano del Seguro Social, Morelia, Mexico

Abstract: Pediatric traumatic brain injuries (TBI) are a significant health concern, yet access to rehabilitation therapies for children remains limited. Environmental enrichment (EE) is a clinical rehabilitation model that promotes behavioral recovery and reduces neuroinflammation after TBI. While the gut microbiota has recently emerged as a potential therapeutic target for treating TBI sequelae in adults, its role in EE-induced recovery after pediatric TBI remains unclear. Therefore, our aim was to assess the effect of EE on gut microbiota and its correlation with cognition and inflammation in a preclinical model of pediatric TBI. Male rats underwent moderate controlled cortical impact or sham procedures at postnatal day 21 and were then randomly assigned to either EE or standard (STD) housing conditions. Cognition was evaluated using the Morris water maze (MWM). Microglial morphology and the caecum microbiome were characterized at post-injury day 21. TBI + STD rats exhibited cognitive deficits in the MWM and

increased microglial activation in the ipsilateral cortex and hippocampus. These effects were not observed in TBI + EE rats. Additionally, EE led to increased microbiota diversity while TBI decreased it. In TBI+STD animals, bacteria from Prevotelleaceae family were more abundant compared to Sham. *Eubacterium* genus and Lachnospiraceae family were more abundant in TBI+EE compared to TBI+STD animals. These are bacterial groups known to be beneficial. In conclusion, our findings suggest that EE mitigates TBI-induced alterations in gut microbiota and that there is a complex interplay between EE, microbiota, and neuroinflammation that predicts behavioral recovery.

Disclosures: J. Zamudio Flores: None. **A. Salinas García:** None. **D. Cerqueda-García:** None. **S. Guerrero Flores:** None. **N. Selem-Mojica:** None. **E. Meléndez:** None. **A.E. Kline:** None. **N. Lajud:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.08/C145

Topic: C.10. Brain Injury and Trauma

Support: NIH NICHD K01 HD083759
NIH NICHD R01 HD099397

Title: Brief apnea and hypoventilation reduces traumatic seizures for several hours.

Authors: *B. COSTINE-BARTELL[1,2];
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Abstract: Objective. Seizures can be difficult to control in infants and toddlers. Seizures with periods of apnea and hypoventilation are common following severe traumatic brain injury (TBI). We previously observed that brief apnea with hypoventilation (A&H) in our severe TBI model acutely interrupted seizures. The current study is designed to determine the effect of A&H on subsequent seizures and if A&H has potential therapeutic implications. **Methods.** Piglets (1 week or 1 month old) received multi-factorial injuries: cortical impact, mass effect, subdural hematoma, subarachnoid hemorrhage, and seizures induced with kainic acid. Apnea and hypoventilation (1 min apnea, 10 min hypoventilation; A&H) was induced either before or after seizure induction, or as a control piglets received subdural/subarachnoid hematoma and seizure without A&H. In an intensive care unit, piglets were sedated, intubated, mechanically ventilated, and epidural EEG was recorded for an average of 18 hours after seizure induction. **Results.** In our severe TBI model, A&H after seizure reduced ipsilateral seizure burden by 80% compared to the same injuries without A&H. In the A&H before seizure induction group, more piglets had exclusively contralateral seizures though most piglets in all groups had seizures that shifted location throughout the several hours of seizure. After 8-10 hours, seizures transitioned to interictal epileptiform discharges regardless of timing of A&H. **Significance.** Even brief A&H

may alter traumatic seizures. In our pre-clinical model, we will address the possibility of hypercapnia with normoxia, with controlled intracranial pressure, as a therapeutic option for children with status epilepticus after hemorrhagic TBI.

Disclosures: B. Costine-Bartell: None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.09/C146

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 NS1030517 to KML

Title: Effects of pediatric traumatic brain injury on microglia, mast cells, and long-lasting cognitive, social, and anxiety-like behavior in rats

Authors: *M. A. SMAIL[1], R. BOLAND[2], M. R. BREACH[1], C. N. DYE[1], J. MCCLOSKEY[2], C. VONDER HAAR[2], O. N. KOKIKO-COCHRAN[2], K. M. LENZ[1];
[1]Psychology, [2]Neurosci., Ohio State Univ., Columbus, OH

Abstract: Traumatic brain injury (TBI) is one of the leading causes of emergency room visits in children under 10. Children are especially vulnerable to the adverse effects of TBI, given that their brains are still developing at the time of the injury. Indeed, this early life insult has been linked to social and cognitive impairments later in life. Sex differences have also been noted, with girls tending to exhibit more severe outcomes than boys. The immune system has been implicated in adult TBI mechanisms and plays numerous key roles in brain development, making it an interesting candidate for linking pediatric TBI and long-term behavioral consequences. Here we developed a rat model of pediatric TBI to investigate the relationship between early life TBI, immune cells, sex, and behavioral outcomes. At postnatal day 15, male and female rat pups were randomly assigned to 3 groups: Naïve, Sham, or TBI (n=12/group). Naïve rats received no surgery or injury, Sham rats received surgery but no injury, and TBI rats received both surgery and injury. Injury was delivered via lateral fluid percussion (2 atm). Rats were either euthanized at 3 days post injury (DPI) for neuroanatomical analyses of microglia and mast cells or aged to adulthood for cognitive, social, and anxiety-like behavioral testing. At 3 DPI, microglia medial to the injury expressed significantly more CD68 in TBI rats than Naïve or Sham rats. Mast cells were more abundant and more degranulated throughout the brain in TBI rats. Both results suggest heighted immune activation acutely following injury. In adult behavioral testing, early life TBI impacted social and cognitive function. These rats exhibited reduced social interaction and increased social avoidance, as well as increased spontaneous alternation on the Y maze and impaired novel object recognition. However, no differences were observed in spatial memory or anxiety-like behaviors. The finding that early life TBI impacts social and cognitive domains more than anxiety domains aligns with human literature, offering face validity. Analyses of more

complex cognitive endpoints in the Rodent Gambling Task are ongoing. No sex differences were observed, although these studies are not yet powered to properly analyze sex effects. Early life TBI leads to enhanced immune activity in both microglia and mast cells. Given the many important roles of microglia in development, greater activation during this critical period has the potential to contribute to long-lasting social and cognitive consequences of this early life insult. Continuation of these studies will seek to better understand the relationship between pediatric TBI, the neuroimmune system, and behavioral outcomes.

Disclosures: M.A. Smail: None. **R. Boland:** None. **M.R. Breach:** None. **C.N. Dye:** None. **J. McCloskey:** None. **C. Vonder Haar:** None. **O.N. Kokiko-Cochran:** None. **K.M. Lenz:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.10/C147

Topic: C.10. Brain Injury and Trauma

Title: Traumatic brain injury induces sex-specific and cell type-specific responses to ferroptosis and neuroinflammation at the acute phase post-injury in juvenile mice

Authors: S. TASEVSKI, Y. MASHAL, S. MOUGHNI, A. GHANNAM, T. ATOUI, ***Z. ZHANG**;
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Abstract: Ferroptosis, a form of programmed cell death, plays an important role in secondary brain damage after traumatic brain injury (TBI). Ferroptosis is induced by iron-dependent excessive phospholipid peroxidation, and is closely related to neuroinflammation. Due to the complex brain cytoarchitecture, TBI can simultaneously affect multiple cell types in the central nervous system (CNS). Moreover, the immature brains exhibit higher vulnerability to ferroptosis, compared with adult brains. However, sex- and cell type-specific responses to ferroptosis after TBI in immature brains remain unclear. The objective of this study is to investigate differential regulation of ferroptosis and neuroinflammation in neurons and microglia during the acute phase post-injury in a mouse model of impact acceleration TBI in both male and female juvenile mice. Male and female mouse littermates were randomized on postnatal day 20-21 into Sham and TBI groups (n=10-12 animals per group). Animals in the TBI groups underwent TBI procedure, while animals in the sham groups underwent anesthesia without injury. Animals in all groups underwent behavioral testing before injury (baseline) and at 4-hour (h) post-injury. The personnel were blinded to the group assignments. Animals were euthanized after behavioral testing. The activation of neuroinflammatory and ferroptosis pathways were analyzed using real-time quantitative PCR (qPCR) and immunohistochemistry. The injured brain regions in the TBI animals and the matching brain regions from the sham animals were micro-dissected. Neurons and microglia were concurrently isolated from the same brain sample of the same animal and used for gene expression analysis. We found that TBI induced significant sensorimotor deficits

in both male and female mice at the acute phase post-injury, accompanied with sex- and cell-type-specific neuroinflammatory responses. Moreover, TBI induced differential regulation of ferroptosis-related genes, e.g., glutathione peroxidase 4 (GPX4) and transferrin receptor (TFRC), in male and female neurons and microglia. In conclusion, TBI results in behavioral deficits, accompanied with sex- and cell-type-specific regulation of ferroptosis and neuroinflammation at the acute phase post-injury in immature mice, which provide a rationale for developing cell specific therapeutics.

Disclosures: S. Tasevski: None. **Y. Mashal:** None. **S. Moughni:** None. **A. Ghannam:** None. **T. Atoui:** None. **Z. Zhang:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.11/C148

Topic: C.10. Brain Injury and Trauma

Title: Multi-metric Evaluation of Isoflurane: Improved Outcomes Following Soman Exposure

Authors: *A. METHVIN[1], J. LEIGHTON[2], J. JANSSEN[1], M. ELLIS[3], J. SAHARGUN[4], E. A. JOHNSON[2];
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Abstract: Organophosphates (OPs) are a class of chemicals with extremely toxic central nervous system (CNS) and peripheral nervous system activity. The most lethal risk of exposure comes in the form of nerve agents, though less potent OPs, such as those found in most insecticides, can prove debilitating or lethal as well. The primary source of long-term deficit following exposure to OPs is CNS damage: OPs bind to acetylcholinesterase and eliminate re-uptake of the highly excitatory neurotransmitter acetylcholine in the synaptic cleft. The result is massive seizure (SZ) and a subsequent neuroinflammatory cascade which damages neural tissue. While rapid use of benzodiazepines offers protection through inhibition at the GABAA receptor, rapid downregulation of this receptor complex during OP-induced SZ reduces drug efficacy. In traditional hospital settings, an alternate approach to address super refractory status epilepticus is the use of volatile anesthetics, such as isoflurane (ISO). While ISO also acts via positive allosteric modulation of the GABAA receptor, inhibition of seizure activity is additionally supported through interaction with the inhibitory glycine receptor pathway and inhibition of excitatory NMDA glutamate receptor subtypes. A major focus of our group is to augment existing treatment capabilities in austere environments to reduce morbidity and mortality after exposure to OPs. ISO is already FDA approved, has a wide therapeutic index, has a quick recovery with few side effects, and has proven effective in terminating paraoxon-induced seizure in rats. We hypothesize that ISO may modulate the mechanisms of SZ control to

widen a treatment timeframe while delivering extended control of negative exposure outcomes. Here we make significant advances in characterizing the realistic efficacy of ISO treatment via utilization of the humanized acetylcholinesterase (C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; KIKO) mouse developed at USAMRICD along with a robust refractory SZ exposure model using the OP poison soman (GD). We clarify the bounds of a treatment window via ISO administration timepoints of 40-, 60-, and 90-minutes post SZ onset. Results show a graded response to ISO administration: when ISO is given at a 40-minute timepoint SZ is fully terminated and remains off for a large observation window; as opposed to at the 90-minute timepoint, where SZ severity is definitively reduced, but not fully terminated. We additionally map pulmonary response (measured via Starr MouseOx), body temperature, and EEG signal to visualize correlating treatment responses as a measure of efficacy and predicted mortality, supported by histology of several brain regions.

Disclosures: A. Methvin: None. **J. Leighton:** None. **J. Janssen:** None. **M. Ellis:** None. **J. Sahargun:** None. **E.A. Johnson:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.12/C149

Topic: C.10. Brain Injury and Trauma

Support: JSPS KAKENHI Grant 20K07971

Title: The microglial changes of mitochondrial functions in the delayed carbon monoxide encephalopathy rat model

Authors: *S. OCHI[1], T. NISHIHARA[2], S. BOKU[3], J. IGA[4], S.-I. UENO[5];
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Abstract: Introduction: Delayed carbon monoxide (CO) encephalopathy may occur following recovery after several weeks from acute CO poisoning. However, the mechanism of delayed neuronal injury remains unknown. We previously reported that the rat model of delayed CO encephalopathy showed cognitive impairment and hippocampal cell death, especially in the lesions of dentate gyrus. Furthermore, delayed CO encephalopathy caused the impairment of neural precursor cells and also the number of microglial cells and the mRNA expressions of several neurotrophic factors in the hippocampus were decreased. Microglia is known to play important roles in the adult neurogenesis and promoting neurotropic factors. Therefore, in the current study, we hypothesized that functional changes of microglia may be affected in delayed

CO encephalopathy and investigated the mitochondrial respiration and glycolysis of microglia in delayed neuronal CO poisoning. Materials and Methods: Wistar male rats (6 weeks old) were exposed to 1000 ppm CO for 40 min and then 3000 ppm for 20 min until they lost consciousness. If not, rats were exposed to 10000 ppm until they lost consciousness. Behavioral effects on learning and memory function were measured by the passive-avoidance test between control and CO treated rats until 3 weeks. After 3 weeks, rats were sacrificed, and we used magnetic cell sorting to obtain microglial cells from the hippocampus with the CD11b/c-microbeads antibody, and flow cytometry was performed to determine the purity of microglia. We investigated oxygen consumption rate and extracellular acidification rate to evaluate mitochondrial respiration and glycolysis, respectively, in rat primary microglial cells using XFp Extracellular Flux Analyzer between CO group (n = 6) and control group (n= 6). Student's T-test or Mann-Whitney U test were used for comparisons between control and CO groups. Significance was determined by P values <0.05. Results: The latencies of the passive avoidance test after 3 weeks were significantly shorter in the CO group than in the control group. Compared to control groups, basal respiration (24.4 pmol/min vs 16.8 pmol/min; p = 0.019), maximum respiration (26.8 pmol/min vs 14.9 pmol/min; p = 0.001) and ATP production (18.5 pmol/min vs 12.5 pmol/min; p = 0.007) in CO group were significantly decreased after 3 weeks. Conclusions: This study suggests delayed CO injury might damage microglia and it was affected at least 3 weeks. Thus, the impairment of functions of microglia may have an important role in the pathogenesis of delayed CO encephalopathy.

Disclosures: S. Ochi: None. **T. Nishihara:** None. **S. Boku:** None. **J. Iga:** None. **S. Ueno:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

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Program #/Poster #: PSTR216.13/C150

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS093073
NIH Grant NS130308

Title: Blocking axon-glial mechanotransduction to prevent concussive brain injury

Authors: *C. GU;
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Abstract: All cells in the central nervous system (CNS) are considered mechanosensitive, but how they collectively respond to a concussive head impact and contribute to the transition from the primary to secondary injury remains unknown. Using a mouse model for mild traumatic brain injury (mTBI) or concussion, we report that blocking the activity of TRPV4 transient receptor potential channels inhibits mTBI-induced sequential changes of neurons and glial cells, as well as behavioral disturbances. A concussive head impact immediately induces axonal

varicosities, preceding glutamate-receptor-mediated microglial activation and cortical demyelination. Afterwards, these changes differentially and partially recover. TRPV4 channel is a key mechanosensitive ion channel. Blocking its channel activity before or after head impact markedly suppresses all these changes or accelerates their recovery, respectively. Using global knockout mice and AAV-Cre-mediated acute and cell-type-specific deletion, we further show that TRPV4 channel regulates the homeostasis of axonal mechanosensation and its hyperactivation causes axonal varicosity formation followed by axon-to-glia mechanotranduction. Taken together, our findings have shown that TRPV4 channel may represent a key target for both preventing and mitigating mTBI.

Disclosures: C. Gu: None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.14/C151

Topic: C.10. Brain Injury and Trauma

Title: Traumatic Brain Injury and Nerve Agent Polytrauma, Behavioral Deficits Alleviated by Experimental Adjuncts to Standard Treatments in a Transgenic Mouse Model

Authors: *M. N. ELLIS[1], J. LEIGHTON[2], J. JANSSEN[3], A. METHVIN[4], E. A. JOHNSON[2]; [1]USAMRICD, Darlington, MD; [2]Neurosci., USAMRICD, Aberdeen Proving Ground, MD; [3]USAMRICD, Gunpowder, MD; [4]Med. Toxocology, USAMRICD, Gunpowder, MD

Abstract: In the line of duty, Service members are potentially at risk for traumatic brain injuries (TBI) and/or chemical warfare nerve agent (CWNA) exposure and the likelihood of both injuries occurring is ever increasing. Both injuries have well established standards of care with countermeasures available to medics and aid station personnel. While the standard of care for each individual injury is established, how these treatments work together for TBI/CWNA polytrauma is largely unknown. The aim of this study was to investigate the synergistic effects of a concurrent TBI and CWNA polytrauma and evaluate the effectiveness of each injury's established standard treatment when used together. This study also determined the efficacy of additional adjunct treatments, drugs already fielded by medics to improve outcomes. To accomplish this, our group created a novel TBI/CWNA polytrauma model using the transgenic human acetylcholinesterase knock-in/serum carboxylesterase knockout (C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; *a.k.a* KIKO) mouse strain. To produce the polytrauma, male KIKO mice received a controlled cortical impact TBI, and its standard countermeasure tranexamic acid (TXA) followed by exposure to CWNA and its standard countermeasures (atropine sulfate, 2-pyridine aldoxime methyl chloride and midazolam. The experimental adjunct treatments investigated were diphenhydramine, hydromorphone, ketamine, levetiracetam, promethazine, or scopolamine. All adjuncts were shown to improve various outcome metrics in either or both individual injury states. All mice were monitored throughout the 72-hour survival

period, a time to mimic prolonged field care, for physiological changes including EEG abnormality, behavioral changes (open field testing), and mortality. Results from the open field test revealed the TBI only mice have minimal deficits while CWNA only mice had more significant deficits. Leading to the polytrauma having minimal synergistic effects on behavioral deficits on distance traveled and immobility suggesting that CWNA exposure plays a major role in the polytrauma deficits. Experimental adjunct treatment following both standard of care treatments revealed differences in both behavioral metrics. Several adjuncts showed an improvement in immobility and distance traveled. Suggesting that polytrauma animals have more severe behavioral deficits than single injury TBI but similar outcomes as the CWNA only exposure animals. Furthermore, these trends suggest that additional treatment can improve behavioral outcomes by using already fielded pharmaceuticals as adjuncts administered with both injury standard countermeasures.

Disclosures: M.N. Ellis: None. **J. Leighton:** None. **J. Janssen:** None. **A. Methvin:** None. **E.A. Johnson:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.15/C152

Topic: C.10. Brain Injury and Trauma

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Department of Science and Technology, Ministry of Science & Technology, Government of India grant [SR/FST/LS-I/2017/05(C)]
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Title: Dantrolene mediated modulation of the NF-κβ/Akt pathway alleviates neuroinflammation and impaired mitochondrial dynamics in traumatic brain injury

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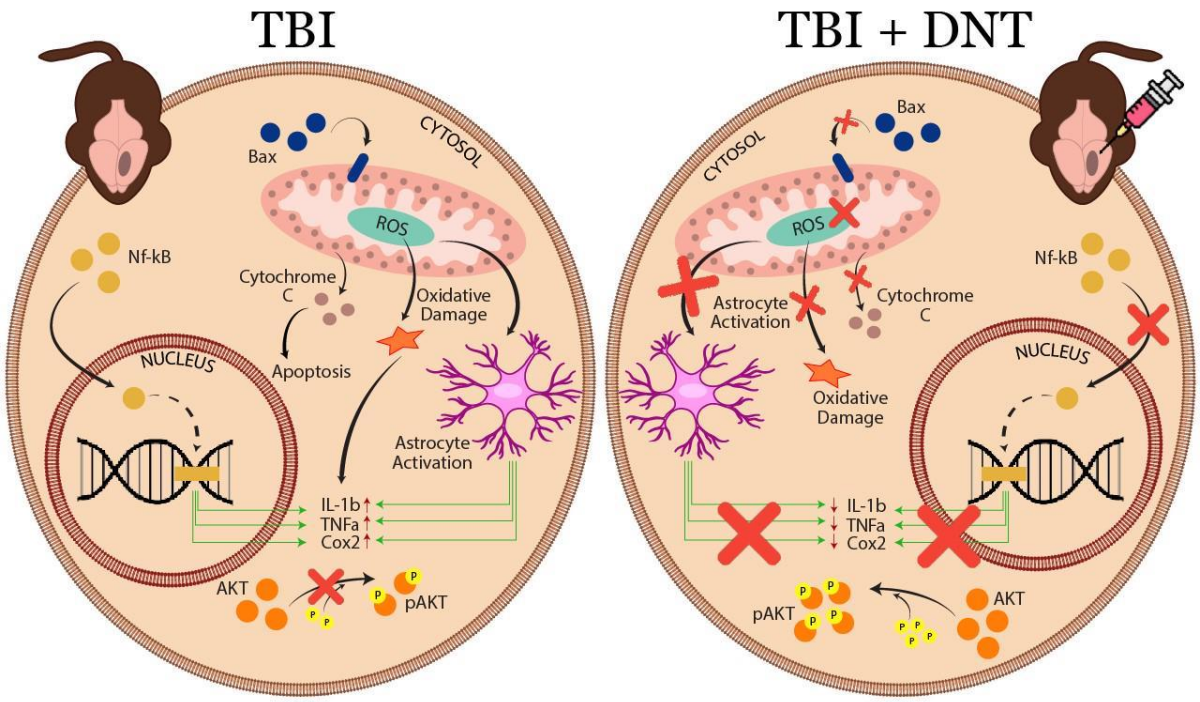
Abstract: Traumatic brain injury (TBI), which precipitates neuronal tissue scarring and mechanical damage to the underlying anatomophysiology, is typically caused by a fall or a severe blow to the head. TBI instigates a multitude of changes viz. neuroinflammation, apoptosis, neurobehavioural impairments, mitochondrial dysfunction, and oxidative stress. The

complex bevy of cellular and molecular changes subsequent to the initial trauma, coupled with the gaping lacunae in our grasp of the underlying pathophysiological derangements results in poor disease prognosis, thereby hampering recovery. Dantrolene (DNT), a postsynaptic muscle relaxant which inhibits intracellular Ca^{2+} signaling, has been repurposed as a potential neuroprotective agent in various neurological diseases. However, there have been limited studies on whether it can mitigate TBI-induced deficits and restore impaired mitochondrial dynamics. Our study sought to evaluate whether Dantrolene can provide neuroprotection in an *in vivo* model of TBI. Male wistar rats subjected to TBI were treated with DNT (10mg/kg) 1h and 12h post-surgery. Animals were assessed 24 h post-TBI to analyze neurobehavioural deficits. We evaluated the protein expressions of apoptotic, autophagic, and neuroinflammatory markers by immunoblotting to ascertain the effects of DNT on TBI. We further performed immunofluorescence staining with Glial Fibrillary Acidic Protein (GFAP) to analyze astrocyte mediated inflammation. H&E staining was also conducted to throw light on the structural integrity of neurons in the cerebral cortex post-TBI. Our findings revealed DNT administration inhibits mitochondria-mediated apoptosis. DNT treatment also reduced cerebral edema, reversed neurobehavioural impairments, and preserved neuronal architecture. We further demonstrated that DNT inhibits neuronal autophagy and alleviates neuroinflammation following TBI by modulating the NF-κβ/Akt pathway. Thus, our results suggest a novel application of DNT in ameliorating the multitude of deficits induced by TBI, thereby conferring neuroprotection.



Disclosures: R. Chakraborty: None. **H. Tabassum:** None. **S. Parvez:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.16/C153

Topic: C.10. Brain Injury and Trauma

Support: NSF BRC-BIO 2313370

Title: Differential immune responses may contribute to varying outcomes between a single, severe TBI and a mild, repeated TBI

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Abstract: Traumatic brain injuries (TBIs) occur when external forces damage the brain, commonly resulting in hospitalization, long-term disability, and death. This neurological disorder accounts for nearly 30% of deaths due to injury in the US. The features of injury that lead to worse outcomes are difficult to discern in the human population due to varying demographics and injury types. To eliminate these confounding variables, we used *Drosophila melanogaster* as a model organism to study the short- and long-term outcomes of mild, repeated TBI (multi-day, MD) compared to a single, severe TBI (single-day, SD). We discovered that the outcomes of the two patterns of TBI differed. We found that flies given a MD TBI showed lower acute mortality (within 48 hours), but the surviving flies displayed a shorter lifespan than flies given a SD TBI. Likewise, flies given a MD TBI exhibited worse long-term locomotor ability. We hypothesized that different immune responses to MD versus SD TBI may mediate differences in short- and long-term outcomes and found evidence of prolonged immune gene expression in MD TBI several weeks post-TBI. To determine if the differences in immune response were causal, we examined flies with mutations in key immune genes and measured their effects in the two injury paradigms. Specifically, we measured acute mortality and lifespan of Imd and Toll mutant flies post-MD and SD TBI. We found that short- and long-term survival in both injury conditions worsened when Imd was absent, suggesting that the Imd immune signaling is likely protective against short- and long-term outcomes from both MD and SD TBI. Interestingly, mutants lacking Toll immune signaling displayed opposite responses to each injury type: worse survival after SD TBI, but better survival after MD TBI. The Toll mutants also displayed increased lifespan following MD TBI, relative to wild type controls, suggesting that Toll signaling is detrimental for both short- and long-term outcomes from a MD TBI. Cell-type specific knockdowns of Imd and Toll signaling suggest that immune signaling in glial cells plays a larger role than in neurons in affecting the outcomes of TBI. Understanding differences in cellular immune responses, and the timing of these responses, to different types of TBI could enable the development of tailored treatments, and ultimately improve outcomes.

Disclosures: D. Tulchinskiy: None. **R. Delventhal:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

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Topic: C.10. Brain Injury and Trauma

Support: NIH MSTP T32-GM145462
K08NS114170
R01NS129609
VA Merit Review Award - 2 I01 BX002745-06A2

Title: Microglia-dependent changes of δ-containing GABA-A receptors in mouse thalamocortical neurons after traumatic brain injury

Authors: *R. P. O'BOYLE[1,2], A. NOLAN[3], A. FEICHTENBINER[3], C. B. RANSOM[4]; [1]Univ. of Miami, Miller Sch. of Med., Miami, FL; [2]University of Washington, Seattle, WA; [3]Univ. of Washington, Seattle, WA; [4]Epilepsy Ctr. of Excellence, VA Puget Sound Hlth. Care Syst., Seattle, WA

Abstract: Traumatic brain injury (TBI) induces cellular and molecular changes that are believed to substantially contribute to neuropsychiatric dysfunction. Alterations of GABAergic signaling and GABA receptor expression/function after TBI have been described for cortical and hippocampal neurons, but little is known about how TBI affects GABAergic signaling in thalamocortical neurons, neurons vital for brain-wide interconnectivity and global state response. We used a mouse model of severe TBI (controlled cortical impact, CCI) and patch clamp techniques to investigate changes in tonic δ-containing GABA-A receptor function in thalamocortical neurons (mediodorsal nucleus). Tonic GABA currents in the presence of the GABA-A receptor δ-subunit selective agonist THIP were significantly reduced 2-4 weeks after TBI compared to sham-treated animals. This reduction in tonic GABA currents was also present at chronic timepoints after TBI (i.e., 3-4 months). In addition to the changes in tonic GABA-A receptor function, there was prominent microgliosis and increased microglial interaction with neurons after TBI. Interestingly, microglial interaction at the neuronal soma inversely correlated with GABA-A δ-subunit expression. Thus, we hypothesized that microglia-dependent neuroinflammation plays a critical role in the observed changes in tonic GABA currents. To test this, we treated animals with the CSF-1 receptor antagonist PLX5622 (PLX) for two weeks to deplete microglia prior to CCI and continued this treatment until electrophysiological assessment; this produced a complete loss of IBA1+ microglia. Functionally, in PLX-treated mice there was also no significant difference in tonic GABA currents between sham and TBI groups. These results show for the first time that TBI-induced changes in tonic δ-subunit-dependent GABA-A receptor function are strongly dependent on microglia.

Disclosures: R.P. O'Boyle: None. **A. Nolan:** None. **A. Feichtenbiner:** None. **C.B. Ransom:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

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Program #/Poster #: PSTR216.18/C155

Topic: C.10. Brain Injury and Trauma

Support: MHC-202402-003
HRF-202405-001
NRF-2021R1C1C2012889

Title: Exploring the Neurological Impact of Microplastic Exposure: Insights into TBI, Gut-Brain Axis, and CTRP9's Neuroprotective Role

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Abstract: Microplastics, due to their microscopic size, have increasingly raised concerns in health sciences because they can penetrate the intestinal barrier, leading to chronic inflammation that contributes to gastrointestinal disorders. Additionally, these particles can disrupt the delicate balance of gut microbiota, essential for digestive health, immune function, and mental well-being. Our study investigates the specific impact of microplastics on Paneth cells in the small intestine, which play a pivotal role in maintaining intestinal health and the body's defense against harmful microbes. Paneth cells are crucial for maintaining zinc homeostasis in the gut, which directly influences antimicrobial pathways. Moreover, we explored the implications of microplastics on traumatic brain injury (TBI), a severe condition often resulting from physical head trauma that causes immediate brain swelling, oxidative stress, and neuroinflammation. We hypothesized that microplastic exposure impairs Paneth cells and disrupts brain zinc balance, thus worsening the neurological complications of TBI. In this study, rats were exposed to microplastics for two weeks to observe their impact on intestinal health, then subjected to a TBI model. We assessed the extent of neuronal death and cognitive impairment post-TBI through histopathological analyses, cognitive behavior tests, and biochemical assays. Results showed that microplastic exposure significantly impaired Paneth cell functionality and brain zinc regulation, exacerbating neuroinflammation and neuronal death following TBI. Additionally, oxidative stress markers were elevated in the TBI-affected brain regions of microplastic-exposed rats. Interestingly, treatment with C1q/TNF-Related Protein 9 (CTRP9), a protein known for its neuroprotective and anti-inflammatory properties, reduced neuronal death, neuroinflammation, and cognitive decline in the TBI model. This underscores CTRP9's therapeutic potential in mitigating TBI-induced neurodegeneration. In conclusion, our study demonstrates the detrimental effects of microplastics on intestinal and brain health, particularly in the context of TBI. Furthermore, it identifies CTRP9 as a promising candidate for developing therapies that

may alleviate TBI-related complications by protecting against neuron death and reducing neuroinflammation. Key Words: Traumatic brain injury, Microplastic, Zinc, Paneth cell, CTRP9, neuronal death

Disclosures: M. Park: None. **B. Kang:** None. **S. Woo:** None. **H. Yang:** None. **D. Kim:** None. **W. Yang:** None. **B. Choi:** None. **S. Suh:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.19/C156

Topic: C.10. Brain Injury and Trauma

Support: HKRGC-CRF C4012-22GF
HKRGC-GRF 14112523
HKRGC-GRF 14115821
Theme-Based Research Scheme T13-605/18-W

Title: The neural circuit basis of the sevoflurane-induced cognitive impairments

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Abstract: The neural circuit basis of the sevoflurane-induced cognitive impairments The administration of general anaesthesia to millions of children for surgery each year raisesconcerns about its potential negative effects on cognitive and memory functions. Despiteextensive research, the exact mechanisms behind how general anaesthesia affects cognition andmemory remains unclear. This study aimed to the role of the pathway from the anteriorcingulate cortex (ACC) to the bed nucleus of the stria terminalis (BNST) in impairing fearmemory due to sevoflurane anaesthesia. By using a repeated sevoflurane exposure model andconducting Pavlovian fear conditioning tests on male wild-type C57BL/6 mice, we examinedchanges in c-fos expressions in the ACC and BNST. Real-time neuronal activities wererecorded from these areas using fiber photometry and chemogenetic manipulation was appliedto assess their roles on freezing behavior during fear memory tests. Results showed thatmultiple exposures to sevoflurane reduced freezing behavior and decreased c-fos expressionsin the ACC and BNST, but did not significantly affect ventilation and oxygenation. Cue-induced freezing led to an increase in GCaMP signals in the ACC and BNST, while activatingthe ACC-BNST pathway via chemogenetics increased freezing behavior after repeatedsevoflurane exposure. These findings suggest that repeated sevoflurane exposure impairs fearmemory by affecting the ACC-BNST neural circuit, although further research is needed toclarify the precise mechanisms involved. Understanding these effects is crucial for developingstrategies to minimize potential cognitive and memory issues in children undergoing generalanaesthesia.

Disclosures: X. zhao: None. **Y. Zhu:** None. **W. Yung:** None. **Y. Ke:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

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Program #/Poster #: PSTR216.20/C157

Topic: C.10. Brain Injury and Trauma

Support: This work was partly supported by the Richard J Traystman professorship endowment (SK).

Title: Anhedonia and learned helplessness in post-traumatic depression in adult rats following repetitive closed-skull traumatic brain injury

Authors: *J. ALLENDE LABASTIDA[1], M. P. AVALOS[2], P. VYAS[2], V. ARUN[3], M. TRIVEDI[4], J. LIU[2], R. C. KOEHLER[2], S. KANNAN[2];
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Abstract: Traumatic brain injury (TBI) is one of the primary causes of death and disability in young adults. Some of the most common long-term disabilities include neuropsychiatric disorders. The prevalence of post-traumatic neuropsychiatric disorders is significant, ranging from 34-50%. Major depressive disorder is the most common sequelae (13-77%), followed by generalized anxiety, and post-traumatic stress disorder (3-28%). The prevalence of the latter doubles in military personnel. Additionally, post-traumatic depression (PTD) increases the risk of suicide. With this study, we aim to determine if repetitive closed-head traumatic brain injury induced with the ratCHIMERA (Closed-head impact model of engineered rotational acceleration) model generates a PTD phenotype in adult rats.
We randomly assigned young adult male Sprague Dawley rats (10-11 weeks old) to sham or repetitive TBI (rTBI) groups and exposed the rats in the rTBI group to four impacts (~10 m/sec) over two days (each day with two injuries and one-hour inter-impact periods). We assessed three main depressive-like symptoms, anhedonia, sociability and social novelty, and learned helplessness/despair, with sucrose preference (SPT), social interaction (SIT), and Porsolt forced swim test (FST), respectively. Rats with rTBI showed anhedonia symptoms presented as a decreased preference for drinking 1% sucrose drink over water. Their preference decreased after the initial novelty of the sweet drink passed being evident on day three of the five-day trial (19-22 days post-injury). To ensure FST results were not influenced by motor deficits we corroborated normal motor function with the open field test. Injured rats also had signs of learned helplessness by decreased attempts to escape from the FST, displaying increased time remaining immobile.
With these results, we conclude that rTBI induced with the ratCHIMERA generates a post-traumatic depression phenotype characterized by anhedonia and learned helplessness/despair.

While further analysis of sociability is warranted, we plan to include female rats and rats from the Flinder's sensitive and resistant lines to generate a more robust phenotype to better test potential therapeutics for PTD with or without resistance to antidepressants.

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Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

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Program #/Poster #: PSTR216.21/C158

Topic: C.10. Brain Injury and Trauma

Support: NeuroID BP-Endure 2R25NS080687-11

Title: Endocannabinoid System Activation Effects in Neuroinflammatory Induced Depressive and Anxiety behaviors and its role on the rat insular cortex.

Authors: *S. SANTOS[1,2], A. RODRIGUEZ LEON[3], D. UMANA[4], A. CORRETJER[5], C. MALDONADO IRIZARRY[6], O. VÉLEZ[7];
[1]UPRRP, GUAYNABO, Puerto Rico; [2]Natural Sciences, University of Puerto Rico, Rio Piedras, PR; [3]Univ. of Puerto Rico, San Juan, Puerto Rico; [4]Univ. de Puerto Rico, Rio Piedras, Guaynabo, Puerto Rico; [5]Biol., Univ. de Puerto Rico Recinto Rio Piedras, Humacao, Puerto Rico; [6]Univ. of Puerto Rico Dept. of Anat. & Neurobio. Grad. Program, Rio Piedras, PR; [7]Univ. of Puerto Rico-Rio Piedras Campus, Rio Piedras, Puerto Rico

Abstract: This study aimed to investigate the effects of lipopolysaccharide (LPS) injection on anxious and depressive behaviors in rats, while examining the potential therapeutic effect of the FAAH inhibitor URB597 on these behaviors. This inhibitor has been proven to increase anandamide (AEA) levels, which can aid in inflammatory processes (Wang J., et al, 2009). This study used the Elevated Plus Maze (EPM) and Forced Swim Test (FST) to evaluate the role of the insular cortex in these processes and gain a better understanding of the underlying mechanisms linking inflammation, endocannabinoids, and depression. The results showed that the rats treated with LPS+VEH exhibited higher anxiety-like behavior and lower swimming behavior, indicating depression-like behavior. The treatment group (LPS+URB597) showed significant mitigation of the negative effects of LPS on behavior. However, the rats that received URB597+VEH exhibited higher anxiety-like behavior and lower swimming behavior, suggesting a positive effect of this FAAH inhibitor only when the animal is in need of decreasing inflammation. These findings indicate that FAAH inhibitors may have therapeutic potential for the treatment of depression and anxiety when the rodent expresses specific inflammatory markers and may contribute to our understanding of the neural circuits involved in these behaviors.

Disclosures: S. Santos: None. **A. Rodriguez Leon:** None. **D. Umana:** None. **A. Corretjer:** None. **C. Maldonado Irizarry:** None. **O. Vélez:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.22/C159

Topic: C.10. Brain Injury and Trauma

Title: Behavioral measures in a long-term goat model of neurodegeneration

Authors: *R. ADAM[1], N. L. ACKERMANS[1], A. OYADEYI[2];
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Abstract: Traumatic Brain Injury (TBI) stands as a global leading cause of mortality and predisposing factor in Alzheimer's disease and related dementias (ADRDs), yet our understanding of how it develops into neurodegenerative disease remains limited. Rodents have led our understanding of neurodegeneration, however, these models are limited to short-term behavioral studies of cognitive impacts of neurodegeneration. This is primarily due to their short lifespan, as well as neuroanatomical differences. Based on previous measures of neurodegenerative pathology observed in wild bighorn sheep and muskoxen, we hypothesize that repetitive head impacts induced by natural headbutting behavior in experimental goats can lead to long-term neurodegeneration. Thus, goats could serve as a long-term large animal model to comparatively represent neurodegeneration in humans. In a pilot study, three adult male goats (Capra hircus) were continuously monitored for six months, T=0 serving as self-control. Continuous video recording paired with an accelerometer measured individual head impact force and frequency over time. Animals were also trained to complete a weekly Y-maze to assess long-term spatial memory. Accelerometer data showed that a high frequency of medium-intensity head impact forces occurred daily. Increased maze latency would suggest that memory impairments related to repetitive mild head impacts could indicate the onset of neurodegeneration in this goat model. In this model, repetitive head injuries combined with behavioral signs of neurodegeneration, may serve as a valuable model for human ADRD. These findings have implications for our understanding of the early stages of neurodegenerative diseases, for which research is lacking.

Disclosures: R. Adam: None. **N.L. Ackermans:** None. **A. Oyadeyi:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

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Topic: C.10. Brain Injury and Trauma

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VA Research Career Scientist Award IK6BX006188
The Veterans Bio-Medical Research Institute

Title: Strain and sex affect acoustic startle response and prepulse inhibition following blast-induced traumatic brain injury in mice

Authors: *K. E. MURRAY[1,2], V. A. STIRITZ[3,2], A. L. SHAIKH[1,2], T. P. COMINSKI[3], A. RAVULA[4], C. MARIN DE EVSIKOVA[5], V. DELIC[1,2,6], K. D. BECK[3,2,6], B. J. PFISTER[7,8], B. A. CITRON[1,2,6];
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Abstract: Over the past two decades, blast exposure has become the most common cause of mild traumatic brain injury (TBI) in military personnel due to the use of improvised explosive devices (IEDs) and heavy weaponry during conflicts in Iraq and Afghanistan. Many combat Veterans with a history of blast-induced TBI (bTBI) fare poorly on measures of global disability, psychiatric symptoms, and neurobehavioral impairment compared to combat-deployed controls, even five years after injury. However, these Veterans show highly variable patterns of recovery, and there is no effective treatment for those who suffer long-term neurocognitive effects following bTBI. We hypothesize that genetic predispositions can influence susceptibility to neuronal damage or, conversely, promote resilience and repair after blast exposure. To examine the effects of genotype and sex on response to bTBI, we exposed both male and female mice from eight genetically diverse strains (129S1/SvImJ, A/J, CAST/EiJ, C57BL/6J, NOD/ShiLtJ, NZO/HlLtJ, PWK/PhJ, WSB/EiJ) to a single 180-kPa blast using a well-established shock tube model. We have also extended our analysis of strain-specific gene dysregulation in response to blast exposure. We assessed the interaction between bTBI and mouse strain or sex on brainstem function and sensorimotor gating by acoustic startle response (ASR) and additional neurocircuits by prepulse inhibition (PPI) at 1 week, 1 month, and 3 months post-injury (n = 11-12/group). Preliminary results demonstrated that both male and female C57BL/6J but not 129S1/SvImJ mice showed significant reductions in ASR at 108 dB at 1 week and 1 month post-injury. Both male and female C57BL/6J as well as male 129S1/SvImJ mice displayed a decrease in response magnitude on paired (prepulse-pulse) trials and enhanced percent PPI at 1 week post-injury with recovery by 1 month post-injury. Additionally, we found that male C57BL/6J mice showed

significant dysregulation of genes in the prepulse inhibition gene ontology (GO) category (p = 0.040). Our findings suggest that cognitive impairment and recovery following bTBI may vary among individuals due to genetic and/or sex-related differences. Future studies will allow us to identify personalized therapeutic targets and identify genetic factors to help predict impairment or recovery for Veterans with a history of bTBI.

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Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

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Program #/Poster #: PSTR216.24/D1

Topic: C.10. Brain Injury and Trauma

Support: I01RX002705

Title: Chronic hippocampal-amygdala circuit alterations underlie TBI induced effects in a contextual fear conditioning paradigm

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Abstract: Traumatic Brain Injury (TBI) and Post-Traumatic Stress Disorder (PTSD) are two of the most common conditions affecting Veterans. However, little is known about the comorbidity of these two disorders, or how they interact and progress following injury. To study the effect of injury on fear memory circuits during acquisition and extinction, we subjected rats to a lateral fluid percussion injury (FPI) of 2.1 atm, creating a unilateral TBI. Animals were then run through contextual fear conditioning (CFC) at a chronic time point 6 months post injury (n=7) or sham surgery (n=8). Two rats from each group were implanted with drivable, active 64 channel probes in the hippocampus (HC) and amygdala to obtain electrophysiology recordings at baseline and during CFC.We saw profound changes in the electrophysiology of the chronic post TBI animals. Our data indicate that single unit activity was greatly affected at this time point, with increases in the HC firing-rate, and a decrease in single unit activity in the amygdala. HC power in the theta range remained significantly decreased at 6 months as it was at acute points. In the amygdala, there was corresponding loss of theta and a loss of gamma, which further decreased post acquisition in both sham and injured animals. Oscillatory coherence between HC and amygdala differed in injured rats at theta, and was greater after acquisition, suggesting loss of HC theta is compounded by a desynchronization of theta between HC/amygdala. The prominent loss of phase amplitude coupling (PAC) between theta and gamma seen in our HC acute cohort was also confirmed at this chronic injury timepoint. PAC between HC theta phase

and amygdala low-gamma amplitude (~30Hz) was present in the sham animals and strengthened post acquisition. A profound difference is present in the injured animals, where theta phase was coupled to high gamma (~85Hz) amplitude instead, which decreased post acquisition.Overall, changes in oscillatory behavior both within and between HC and amygdala suggests a loss of coordination of theta and gamma at chronic time points post TBI. These alterations may underlie our observed behavioral differences in CFC extinction, and suggest dysfunction in limbic circuitry in chronic TBI. Profound changes in HC theta-gamma coupling, and possible downstream effects in the amygdala, suggest that neuromodulatory restoration of PAC in the HC may aid extinction. We have also piloted the use of female rats for this injury/behavioral paradigm. Early analysis suggests modifications are needed to account for size differences between sexes. Females also show several base behavioral differences pre-injury that need to be accounted for in comparative results.

Disclosures: E. Halter: None. **E. Mirzakhalili:** None. **C.D. Adam:** None. **J.A. Wolf:** None.

Poster

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.25/D2

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS112642

Title: Identifying and Comparing Learning and Memory Deficits in Two Different Models of Repetitive Traumatic Brain Injury

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Abstract: Identifying and comparing learning and memory deficits in two different models of repetitive traumatic brain injury
Moiz Hasan[1] and Zahoor A. Shah[1,21]Pharmacology and Experimental Therapeutics,[2]Department of Medicinal and Biological Chemistry, College of Pharmacy and Pharmaceutical Sciences, Toledo, Ohio US
Many pastimes and sporting events today involve regular or repetitive hits or blows to the head, resulting non-symptomatic traumatic brain injury (rTBI) and the long term, chronic traumatic encephalopathy (CTE). These sports include American Football, boxing, martial arts, hockey, lacrosse, basketball, soccer, rugby, and many more. It has been reported that players who play these sports will undergo significant behavioral change over time and molecular abnormalities such as increased risk of neuroinflammation and tauopathy. Understanding these changes and potentially developing a treatment for them remains a crucial mystery researchers could solve. Our lab has investigated the relationship between adverse behavior in the form of memory and

learning, which is associated with repetitive traumatic brain injury. While many studies are associated with rTBI, there seems to be no agreement on optimal experimental conditions. Therefore, in this study, two different methodologies will be compared to optimize the effects and safety of experimental subjects. The first method is a repetitive TBI with 48 hours in between each TBI for one week. The second comparative method involves one weekly TBI for four weeks. As a comparison, both methods have two groups: a surgery group where the TBI will be administered and a sham surgery group mimicking the surgery group in all conditions except TBI. Memory and learning were determined through a T-maze and Open Field task. In the 48-hour repetitive TBI, the surgery group alternated significantly less (30% ± 12.25 vs 90% ± 6.124) than the sham group, implying a significant difference in cognition. Further, the open field heat map showed that the surgery group would cluster towards one side of the open field. In contrast, the sham group adequately explored more sides of the open field, implying a large significant difference in learning between the rTBI group and the sham surgery group again. Key words, Repetitive TBI; Learning and Memory deficits; Neuroinflammation; T-maze; open Field task Funding: National Institute of Neurological Disorders and Stroke of the National Institutes of Health #R01NS112642 to ZAS

Disclosures: S. Hasan: A. Employment/Salary (full or part-time):; University of Toledo. **Z.A. Shah:** A. Employment/Salary (full or part-time):; University of Toledo. 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

PSTR216: TBI Inflammation, Emotion, Learning, Memory, and Early Life

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR216.26/D3

Topic: C.10. Brain Injury and Trauma

Support: DoD ERP CDMRP W81XWH-20-1-0901
VA MERIT I01RX002705
VA I01RX003498-01

Title: Touchscreen visuomotor associative learning tasks for cognitive assessment following TBI in pigs

Authors: *S. PENNACCHI[1], T. A. ALLEN[2], O. E. FRUCHET[3], E. R. HALTER[1], C. D. ADAM[4], E. MIRZAKHALILI[1], J. A. WOLF[1];
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Abstract: We have designed and adapted two touchscreen tasks for use with Yucatan mini pigs. Pigs are trained to associate visual stimuli with specific touch responses. Both tasks are visuomotor associative learning paradigms developed to assess cognitive changes post-traumatic brain injury (TBI). We've tested these paradigms in pigs that have received controlled cortical impact (CCI) and have seen early deficits between injured and sham animals. In the Conditional Association Task (CAT), the pig is presented with one of three random stimuli, then forced to choose the right or the left box on the touchscreen. Two images are “fixed” trial stimuli associated with either the left or the right box responses. The third image is a conditional trial where the pig must remember the result of the previous “fixed” trial and choose the opposite response. Performance is determined by the percentage of correct responses (fixed and conditional) and sessions to reach criteria (70% correct on conditional trials, 95% correct for fixed trials). After reaching criteria, pigs underwent CCI (n = 2) or sham injury (n = 1). Injured animals took over 50% longer to reach criteria on conditional trials in the first month post injury compared to the sham animal. At 3 months post injury, injured animals took 62% longer to reach conditional criteria compared to the sham. Animals were also trained on the Delayed Match to Sample Sequence (DMTSS) task. Pigs are trained initially to choose a random stimulus image out of a group of four image choices. Once an animal is fully trained on a simple sample matching task (>70% correct answers), there are two ways to increase the difficulty of the task. First, a variable time delay can be added between the stimulus presentation and the response screen. Second, the animal must remember and reproduce a sequence of two stimuli instead of a single stimulus image. Finally, both these items can be combined into a delayed sequence matching task. Pigs were trained on a delayed sample matching task with a variable delay from 0.5 to 6 seconds. Injured animals trained on the DMTSS task (n = 4) had a drop of over 10% in their percent of correct responses post-injury, which took a week of training to recover. This preliminary data suggests these spatial, episodic, and frontal-hippocampal dependent memory tasks may detect changes in hippocampal/limbic circuitry after CCI.

Disclosures: S. Pennacchi: None. **T.A. Allen:** None. **O.E. Fruchet:** None. **E.R. Halter:** None. **C.D. Adam:** None. **E. Mirzakhalili:** None. **J.A. Wolf:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.01/D6

Topic: C.10. Brain Injury and Trauma

Title: Concurrent *Toxoplasma gondii* infection induced Neuroinflammation in traumatic brain injury patients in a referral hospital in Cameroon, Sub-Saharan Africa

Authors: *F. C. BUH[1], G. S. TAIWE[2], I. N. SUMBELE[3], K. W. WANG[4], I. ESENE[5], P. J. HUTCHINSON[6];
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Abstract: Introduction: *Toxoplasma gondii* infection reduces cerebral microvascular perfusion and induces neuro-inflammation through activation of cerebral endothelial cells, which could affect traumatic brain injury (TBI) outcomes, but whether TBI inflammatory profile and outcomes differ in persons with concurrent latent infection with *Toxoplasma gondii (T. gondii)* have not yet been elucidated in humans, and therefore constituted the core of this study.**Objective:** The aim of this study was to explore the inflammatory profile in TBI patients and the influence of concurrent *T. gondii* infection on neuroinflammation and TBI outcomes.**Methods:** Inflammatory markers [interleukin (IL)1β, IL6, IL10, interferon-gamma (INF-ɣ), Tumor necrosis factor-alpha (TNF-α), and *Toxoplasma gondii* infection were detected in serum obtained <24 h post injury. Glasgow outcome scale-extended (GOSE) was used to evaluate the 6-month outcome post-discharge. The Wilcoxon and Kruskal-Wallis's rank sum tests were used to compare concentrations of inflammatory markers to *T. gondii infection* and TBI outcome.
Results: *Toxoplasma gondii* infection was detected in 33% (52/160) of TBI cases. There was a significant increase ($p<0.001$) in the concentrations of all neuroinflammatory markers (NIM) in TBI patients when compared with the controls. These increases in levels of NIM did not vary significantly with the severity of TBI ($p>0.05$). No statistically significant association was found ($p>0.05$) between inflammatory marker levels and outcome. However, IL6, INF-ɣ, TNF-α had higher values with mortality. Interestingly, there was increase in NIM levels for all the *T. gondii* positive TBI cases, albeit significant for IL1β ($P<0.001$) and TNF-α ($P<0.001$). IL1β and TNFα levels were significantly elevated in unfavorable [94 (92, 96), 93 (91, 96), $p<0.001$] and favorable [33 (31, 37), 34 (28, 44)], $p<0.001$] outcomes for TBI cases seropositive to *T. gondii* compared to TBI cases without *T. gondii* infection [73 (63, 78), 73 (62, 81)] [27 (22, 31), 25 (23, 28)], respectively. **Conclusion:** Concurrent *Toxoplasma gondii* infection in TBI significantly influenced neuroinflammation profile of patients. Further multicenter studies with larger sample sizes will provide more insights into *T. gondii* induced neuropathology in TBI.Keywords: Neuroinflammation, Traumatic brain injury, *Toxoplasma gondii*, Sub-Saharan Africa
Indexinfection ... 1, 2, 3neuro-inflammation ... 1oxidative stress ... 1T. gondii ... 1, 2, 3TBI ... 1, 2Toxoplasma ... 1

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.02/D7

Topic: C.10. Brain Injury and Trauma

Title: Exploring Connections to Broader Neurodegenerative Diseases: Glial Senescence in Chronic Traumatic Encephalopathy

Authors: *A. GAMBOA[1], P. KOTA[2], B. T. HARRIS[3,4];
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Abstract: Objectives: This study aims to investigate the role of Δ133p53α, a modulator of cellular senescence, in Chronic Traumatic Encephalopathy (CTE) and its potential relevance to other age-related neurodegenerative diseases (NDDs), like Alzheimer's Disease (AD), Amyotrophic Lateral Sclerosis (ALS), and Parkinson's Disease (PD). By analyzing Δ133p53α levels and cell types across these conditions, we hope to identify potential shared mechanisms of neurodegeneration and explore Δ133p53α as a diagnostic and therapeutic target for a broader range of these diseases. **Methods:** Human postmortem CTE brain samples were obtained from Boston University CTE Center (n = 10). Additionally, other NDDs (n = 10) and control (n = 10) tissue was obtained from Georgetown University's Brain Bank. To investigate protein markers associated with the NDDs, we performed western blot analysis. PHF-1 was used to confirm abnormal tau accumulation, P21 for presence and extent of cellular senescence, and MAP4 for Δ133p53α-specific levels. **Results:** A Mann-Whitney U test revealed significant changes when comparing disease groups to a control group. Both AD and ALS showed a significant decrease in Δ133p53α levels (p = 0.015 and 0.002 respectively) compared to the control group. Interestingly, neither AD nor ALS displayed a significant difference in p21 levels compared to controls (p = 0.329 and 0.536 respectively). However, both diseases did show a significant increase in PHF-1 levels compared to controls (p = 0.008 and 0.021 respectively). PD also showed a significant decrease in Δ133p53α (p = 0.009) compared to the control group. However, unlike AD and ALS, PD showed a significant increase in p21 levels compared to controls (p = 0.004). **Conclusions:** Our findings confirm that cellular senescence plays a role in CTE pathogenesis and Δ133p53α could be targeted as a biomarker for age-associated neurodegenerative conditions and could serve as a potential therapeutic target. Importantly, we are currently identifying and quantifying senescent cell types with immunohistochemistry and QuPath image analysis. Additionally, we plan to further study Δ133p53α and cell types using RNAscope. **Scientific Rigor:** Sample sizes were 10 and all groups of tissue were anonymized, age-matched brain samples. Sex as a biological variable was considered and is currently being assessed. A Mann-Whitney U test, a non-parametric test, was employed to compare the groups.

Disclosures: A. Gamboa: None. **P. Kota:** None. **B.T. Harris:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.03/D8

Topic: C.10. Brain Injury and Trauma

Support: NIH T32 Training Grant NC State CVM

Title: Dynamic and Static Resting-State Functional Connectivity of Canonical Networks in Military and Civilian Populations with Posttraumatic Stress Disorder and Mild Traumatic Brain Injury

Authors: *A. K. DWULIT[1,2], C. C. HASWELL[3], E. L. DENNIS[4,5], P. M. THOMPSON[6], J. U. BLACKFORD[7], A. P. KING[8], I. LIBERZON[9], M. ANGSTADT[10], H. WALTER[11], S. KOCH[12,13], D. VELTMAN[14], L. NAWIJN[15,16], R. MOREY[3,17,18];
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Abstract: Posttraumatic stress disorder (PTSD) and mild traumatic brain injury (mTBI) are associated with insults to the functional connectome. Alterations in the default mode (DMN), central executive (CEN), and salience networks (SN) are implicated in the triple network model of PTSD. By contrast, alterations associated with mTBI are not restricted to particular functional networks. Frequently co-occurring, comorbid mild TBI and PTSD are linked to worse functional outcomes. Nevertheless, knowledge about the functional connectome in comorbid mTBI and PTSD is scant. We investigated brain phenotypes from resting-state fMRI associated with PTSD, mTBI, and comorbid PTSD+mTBI in US veterans and civilians (n=1526) from ENIGMA-TBI and ENIGMA-PTSD. We examined static functional connectivity (SFC) and dynamic functional connectivity (DFC) within the CEN, SN, and DMN and between networks (i.e. SN-DMN). We used ComBat-GAM to harmonize FC across sites. One-way ANCOVA was followed by post-hoc linear regression to test main effects of diagnosis and interaction effects of diagnosis and age, diagnosis and military status, diagnosis and sex, diagnosis and anxiety severity, and diagnosis and depression severity. Co-variates included age, sex, military status, depression severity, PTSD severity, anxiety severity, number of loss of consciousness events, time since most recent TBI, and Major Depressive Disorder (MDD) diagnosis. We found that comorbid PTSD+mTBI individuals, relative to healthy controls, had significantly ($p_{FDR}<0.01$) different DFC between SN and CEN, DMN and CEN, and SN and DMN, and within SN. Comorbid PTSD+mTBI individuals, relative to individuals with mTBI-only, had significantly ($p_{FDR}<0.05$) different DFC between SN and CEN, DMN and CEN, SN and DMN, and within SN. Comorbid individuals, relative to individuals with PTSD-only, had significantly ($p_{FDR}<0.01$) different DFC within SN. Thus, we conclude that DFC is a more accurate diagnostic predictor than SFC. Between-network DFC of the triple network (DMN, CEN, SN) is better than within-network

DFC at relating to diagnostic grouping. Increased DFC in comorbid PTSD+mTBI may be a compensatory mechanism to meet cognitive demand in the setting of damage to the functional connectome. Increased DFC may reflect unsuccessful recruitment of brain regions that are working less efficiently to compensate for damaged networks.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.04/D9

Topic: C.10. Brain Injury and Trauma

Title: Autonomic dysfunction in adults with mild traumatic brain injury

Authors: *E. BAT-ERDENE[1,3], T. HIRAMOTO[4], E. TUMURBAATAR[2], G. TUMUR-OCHIR[5,6], K. MUNKHJARGAL[7], *E. ERDENEBAT[7], B. SELENGE[7], H. ADACHI[8], B. LKHAGVASUREN[1,7,9];
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Abstract: Autonomic dysfunction may occur following mild traumatic brain injury (MTBI), contributing to increased mortality. The effects of MTBI on autonomic nervous system (ANS) functions and mental health in the general population are less well-studied. Therefore, we aimed to investigate the autonomic dysfunctions and psychological symptoms of those who reported a lifetime MTBI in the general population. This study was part of a nationwide, population-based cohort study conducted in the adult population of Ulaanbaatar, Mongolia. We collected demographic characteristics, lifestyle information, history of MTBI, and conducted medical examinations. The dysfunction of the ANS was evaluated by a noninvasive analysis of the heart rate variability (HRV). Anxiety, depression, sleep quality, and quality of life were examined using the Hospital Anxiety and Depression Scale (HADS), the Pittsburgh Sleep Quality Index (PSQI), and the brief version of World Health Organization Quality of Life (WHOQOL-BREF), respectively. This study included a total of 177 participants (46 males and 131 females) aged 18 – 65 years with a mean of 39.85 ± 9.82 years. Among them, 16 participants reported a history of MTBI. The age and gender-adjusted prevalence of MTBI was 2.4%. Body temperature, heart rate, and the anxiety score was higher (P=0.028, P=0.039, and P=0.04, respectively), whereas pNN50, and RMSSD were lower in participants with MTBI history compared to participants

without MTBI history ($P<0.001$ and $P<0.001$, respectively). Overall, pNN50 was correlated with the WHOQOL-BREF domains (psychological: $r=0.194$, social: $r=0.156$, and environmental: $r=0.204$), and inversely correlated with BMI, waist circumference, and the HADS anxiety score ($r=-0.215$, $r=-0.272$, $r=-0.218$, and $r=-0.160$, respectively). The LF/HF ratio was correlated with the neck and waist circumferences ($r=0.229$ and $r=0.185$) and inversely correlated with depression ($r=-0.167$). In participants with MTBI history, the pNN50 was correlated with the systolic blood pressure ($r=0.629$), and inversely correlated with age and heart rate ($r=-0.631$, $r=-0.582$). In contrast, LF/HF was correlated with the neck circumference ($r=0.650$) and inversely correlated with depression ($r=-0.567$). Logistic regression analysis suggests that decreases in pNN50 and WHOQOL-BREF, and increases in body temperature and anxiety were the contributing predictors of MTBI.Participants with the MTBI history had decreased HRV and increased anxiety compared with the general population with no lifetime history of MTBI. Furthermore, these results suggest that MTBI negatively affects the autonomic functions, mental health, and quality of life.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.05/D10

Topic: C.10. Brain Injury and Trauma

Support: NIH 1R01NS094444
NIH UL1TR001412
NIH 1R42NS119119

Title: Diffusion magnetic resonance imaging analysis after sports-related concussion in adolescents

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Abstract: Sport-related concussions (SRC) are a public health concern, prompting their investigation using advanced neuroimaging techniques such as diffusion magnetic resonance imaging (dMRI). Studies in the past have used dMRI to investigate SRC and traumatic brain injury in adolescents with mixed results, but importantly, none have included the brainstem in their analyses. Autonomic nervous system (ANS) dysfunction during SRC is thought to be a cause of exercise intolerance, defined as symptom exacerbation that limits exercise performance,

which can be experienced following an SRC. Many of the crucial ANS centers are in the brainstem, therefore currently not much is known about the pathophysiology of exercise intolerance in adolescent athletes. Recent work has developed five different SRC symptom subtypes to deal with the variety of SRC symptoms. However, no studies have correlated these symptom subtypes with structural brain analyses, which would allow for examination of the pathophysiology of symptom subtypes in SRC. Here, will fill this gap by characterizing the neurological profiles associated with SRCs in adolescent athletes through integration of structural brain analyses, including diffusion and network analyses, and the association of those analyses with ANS dysfunction and symptom subtypes. We collected dMRI, cognitive, physiological, and clinical data from 13-18-year-old adolescent athletes within 10 days of experiencing an SRC (baseline) and after recovery (follow-up). Healthy, age and sex-matched controls had the same data collected at baseline and follow-up (4-6 weeks later) visits. The dMRI data are multishell (b=1000, b=2000, 46 directions) whole brain scans. A total of 35 SRC and 34 controls with dMRI data were collected. The dMRI data was preprocessed, a population specific atlas was chosen, and generalized q-sample imaging (GQI) was used for reconstruction and tractography. We find structural brain differences, seen in diffusion and network measures, between adolescent athletes with an SRC and healthy controls, specifically in the brainstem regions. Further, these differences correlate with injury symptoms experienced by the adolescents. By incorporating the brainstem, exploring the intricate relationship between exercise intolerance and ANS dysfunction, and leveraging advanced dMRI methodologies, this proposal offers a novel approach to filling critical gaps in the existing literature.

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Poster

PSTR217: Brain Injury: Human Studies

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Program #/Poster #: PSTR217.06/D11

Topic: C.10. Brain Injury and Trauma

Support: K23NS112596-01A1

Title: Exploring the power of the glasgow coma scale and the modified rankin scale when compared to resting state functional connectivity in acute and subacute traumatic brain injury patients

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Abstract: Traumatic brain injury (TBI) is a leading cause of disability worldwide. Even if MRI is increasingly used post-TBI, the utility of functional connectivity (FC) scans is less well-defined in the clinical setting. Also, the association of sub/acute FC scans and common clinical assessments, like the Glasgow Coma Scale (GCS) and modified Rankin scale (mRS) are not well described. Here, we explored if such standard tests can be predicted by resting state FC. Methods: We retrospectively (2019-2022) collected resting fMRI and clinical data from 50 patients (19F, mean age = 48y) across all TBI severities, after excluding those with prior neurologic deficits. Anatomical and functional preprocessing included standard procedures alongside regression of mean time-courses in WM, CSF and GM, global signal regression and low-pass Gaussian filter. We used a 268-node FC atlas to calculate the mean time-course of each region (node) and the correlation for each pair of nodes (edges). Fisher transformed Z-scores for 35,778 unique edges were derived. We used regions of interest (based on 10 resting state networks) and whole-brain analysis to determine if FC and GCS (at admission and at scan) and mRS (at 3months) are associated. We also compared FC values with TBI severity based on GCS (i.e. mild, moderate and severe). The Benjamin-Hochberg procedure was applied to control for statistically notable edges. Results: For all TBI severities together, patients showed a significant correlation between: [A] admission GCS and FC of the default mode network (DMN); rho = 0.36(*)), motor (rho = 0.3(*)) and subcortical networks (rho = 0.37(**)); [B] GCS at scan - DMN (rho = 0.35(*)), motor (rho = 0.44(*)), subcortical (rho = 0.41(**)) and cerebellar networks (rho = 0.32(*)); [C] mRS - DMN (rho = -0.28(p = 0.053)) and subcortical networks (rho = -0.29(*)). Further, Kruskal-Wallis was significant for the severe-mild for DMN and subcortical (scan/admission GCS) and motor networks (admission GCS). For total GCS at admission/scan, whole brain analysis did not show notable edges between the severe and mild groups. Correlation-wise, all TBI patients, together or by severity, had a high number of significant positive and negative edges (thr 0.001). Discussion: GCS at scan had a slightly stronger correlation to FC vs admission GCS, though all correlations remain weak. Also, the data suggest some FC networks collected sub/acutely may reflect prognostication of 3-month mRS. Taken together, acquiring sub/acute FC scans post-TBI may provide complementary prognostic information beyond GCS at admission/scan providing better care for TBI patients. Further clinical studies are needed to address this assumption.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.07/D12

Topic: C.10. Brain Injury and Trauma

Title: Does dual-task walking affect cognitive performance in individuals with/out concussion 'negatively or positively'?

Authors: T. MUSSIE[1], *P. ACHARYA[1], M. DALECKI[2];
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Abstract: Some studies reported positive effects of priorly executed slight physical activity (SPA) on cognitive performance (CP) in various groups. Others showed CP declines in individuals post-concussion in single-task conditions without SPA. Not many studies have examined whether concurrent cognitive and motor tasks (CCMT) with SPA, such as dual-task (DT) walking, may affect young individual's CP post-concussion. Here, we investigated whether CP of young individuals with (CH) and without concussion history (NoH) was affected in a CCMT scenario with SPA during DT walking compared to CP while standing. Thirty-five college students (M=20 yrs., 68-inch, 180 lbs), including 18 CH (8 females; 45 mths post-injury) and 17 NoH students (11 females) participated in the study. Participants performed two computerized cognitive tasks while standing or walking on a treadmill and were randomly assigned to start in one of the two body positions. The cognitive tests were performed on a laptop: 1) a Stroop Color word test (48 congruent, 48 incongruent trials) and 2) a D2 sustained attention test (computerized version of the D2 test, with varying sequences of d and p letters; participants had to select d´s framed by two vertical lines). The laptop was placed at eye level on top of the treadmill. Participants' comfortable walking speed (CWS) was determined before testing (M=1.7 mph) and used in the walking condition. 2 x 2 ANOVAs (standing, walking; CH, NoH) were used to analyze response time (RT; milliseconds) and error rate (ER; %) in both Stroop test conditions, as well as RT, ER and Sustained Attention Score (CS) of the D2 test. For the D2 test, ANOVA revealed a significant effect of body position on ER ($p<0.05$), suggesting a higher error rate in walking than standing, independent of groups. No other significant effects for D2 test variables were found (all $p>0.05$); descriptively, CS was slightly lower and RT slightly higher during DT walking. For the Stroop test, no significant effects nor interactions were found (all $p>0.05$), suggesting Stroop performance, in contrast to sustained attention levels, was not negatively affected during DT walking compared to standing. Our results suggest CP declines during DT walking only for sustained attention levels but not for Stroop performance and no extra CP declines in individuals with CH compared to NoH. The latter outcome stays in contrast to previous work in a similar CH cohort without concurrent SPA such as DT walking. Thus, concurrent SPA may have been a factor in masking potential CP declines in the CH group in the present study. However, further research is needed to better understand the effect of CCMT scenarios with SPA on CP in CH and NoH individuals.

Disclosures: T. Mussie: None. **P. Acharya:** None. **M. Dalecki:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.08/D13

Topic: C.10. Brain Injury and Trauma

Support: NINDS U01NS093334
NINDS K00NS113419
NIMHD L32MD016519
Burroughs Wellcome Fund Postdoctoral Diversity Enrichment Program

Title: Shape analysis of the amygdala, thalamus, and hippocampus of former American football players: Findings from the DIAGNOSE CTE Research Project

Authors: O. JOHN[1,2], A. WICKHAM[3], L. JUNG[4,5], C. HELLER[6,2], K. BREEDLOVE[3], T. WIEGAND[4,5], N. KIM[3], D. DANESHVAR[7], T. BILLAH[3], O. PASTERNAK[3], C. ADLER[8], C. BERNICK[9,10], M. ALOSCO[11], J. CUMMINGS[12], I. KOERTE[3,13], A. LIN[3], E. M. REIMAN[14,15,16,17], R. A. STERN[18], M. SHENTON[3], S. BOUIX[19], *H. ARCINIEGA[20,2];
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Abstract: Research Objective and Rationale: Exposure to repetitive head impacts (RHI) is associated with brain changes and the potential development of chronic traumatic encephalopathy (CTE). Given the critical roles of the amygdala, thalamus, and hippocampus in cognitive and emotional functioning, detecting alterations within these structures may provide valuable insights into the effects of RHI exposure and possible CTE development. Here, we use a neuroimaging approach to assess group-level differences in the amygdala, thalamus, and hippocampus, while also exploring the influence of age and RHI exposure factors. Methods: Using data from the DIAGNOSE CTE Research Project, we compared the subcortical structural morphology of the amygdala, thalamus, and hippocampus between former American football players (n = 164) and unexposed asymptomatic controls (n = 53). Structural segmentation of these regions was performed with FreeSurfer 7.1, and the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) shape analysis pipeline was employed to generate structural shape reconstructions. Vertex-level measures of shape morphometry, derived from the logarithm of the Jacobian determinant (JD), were used to assess local surface contraction or expansion relative to our study template. Linear regression models were used for group comparisons, while partial correlation analyses were conducted on former football players to explore the effects of age and exposure factors such as the age of first exposure to football and cumulative head impact index (CHII) scores of frequency, linear acceleration, and rotational force. Age, body mass index, education, race, imaging site, Apolipoprotein E4 carrier status, and total intracranial

volume were used as covariates. Results: Former football players exhibited lower JD values in the amygdala and hippocampus bilaterally, compared to controls, indicating local surface area contractions. Older age was associated with lower JD values in all regions bilaterally. A younger age of exposure to football was associated with lower JD values in the thalamus bilaterally and in the left hippocampus. Increased linear acceleration was associated with decreased JD values in the hippocampus bilaterally. Conclusion: These findings build upon our prior volume-based analysis, illustrating the enduring impact of RHI on subcortical structures. Here, we identify local surface area contractions in former American football players and indicate the influence of age and exposure factors. This study sheds light on the specific effects of RHI on subcortical structures, offering valuable insights into their long-term consequences.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.09/D14

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 1015815

Title: Association between cerebral respiratory pulsations and symptom severity in individuals with concussion: a pilot study

Authors: *M. LUTHER[1], L. DENNIS[1], A. SCATENA[1], C. WONG[1], S. KHAMBADKONE[1], E. YAMAMOTO[2], J. PIANTINO[1];
[1]Dept. of Pediatrics, Div. of Child Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Dept. of Neurolog. Surgery, Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Objective: Mild traumatic brain injury, or concussion, is a leading cause of disability worldwide. Approximately one-third of individuals after concussion experience prolonged post-concussive symptoms, but the biological mechanisms behind this remain poorly understood. Cerebrospinal fluid (CSF) dynamics and CSF/interstitial fluid exchange are thought to be critical in removing injury-generated waste from the cerebral parenchyma, thus alleviating the deleterious effects of concussion. Respiratory cerebral oscillations are major drivers of CSF flow. BOLD fMRI pulsation has been shown to accurately reflect respiratory cerebral oscillation. We hypothesized that in participants with concussion, decreased respiratory cerebral oscillation as measured by fast functional MRI associates with worse post-concussive symptoms.
Methods: This pilot study included 11 healthy controls (4 female) and 26 participants diagnosed

with concussion within the last three years (7 female), who provided informed consent. Participants underwent fast functional MRI (TR=400 ms) with concurrent cardiorespiratory monitoring. To standardize respiratory frequencies, participants were instructed to synchronize their breathing to a visual cue, i.e., a ball oscillating at 0.2 Hz (~12 breaths per minute). Voxel-wise amplitude spectra were generated using fast Fourier transform, and fractional amplitude of low frequency fluctuation (fALFF) was used to measure differences in the magnitude of respiratory oscillations ($fALFF_{resp}$) between controls and cases. Linear regression was used to estimate the effect of concussion on $fALFF_{resp}$ in different brain regions, adjusting for sex and age. The association between $fALFF_{resp}$ and post-concussive symptom severity (assessed with the Sports Concussion Assessment Tool, SCAT) was assessed with Pearson's correlation coefficient.
Results: Controls had higher $fALFF_{resp}$ values compared to the concussion group in the caudate ($p=0.31$), putamen ($p=0.02$), and thalamus ($p=0.01$). In participants with concussion, post-concussive scores negatively correlated with fALFF in the cerebral cortex ($r(26)=-0.47$, $p=0.03$) and the caudate ($r(26)=-0.45$, $p=0.04$) such that participants with lower fALFF had higher SCAT scores.
Conclusions: Participants with concussion exhibited decreased regional respiratory cerebral oscillations to controls and in association with worse post-concussive symptoms. Decreased respiratory cerebral oscillations after concussion may be an important neuroimaging-based biomarker of post-concussive symptom severity and recovery.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.10/D15

Topic: C.10. Brain Injury and Trauma

Title: Evaluating eye movements in concussed individuals to explore executive function

Authors: *L. J. STEINKE[1], E. POWNALL[2], L. E. BROWN[2];
[1]Psychology, Trent Univ., Peterborough, ON, Canada; [2]Trent Univ., Peterborough, ON, Canada

Abstract: People who have sustained a concussion often complain of brain fog, a type of executive dysfunction that leaves people feeling slow and forgetful, easily distracted, and unable to bring the current task into focus. Because both executive function and purposeful eye movement performance are dependent on intact frontal-cortex systems, we aimed to determine whether post-concussion eye-movement performance is related to post-concussion executive function. ***Objectives:*** 1) Are subtle differences in eye-movement performance in concussed individuals related to their experience of executive dysfunction or brain fog? 2) Does this relationship vary with recovery time? ***Method:*** Twelve concussed and 11 lower-body injury-

control participants (injuries occurring during the past 5 years) completed a standardized battery of tests regarding concussion experience (e.g. SCAT-5), experience with depression (Beck Depression Index), executive function (Trail-Making, Verbal Fluency, Stroop, and BRIEF-A), and eye-movement performance (pro- and anti-saccade tests, continuous sinusoidal smooth pursuit tracking test). ***Results:*** Concussed individuals were significantly more likely to report ongoing concussion-related symptoms than injury controls. The two groups did not differ on neuropsychological measures of executive function nor on performance measures of pro- vs anti-saccades. Concussed participants showed significantly more horizontal and vertical smooth pursuit spatial variability than injury controls while tracking a sinusoidal target. Interestingly, smooth pursuit spatial variability was significantly negatively related to time since injury, and while not related to tests of executive function, was explained by concussed participants' scores on the Beck Depression Inventory (R^2= .33). ***Conclusions:*** These findings suggest that people who have sustained a concussion experience differences in eye-movement performance that are sensitive to time since injury.

Disclosures: L.J. Steinke: None. **E. Pownall:** None. **L.E. Brown:** None.

Poster

PSTR217: Brain Injury: Human Studies

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.11/D16

Topic: C.10. Brain Injury and Trauma

Support: Mangasar M. Mangasarian Award Fund
Eugene V. Cota-Robles Award

Title: The neural consequences of mild traumatic brain injury and cognitive impairment

Authors: *S. A. ASHIKYAN[1], A. HOLDER[1], M. M. MONTI[2];
[1]Psychology, UCLA, Los Angeles, CA; [2]UCLA, Los Angeles, CA

Abstract: Worldwide, about 42 million individuals endure a mTBI every year with over 5.3 million individuals currently living with disability that was caused by a TBI in the United States alone. The vast majority of studies tend to focus on one Magnetic Resonance Imaging (MRI) modality/type only, which is an important limitation since different MR images can capture different aspects of the brain. We have designed a study which will allow us to leverage multiple types of scans and fuse them using FSL-PALM (Permutation Analysis of Linear Models) coupled with non-parametric combination (NPC) to correlate MRI scans to behavioral variables. We used a total of four modalities; T1-weighted, T2-weighted echo 1, T2-weighted echo 2, and Fluid-Attenuated Inversion Recovery (FLAIR) imaging. Data from 136 U.S. military personnel was obtained across two timepoints (baseline and 3-months post-injury) and two groups (mTBI and control). Principal component analysis (PCA) was performed on the 12 behavioral variables selected and both sex and age were accounted for. We aimed to answer two questions: (1) What

is the relationship between neural damage and cognitive impairments; (2) Can the fusion of multiple types of MRI images be leveraged to predict outcomes at three-months post-injury on the basis of behavioral data? This study will be one of the first to leverage the power of fused multimodal MRI data to assess the link between brain and mind in the context of mTBI. Our preliminary findings suggest that within 24-hours of obtaining a mTBI, patients who report a lack of resilience also tend to have significant structural differences in the thalamus, right caudate putamen, hippocampus, pallidum, insular cortex, amygdala ($P<0.05$) across all MRI modalities. The structural integrity of these regions implies potential impacts of white matter lesions, changes in brain volume associated with atrophy, and changes in intracranial volume and blood flow. On the other hand, our 3-month post-injury analysis suggested that patients who reported more symptoms of depression and performed higher on both recall and delayed recall visuospatial memory tasks also tended to have an increase in the presence of white matter lesions in cerebral white matter, posterior and anterior cingulate gyrus, caudate, corpus callosum, and thalamus ($P<0.05$) only in the FLAIR modality. We aim to better understand mTBI because it is crucial for the development of effective discharge and return to active-duty guidelines for military personnel, return-to-play protocols, and sport gears (helmets) for athletes, and motor vehicle airbag advancement for the general civilian population.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.12/D17

Topic: C.10. Brain Injury and Trauma

Support: CIHR grant PJT-175063

Title: Estrogen levels alter cognitive-motor integration-related brain network function and symptom severity in working-aged adults with persisting symptoms after concussion

Authors: T. SHAHZAD[1], K. K. BUMRA[2], M. OZZOUDE[2], N. SMEHA[1], S. E. WEINBERG[1], D. J. GORBET[1], A. K. MACPHERSON[1], *L. E. SERGIO[1];
[1]Sch. of Kinesiology & Hlth. Sci., York Univ., Toronto, ON, Canada; [2]Psychology, York Univ., Toronto, ON, Canada

Abstract: Background: Often we interact with our environment indirectly, where rules dictate the relationship between perception and action and require cognitive-motor integration (CMI). The underlying CMI control networks are often compromised following concussion, resulting in an impaired ability to engage in complex movements. Our group has previously found relationships between white matter integrity and visuomotor performance in working-aged adults with persisting post-concussion symptoms (PPCS). We have also observed altered functional connectivity between brain regions as a function of sex hormones when performing a CMI task.

Here, we addressed the knowledge gap on the relationship between sex hormones and movement control in working-aged adults affected by PPCS. Methods: 22 working-aged participants with PPCS (17 female, mean age 47.2±9.3 years old) completed two visuomotor assessment tasks, one with a direct interaction between visual feedback and motor output, and one with CMI (hand motion in a different spatial plane/visual feedback reversal between viewed target and cursor feedback). PPCS symptom severity was gathered using validated questionnaires. Resting state fMRI was collected to analyze brain network functional connectivity in CMI-related brain regions. Hormone concentrations of progesterone, estrogen, and testosterone were collected through a saliva sample and analyzed using ELISA. Results: After controlling for age, we observed a positive association between estrogen levels and the visual attention resting state network functional connectivity (RSFC, $p<0.05$). Further, we observed that elevated estrogen was significantly predictive of early symptom severity (RPQ-3; $p<0.05$). Lastly, we found significant correlations between both the CMI performance kinematics (accuracy, precision, and path length composite score) and the RPQ-3 score with the somatomotor RSFC ($p<0.05$) Conclusions: Our findings indicate that symptom severity and complex skill-related resting state functional connectivity vary as a function of sex hormone level in adults with persisting post-concussion symptoms. We suggest that an individual's sex and gender may affect their experience of, and functional recovery from, concussion.

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Poster

PSTR217: Brain Injury: Human Studies

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Program #/Poster #: PSTR217.13/D18

Topic: C.10. Brain Injury and Trauma

Support: National Natural Science Foundation of China 81925031
National Natural Science Foundation of China 81820108026

Title: Regulatory CD4+ T cells highly expressing TIGIT play a protective role in radiation-induced brain injury

Authors: *Z. DENG, Y. ZUO, Y. TANG;
Neurol., Sun Yat-sen Mem. Hosp., Guangzhou, China

Abstract: Radiation-induced brain injury (RIBI) is a common and severe complication after radiotherapy for head and neck cancers. Much is still unknown regarding the underlying pathogenesis mechanism, impeding the development of effective treatment. Our group has shown that the infiltration of $CD8^+$ T cells into brain mediates brain injury after radiation. However, it remains unclear whether $CD4^+$ T cells are involved in RIBI. Using single-cell RNA

sequencing (scRNA seq) of the paired brain lesions tissues and PBMCs of RIBI patients, we identified the infiltration of $CD4^+$ T cells, further validated by immunohistochemical staining on the brain lesion slices of patients. In a RIBI mouse model with gamma-ray irradiation, we also observed the increase of $CD4^+$ T cells in brain lesions through immunofluorescent staining and flow cytometry. To assess the pathological role of these $CD4^+$ T cells in RIBI, we administered CD4 neutralizing antibodies to deplete $CD4^+$ T cells in the RIBI mouse model for eight weeks after irradiation. We found that brain lesion was significantly increased, accompanied by the aggravated activation of $IBA1^+$ microglia, suggesting that these $CD4^+$ T cells exert an overall protective role in RIBI. $CD4^+$ $CD25^+$ regulatory T cells (Tregs) are classical immune modulators to control excessive immune response. Further exploring the infiltrated $CD4^+$ T cells with scRNA-seq data, Tregs were significantly enriched and highly expressed immunosuppressive-related genes (*CTLA4*, *TIGIT*, and *LAG3*). We also confirmed the high and specific expression of TIGIT in infiltrated Tregs in the RIBI mouse model. To further demonstrate whether the protective effect of $CD4^+$ T cells was attributed to these TIGIT+ Tregs, we administered CD25 neutralizing antibody to deplete Tregs in the RIBI mouse model. The results showed that the brain lesion was significantly aggravated, suggesting that $TIGIT^+$ Tregs are involved in the pathogenesis of RIBI with a protective role. To identify the cell type that secret chemokines attracting Tregs, we used CellChat to infer cell-cell communication with scRNA seq. Microglia exhibited the strongest interaction with Tregs. We noted that *CXCR6* was specifically expressed on the infiltrated Tregs. CXCL16, the ligand for CXCR6, was upregulated in microglia with a higher level in the proximal site of the brain lesion than the distal site. We infer that microglia attract Tregs to infiltrate into the brain through CXCL16-CXCR6 axis. Together, these findings illustrate that Tregs highly expressing TIGIT infiltrate into brain through CXCL16-CXCR6 axis and play a protective role in RIBI. Selectively modulating Tregs may be a potential strategy to treat RIBI.

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Poster

PSTR217: Brain Injury: Human Studies

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.14/D19

Topic: C.10. Brain Injury and Trauma

Support: National Key R&D Program of China, 2022YFB4700101

Title: Thalamic spiking activity signals consciousness level and recovery in patients with disorders of consciousness

Authors: *H. WANG[1], Y. YANG[2];
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Abstract: Thalamic spiking activity signals consciousness level and recovery in patients with disorders of consciousness Huan Wang[1], Yongxiang Hu [1,2], Qianqian Ge[3], Yuanyuan Dang[4], Yi Yang[3], Long Xu[3], Peng Zhang[1,5,6], Sheng He[1,5,6], Yan Yang[1,5,6], Jianghong He[31] State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences; Beijing, China[2] School of Life Science, University of Science and Technology of China; Hefei, China[3] Department of Neurosurgery, Beijing Tiantan Hospital, Capital Medical University; Beijing, China [4] Department of Neurosurgery, the First Medical Center of Chinese PLA General Hospital; Beijing, China [5] University of Chinese Academy of Sciences; Beijing, China[6] Institute of Artificial Intelligence, Hefei Comprehensive National Science Center; Hefei, China
Patients with disorders of consciousness exhibit severe declines in arousal and awareness, as well as anomalous functional brain connections and aberrant neuronal activities. Yet, the diagnostic error of patients' consciousness states can reach up to forty percent, resulting in a worse prognosis. Neuronal mechanisms underlying the disorders are indispensable for identifying objective and intrinsic markers of consciousness. As the primary relay station between the brainstem arousal nuclei and the cerebral cortex, the thalamus is responsible for maintaining consciousness and wakefulness. In this study, we examined thalamic spiking activities, thalamocortical connections, consciousness levels, and deep brain stimulation outcomes in patients. We found that thalamic activities can be correlated with consciousness levels: patients with higher consciousness levels had more active thalamic neurons. Furthermore, as a direct treatment site, thalamic profiles of the centromedian/parafascicular complex signal whether stimulation here improved outcomes: stronger and more stable neural discharge was associated with enhanced thalamocortical connections and better consciousness-altering outcomes within a year. We suggest that thalamus plays critical roles in the representation and alternation of consciousness.

Disclosures: H. Wang: None. **Y. Yang:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.15/D20

Topic: C.10. Brain Injury and Trauma

Support: NSERC SMART CREATE Program at the University of Albert
Alberta Innovates

Title: Interhemispheric EEG disparities in poor outcome prognostication after cardiac arrest

Authors: *S. RAIESDANA[1], V. K. MUSHAHWAR[2], S. AHMED[3];
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Abstract: Due to post-cardiac arrest anoxic brain injury, prognostication becomes uncertain. Predicting poor neurological outcomes accurately avoids premature withdrawal of life-sustaining devices, and also futile treatments. Cardiac arrest typically induces global brain injury as a result of reduced blood flow, affecting both hemispheres. However, regional vulnerabilities to hypoxic-ischemic insults may cause asymmetrical patterns and functional impairment. Damage to specific regions within each hemisphere can affect vital functions necessary for recovery post-cardiac arrest. Additionally, asymmetrical damage and hemispheric differences may hinder the brain's capacity to compensate for damage following such an event, ultimately leading to a poorer prognosis. The objective of this study was to identify interhemispheric variations in comatose patients with anoxic brains using EEG activity. We hypothesized that poor outcomes can be correlated to hemispheric disparities in EEG patterns. EEG data were analyzed for 30 comatose patients with anoxic brain injury admitted to the Intensive Care Unit of the University of Alberta Hospital following cardiac arrest. Neurological outcomes at 3 months were prospectively evaluated, with poor outcomes defined as death, vegetative state, or severe disability. The Laterality Index (LI) calculating the lateralized EEG power, the magnitude of squared coherence (MSC) as an index of interhemispheric EEG interdependence in phase and amplitude, and functional connectivity analysis examining interactions and synchrony among regions within and across hemispheres, were utilized. LI, MSC and phase locking value (PLV) measures were calculated for C3-C4 and F3-F4 signals across the delta, theta, and alpha frequency bands. Group analysis revealed an increased delta LI, a significant ($p<0.05$) right interhemispheric MSC and a reduced alpha interhemispheric PLV associated with poor outcomes. Group analysis further revealed that intrahemispheric PLV indicated a more pronounced disruption of neural networks in the left hemisphere across all patients in this study due to the lack of oxygen supply. In conclusion, our findings underscore the possible role of interhemispheric variations in EEG patterns as potential prognostic indicators for poor neurological outcomes in comatose patients with post-cardiac arrest anoxic brain injury. This study sheds light on the search for complementary viewpoints in evaluating comatose EEGs, which can be considered alongside the presence of malignant patterns for outcome prediction.

Disclosures: S. Raiesdana: None. **V.K. Mushahwar:** None. **S. Ahmed:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.16/D21

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant # D43TW009345

Title: Cranial Moulding Practices, Plagiocephaly and Brachycephaly among Young Children in Southern Ghana

Authors: *F. T. DJANKPA[1], G. WIAFE[2], K. O. AFFRAM[3], M. ULANJA[4], E. DORDOYE[5], S. ADJENTI[6], M. S. VAVILALA[7];
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Abstract: Background Hot formentation and cranial molding practices in infants range from 64%-74% and are performed to achieve a "round" head shape. This practice may result in head deformities, brain injury and neurological deficits. Yet, the relationship between cranial moulding and head deformities is poorly studied.**Aim**To determine the prevalence and severity of deformational plagiocephaly and brachycephaly (DPB) and its association with cranial moulding practices.**Methodology**Using a cross-sectional survey approach, we sampled nursing mothers with infants and children 0-2 years in southern Ghana. We determined the prevalence of cranial molding practices, DPB by craniometer and a plagiocephalometer, and their association. DPB severity was scored as mild, moderate or severe using the cranial vault asymmetry index (plagiocephaly) and cephalic index (brachycephaly).**Results**Of the 304 nursing mothers interviewed, 203 (66.8%) endorsed cranial moulding practices. Children with mean age 36 ± 5 weeks were examined. Plagiocephaly (26%) and brachycephaly (3%) were both diagnosed, respectively. DP was severe in 2.5% of infants and children with plagiocephaly. The presence of DPB was associated with caregiver cranial moulding practices (Chi Sq. test, *p = 0.046*, *Cramer V = 0.48*).**Conclusion**Cranial molding practice was common and associated with DPB, which has the potential to cause traumatic brain injury. Education of mothers and infant caregivers to prevent cranial moulding is urgently needed.

Disclosures: F.T. Djankpa: None. **G. Wiafe:** None. **K.O. Affram:** None. **M. Ulanja:** None. **E. Dordoye:** None. **S. Adjenti:** None. **M.S. Vavilala:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.17/D22

Topic: C.10. Brain Injury and Trauma

Support: CIHR PJT-175063
NSERC CREATE DITA

Title: Using Kinematics Measured During an Augmented Reality Task Sensitively Classifies Individuals Affected by Concussion

Authors: *A. MACHULA[1,2], A. KHAN[1], D. J. GORBET[2], L. E. SERGIO[2];
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Abstract: Background: We interact indirectly with our environment daily in which rules dictate the relationship between perception and action, requiring cognitive-motor integration (CMI). Daily-life interactions also typically involve dynamic postural control, where one must maintain balance while performing activities. The neural networks that control CMI and postural stability rely on intact frontal, parietal, and subcortical brain connectivity that may be compromised following sport-related concussion, resulting in an impaired ability to control complex, multi-domain (sensory-cognitive-motor) tasks. Here we explore the use of a whole-body multi-domain integration augmented reality (AR) task to assess whether an individual's coordination may be classified using deep learning (DL) as impaired, in a non-invasive way. Our hypothesis was that such an approach would successfully distinguish individuals with a history of concussion from those without, moreso than more standard linear statistical analyses. **Methods:** To probe CMI and dynamic postural control, 16 participants (5 with concussion history), engaged in an AR (Magic Leap, Inc.) whole-body task with 8 conditions of increasing complexity. First, participants walked naturally while moving virtual objects to colour-matched targets with direct hand-to-object interaction. Subsequent levels introduced vestibular and cognitive challenges. Kinematic measures included head and handheld controller position (pitch/roll/yaw, X-Y-Z, 60Hz sampling rate). Analysis of head/hand path length variability and velocity profiles were done using standard univariate methods. Data were preprocessed using a fast Fournier transform for feature extraction and noise reduction. To classify individuals with concussion history, a deep learning (DL) model was developed using a time-series split k-fold-cross validation method with five segments. **Results:** Standard statistical analysis on the kinematic data did not yield any group significance ($p>0.05$). However, we observed that our DL model distinguished concussed and non-concussed participants with a classification accuracy of 91%. **Conclusions:** These data suggest that a sophisticated DL model of head and upper limb motion during whole-body goal-oriented tasks can sensitively classify concussed and non-concussed individuals. The ability to identify concussion-related impairments in CMI performance offers a readily accessible, non-invasive method to assess neural functioning and brain network integrity. Such an approach holds promise as an ecologically valid means of recovery monitoring and rehabilitation following brain injury.

Disclosures: A. Machula: None. **A. Khan:** None. **D.J. Gorbet:** None. **L.E. Sergio:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.18/D23

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant K08NS119882

Title: Characterizing the spectrum of neurological outcomes of prenatal zika infection across the americas

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Abstract: Background: Prenatal Zika infection can cause brain injury, which is associated with a spectrum of neurological disorders. Exposed infants are at risk for conditions such as developmental delay, seizures and blindness. However, the spectrum of clinical outcomes varied across the Americas. It appears that clinical trends cluster by geographic region. We have developed a research program to evaluate whether genetic variation or environmental factors offer an explanation for these disparities. Here, we present a comparative analysis of the clinical profiles from six distinct studies conducted in three Latin American regions brought together by the Prenatal Infection and Neurodevelopmental Genetics (PING) Consortium.
Methods: The six cohorts included in this analysis were part of a case-control, retrospective or prospective longitudinal study. While the specific research question varied between study sites, all were constructed with a goal of investigating the effect of prenatal Zika infection on development. Among all sites, Zika-exposed mothers were enrolled between 2015 to 2017 and infants were followed from 18 months to eight years after birth. The exposure status of women in the respective studies were determined through presence of Zika-like symptoms or confirmed with laboratory testing. Brain injury was assessed using growth measurements and brain imaging, including ultrasound, computerized tomography, and magnetic resonance imaging at various time points.
Results: We evaluated the impact of Zika infection on the developing brain among cohort participants. Specific outcomes evaluated included microcephaly, intracranial calcifications, cortical malformation, ventriculomegaly and hydrocephalus. Our results highlight striking disparities in the severity of outcomes among infants in the Brazilian, Colombian, and Puerto Rican cohorts exposed to the Zika virus. The Brazilian cohorts exhibit a notably high severity burden, with 85.6% (178/208) to 100.0% (60/60) of infants categorized as severely affected. This contrasts with the Colombian cohorts, where a considerably lower proportion of infants experienced severe neurological outcomes, ranging from 4.1% (25/612) to 8.3% (44/528). The Puerto Rican cohort displayed an intermediate severity level, with 7.5% (3/40) of children identified as severely affected.

Disclosures: B. Fasil: None. **L. Tran:** None. **Y. Kousa:** None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.19/D24

Topic: C.10. Brain Injury and Trauma

Support: CHS Scholarship

Title: The Potential of S100B and GFAP as biomarkers for diagnoses and prognoses of traumatic brain injury.

Authors: *S. MAFUIKA;
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Abstract: Background: Traumatic brain injury (TBI) occurs as a result of a severe head injury caused by an external force during a vehicle accident, fall, domestic violence, or explosion. The existing data shows that approximately 69 million individuals per year are projected to sustain TBI worldwide. In sub-Saharan Africa, the prevalence of TBI is 150-170 per 100 000 individuals. It is estimated that by the year 2050, the TBI prevalence in Africa will increase to 14.25±0.75 million due to increased urbanization. TBI is evaluated & classified clinically according to the Glasgow Coma Scale (GCS), associated with Computed Tomography (CT) & Magnetic Resonance Imaging (MRI). However, there are many limitations associated with GCS. Therefore, the aim of this study was to investigate the role of serum S100 calcium-binding protein B (S100B) & Glial Fibrillary Acidic Protein (GFAP) as potential biomarkers for detection & diagnosis for human TBI. This pilot study utilized purposive sampling of serum fluid of 78 adult patients with moderate to severe TBI. The S100B & GFAP concentrations were determined by multiplex immunoassay technique (ELISA) following the manufacturer's instructions. The data generated were subjected to statistical analysis using a one-way analysis of variance & Dunn's multiple comparisons post hoc tests using Graph pad prism software version 9.00. The results obtained were expressed as mean ± standard error of the mean at $P< 0.05$. There were no significance differences ($P < 0.2162$) between the serum concentrations of S100B protein in the moderate TBI compared with control. However, a significant increase in the serum concentration of S100B protein was observed in the severe TBI compared with the control ($P< 0.0002$) & moderate ($P< 0.0011$) TBI. The serum concentrations of GFAP proteins were significantly increased in the moderate ($p<0.0015$) & severe ($p<0.0083$) categories of TBI as opposed to the control. However, there was no significance difference ($p<0.9999$) between the serum concentrations of moderate & severe TBI categories. This finding confirms that the significant increased concentration of S100B & GFAP proteins increase the severity & worsen the progression of TBI. Hence, S100B & GFAP can be utilized as biomarkers for better prediction of severity and progression of TBI from moderate to severe categories, combination of these two biomarkers & the current GCS system may provide better diagnostic results.

Disclosures: S. Mafuika: None.

Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.20/D25

Topic: C.10. Brain Injury and Trauma

Title: The influence of repetitive head impact exposure on white matter integrity in former American football players: findings from the DIAGNOSE CTE Research Project

Authors: *A. S. WICKHAM[1,3], N. KIM[4], O. JOHN[5], C. HELLER[6], K. BREEDLOVE[7], L. JUNG[9], T. WIEGAND[9], F. TUZ-ZAHRA[10], D. DANESHVAR[11], T. BILLAH[4], C. BERNICK[12], M. ALOSCO[13], J. CUMMINGS[14], E. M. REIMAN[15], R. A. STERN[5], A. LIN[8], I. KOERTE[9], M. E. SHENTON[16], S. BOUIX[17], H. ARCINIEGA[2];
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Abstract: Research Objective and Rationale: Exposure to repetitive head impacts (RHI) has emerged as a potential factor associated with the development of neurodegenerative disorders, notably Chronic Traumatic Encephalopathy (CTE), as well as injuries related to white matter shearing and myelin degradation. Conventional structural magnetic resonance imaging (MRI) has revealed widespread white matter atrophy following RHI, but its resolution is limited when attempting to explore alterations at the axonal level. In our study, we utilize diffusion-tensor imaging (DTI) to identify *in vivo* alterations of white matter microstructure associated with RHI in former American football players. We evaluate how chronological age, the age of first exposure to tackle football, and the severity of forces associated with head impacts over one's career influence white matter integrity later in life.
Methods: We analyzed data from the DIAGNOSE CTE Research Project, focusing on former American football players (n = 166). The DTI measures included whole-brain Fractional Anisotropy (FA), reflecting water diffusion directionality in white matter, and FreeWater-corrected FA (FAt) accounting for extracellular free water, using FSL Tract-Based Spatial Statistics (TBSS). We performed linear regressions on FA and FAt with chronological age, age of first exposure to tackle football, and estimates of cumulative head impact index (CHII) scores of frequency, linear acceleration, and rotational force. We controlled for several covariates in our analysis, including age, body mass index, race, education, and Apolipoprotein E4 allele carrier status.
Results: Both whole-brain FA ($p < 0.00001$) and FAt ($p < 0.00001$) decreased as age increased. Moreover, football players who initiated tackle football at a younger age exhibited significantly lower whole-brain FA ($p < 0.01$) and FAt ($p < 0.01$). We also found that a decrease in FAt correlated with increased CHII-linear acceleration ($p < 0.04$) and CHII-rotational forces ($p <$

0.02).
Conclusion: Our study reaffirms the well-established research of decreasing FA and FAt across the lifespan, which here we observe in former American football players exposed to extensive periods of RHI. Moreover, our findings highlight that earlier exposure to tackle football and greater intensity of RHI during one's career have a significant impact on white matter microstructure later in life. Overall, these results underscore the profound impact of prolonged RHI exposure on underlying neuropathology and suggest that DTI may serve as a valuable tool in detecting these alterations following sport-related head impacts.

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Poster

PSTR217: Brain Injury: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR217.21/D26

Topic: C.10. Brain Injury and Trauma

Title: The Influence of Multiple Concussions and Time Since Last Concussion on Functional Outcomes in Adult Females

Authors: *J. R. DETERS[1,2], E. ANDERSON[3], P. E. GANDER[4];
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Abstract: Mild traumatic brain injury (mTBI), synonymous with concussion, accounts for between 70% and 90% of all forms of traumatic brain injury (TBI). Previous research posited that a vast majority of patients recover within 3 months, however, a recent report suggests that 50% of TBI patients may have ≥ three symptoms worse at one year post-injury than pre-injury, despite only 13% of TBI patients showing objective cognitive deficits. Furthermore, postural instability is present in ~ 50% of mTBI patients at 3 months post-injury. It is widely reported that females tend to have a higher symptom burden than males, however there is a paucity of investigations into objective cognitive deficits and postural control (PC) in this population and how symptom burden, number of concussions, and time since concussion influence these outcomes. Therefore, we assessed cognitive outcomes and PC in women with persistent post-concussion symptoms (WwPPCS). 21 WwPPCS who had least 1 confirmed concussion suffered ≥ 1 month previous to enrollment were recruited. All participants answered the Rivermead Post-concussion Questionnaire (RPQ), performed the simple and choice reaction tasks, the flanker inhibitory control and attention test, as well as static posturography in four conditions (eyes

open/closed, firm/foam surface). Pearson's correlations were used to assess the influence of number of concussions, time since concussion, and symptom severity (RPQ Score) on cognitive and PC outcomes. Higher RPQ score was associated with increased sway in the mediolateral direction during both eyes open ($p = 0.02$; r = 0.49) and eyes closed ($p = 0.04$; r = 0.45) firm conditions and center of pressure area in the eyes closed foam condition ($p = 0.05$; r = 0.44). Higher number of concussions was also associated with increased flanker effect ($p = 0.02$; r = 0.52). All other associations were non-significant. These results suggest that increased symptom severity may negatively impact PC, and imply a potentially cumulative negative effect on inhibitory control in WwPPCS who experience multiple concussions. Thus, future investigations into WwPPCS should consider both number of concussions and symptom severity as important factors to consider.

Disclosures: J.R. Deters: None. **E. Anderson:** None. **P.E. Gander:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.01/D27

Topic: D.01. Somatosensation – Pain and Itch

Title: Using cold pretreatment to lower thermal block in small-diameter unmyelinated Aplysia californica nerves

Authors: *R. MUZYCHUK[1], H. J. CHIEL[2], E. D. JANSEN[3], M. W. JENKINS[4];
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Abstract: Small diameter unmyelinated C-fibers carry nociceptive information and are implicated in chronic pain. Current treatments for pain either involve systemic drugs that may be addictive, or external treatments (e.g., neuromodulators or heating pads) that lack selectivity and/or could damage tissue if applied chronically. Prior studies have shown that pre-treating nerves with heat can raise the temperature at which cold block occurs. We explored the possibility that pre-cooling unmyelinated nerves could lower the threshold for heat block over an extended period of time. The model system that we have used are the unmyelinated axons in the nerves of the marine mollusk *Aplysia californica*, which does not produce myelin. Preliminary data has shown that pretreatment with cold reduces the threshold for heat block by 1.78 +/- 0.73° C ($N = 5$, $p < 0.05$ using a paired t-test). Future studies will determine the duration for which this change of threshold occurs and will determine the reliability of the effect. If cold pre-treatment does reduce the temperature for thermal block, it is possible that these results could have useful translational implications.

Disclosures: R. Muzychuk: None. **H.J. Chiel:** None. **E.D. Jansen:** None. **M.W. Jenkins:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.02/D28

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH RM1NS128787

Title: Transcutaneous auricular neurostimulation demonstrates brain activation of pain-related neural networks in humans

Authors: M. MCWADE[1], N. KHODAPARAST[1], *A. COVALIN[2], F. SUTTON[3], D. CONNOLLY[3], X. PENG[3], B. BAKER-VOGEL[3], E. SHORT[3], E. ASHLEY[3], M. WOOD[3], J. J. BORCKARDT[3], V. RAMAKRISHNAN[3], B. BADRAN[3];
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Abstract: The United States is experiencing an epidemic for prescription and non-prescription opioids. In 2022, more than 131M opioid prescriptions were dispensed in the US, and the Center for Disease Control reported 107,941 substance use overdose deaths. There is a need for nonpharmacological pain interventions. Transcutaneous auricular neurostimulation (tAN) is a non-invasive form of neuromodulation targeting the vagus (via the auricular branch of the vagus nerve; ABVN) and trigeminal (via the auriculotemporal nerve; ATN) nerves on/around the ear that was recently proven to be an efficacious non-pharmacologic treatment for reducing opioid withdrawal symptoms (OWS). In 2021, tAN received FDA clearance as an adjunct treatment for OWS in adults. In this study, we explored the anti-pain effects of tAN versus sham stimulation in healthy individuals. To understand the mechanism, an opioid blockade (naloxone) was administered during stimulation to elucidate if endogenous opioids are responsible for antinociception. We conducted a blinded analysis on initial completers (n=5 of planned 136, tAN=2, sham=3). Participants attended two experimental visits at least one week apart during which they received stimulation to the targeted region while also receiving naloxone (0.15mg/kg) or saline (matched volume) intravenously. The stimulation condition stayed constant for both visits, and the only variable changed was the drug given. We measured heat sensation with Quantitative sensory thresholds (QST) before and after stimulation. This analysis investigates the anti-pain effects of tAN and sham stimulation paradigms under blinded drug conditions assigned Y and Z. tAN induced anti-pain effects in participants receiving Drug Y, rather than Drug Z (mean threshold change post-pre stimulation, °C) Pain, Drug Y: 3.35, Drug Z:-0.75; Tolerance, Drug Y: 0.71, Drug Z: -0.43. Sham demonstrated no anti-pain effects with Drug Y; Pain, Drug Y: 0.1, Drug Z:-2.44; Tolerance, Drug Y: -0.38, Drug Z: -0.78. Brain

activation was measured with fMRI demonstrating differences in activation profiles when stimulating the ABVN, ATN, tAN, or sham. We aim to determine whether these antinociceptive effects are caused by the release of endogenous opioids, which will set the stage for tAN to be utilized as neuromodulatory approach for pain mitigation.

Disclosures: M. McWade: A. Employment/Salary (full or part-time):; Spark Biomedical. **N. Khodaparast:** A. Employment/Salary (full or part-time):; Spark Biomedical. **A. Covalin:** A. Employment/Salary (full or part-time):; Spark Biomedical. **F. Sutton:** None. **D. Connolly:** None. **X. Peng:** None. **B. Baker-Vogel:** None. **E. Short:** None. **E. Ashley:** None. **M. Wood:** None. **J.J. Borckardt:** None. **V. Ramakrishnan:** None. **B. Badran:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.03/D29

Topic: D.01. Somatosensation – Pain and Itch

Support: Stratton VA Medical Center EB018783
Union College Student Research Grant

Title: Assessing the immediate effect of transcutaneous auricular vagus nerve stimulation (taVNS) on the P300 event-related potential across modalities

Authors: *M. A. RUSGROVE[1,2], T. M. VAUGHAN[3], S. HALDER[4], R. DOWMAN[5], K. FELLER[6], J. S. CARP[7], R. SCHERER[8];
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Abstract: Invasive vagus nerve stimulation (VNS) is an FDA-approved approach to treating drug-resistant epilepsy and depression, and is being investigated for use in ischemic stroke rehabilitation, Parkinson's disease, chronic pain, migraines, and tinnitus. The vagus nerve influences the locus coeruleus (LC), a small brainstem nucleus that serves as the brain's primary source of norepinephrine (NE), and which plays an essential role in arousal, stress, cognitive function, and attention. An imbalance of norepinephrine is associated with a number of neurological conditions, including depression and epilepsy; thus, VNS-induced change in LC-NE activity is considered to be a plausible explanation for the mechanism of VNS treatment in those conditions. Considering the cost and risks associated with invasive procedures, there is growing interest in studying non-invasive techniques for VNS as alternative treatments. The present study, conducted at the National Center for Adaptive Neurotechnologies, focuses on

transcutaneous stimulation of the auricular branch of the vagus nerve (taVNS) that innervates portions of the outer ear. Recent studies investigating LC-NE activity in taVNS treatment lack consensus on which biological marker should be used to assess taVNS efficacy. Some studies suggest use of the P300 biomarker, an event-related potential (ERP) recorded by electroencephalography (EEG), that is detected 250-600 milliseconds after presentation of a low-probability "oddball" stimulus, but the findings are inconsistent. The P300 is generally associated with higher cognitive functions such as attention, with classifications for task and memory processing. This study aims to replicate and extend study of the P300 as a biomarker for adjusted attention in response to taVNS. We hypothesize that taVNS causes an immediate and short-term change in the P300 ERP, independent of the stimulus modality presentation (i.e. auditory or visual). Preliminary results from six participants demonstrate that taVNS has a significant effect on the P300 amplitude in the visual modality, though in both active and sham conditions. Further findings from this study may help guide the selection of monitoring tools for more widespread use of taVNS treatment in the variety of clinical applications.

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Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.04/D30

Topic: D.01. Somatosensation – Pain and Itch

Title: Treadmill training attenuates contralateral post-thoracotomy pain in rats

Authors: *A.-K. CHOU[1], Y.-W. CHEN[2];
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Abstract: Background: The goal of the experiment was to utilize treadmill training as therapy to assess contralateral pain after thoracotomy and anti-inflammatory cytokines.Methods: Rats in the thoracotomy and rib traction (TRR) group were subjected to surgery and rib traction; the control and sham groups underwent no surgery and underwent surgery other than rib traction, respectively; both TRR and sham rats received treadmill training for 6 wk. Mechanical sensitivity and cold (acetone) allodynia were performed at the skin wound and contralateral locations of the rats. Right and left intercostal nerves were obtained for cytokine analysis.Results: Beginning on postoperative day 10 and continuing through postoperative day 52, TRR rats showed incremental scratching and mechanical hypersensitivity on both the ipsilateral and contralateral sides exercise training relieved the upper two pains. In the ipsilateral and contralateral intercostal nerves, TRR rats with exercise showed an improvement in increased expression of tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) compared with

TRR rats without exercise (P < 0.01).Conclusions: We demonstrate that treadmill exercise improves ipsilateral scratching behavior and mechanical allodynia caused by TRR, with the same effect on the contralateral side. The efficacy of exercise in treating pain coincided with the inhibition of up-regulation of pro-inflammatory cytokines (IL-1ß and TNF-α) observed in contralateral and ipsilateral intercostal nerves.

Disclosures: A. Chou: None. **Y. Chen:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.05/D31

Topic: D.01. Somatosensation – Pain and Itch

Title: Effects of video-guided imagery treatment on chronic low back pain symptoms

Authors: *S. HODGES[1], A. URSITTI[1], M. CANNISTRA[2], S. REDDY[3], J. KONG[3];
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Abstract: Background: Chronic low back pain (cLBP) is a widespread disabling disorder with few satisfactory treatment options. In a previous study, we combined acupuncture and imagery to develop a new treatment method, video-guided acupuncture imagery treatment (VGAIT), which showed promise in reducing pain severity in cLBP patients. The aim of this study is to further investigate the potential of VGAIT in managing symptoms of cLBP when administered remotely.
Methods: 53 participants with chronic low back pain were enrolled and randomized to two groups: video-guided acupuncture imagery treatment or video-guided imagery of a cotton swab rotating on the back and legs. In the videos, the acupuncture needle and cotton swab stimulation were shown on an animated body. Both groups completed eight online guided imagery sessions over four weeks on Zoom. Pain bothersomeness and average pain intensity (in past 7 days) data were collected at baseline and after the last imagery treatment.
Results: 48 participants completed the study (VGAIT n = 23, Swab n = 25). Within group comparisons showed a significant reduction of pain bothersomeness in both groups (VGAIT p = 0.01, Swab p = 0.03). The VGAIT group also had to a significant reduction in average pain intensity over the past seven days (p = 0.02). The swab imagery group showed a nonsignificant trend toward reduced average pain intensity over the past seven days (p = 0.08). Between-group comparison revealed no significant findings.
Conclusion: Our findings suggest that both guided imagery of acupuncture needle stimulation and swab touch stimulation may reduce symptoms of cLBP, indicating the potential of online guided imagery of body stimulation as a treatment for cLBP.

Disclosures: S. Hodges: None. **A. Ursitti:** None. **M. Cannistra:** None. **S. Reddy:** None. **J. Kong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); holds equity in two startup companies (MNT, BTT), a patent on applying neuromodulation and a provisional patent application on video guided imagery peripheral stimulation for chronic pain..

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.06/D32

Topic: D.01. Somatosensation – Pain and Itch

Support: NSTC113-2320-B-038-017

Title: Investigating the role of focused ultrasound in modulating pain response by altering CGRP-mediated cascades in dorsal root ganglia

Authors: *Y.-T. LIN[1], P. S. CHAN[2], C.-C. HSU[3];
[1]Grad. Inst. of Metabolism and Obesity Sci., Taipei Med. Unversity, Taipei, Taiwan; [2]Dept. of occupational therapy, Col. of medicine, Chang Gung Univ., Taoyuan, Taiwan; [3]Sch. of Resp. Therapy, Col. of Med., Taipei Med. Univ., Taipei, Taiwan

Abstract: Pain represents a significant public health issue and serves as a common reason for seeking medical consultation. Chronic pain significantly disrupts daily life, often leading to difficulties in performing routine activities, adversely affecting mental health, and reducing quality of life. Several types of pharmacological medications are available for managing chronic pain, such as opioids. Opioids like morphine are recognized as potent analgesics; however, their narrow therapeutic window, the risk of addiction, and the development of tolerance significantly limit their applications. Non-invasive, non-pharmacological therapies provide alternative strategies for pain management by targeting peripheral, spinal, and supraspinal mechanisms. Many studies indicate that the dorsal root ganglia (DRG) is an effective target for managing chronic pain through ganglionectomy, lesioning, drug injection or electrical stimulation. However, most of these treatments require surgically invasive procedures to access the DRG. The acoustic effects of Focused Ultrasound (FUS) have been utilized in modulating the activity of tissues, including the central and peripheral nervous systems. Unlike high-intensity FUS used for tissue thermal ablation, low-intensity FUS (LIFU, 30-1000 mW/cm2) does not produce significant heat and enables precise targeting of acoustic energy to specific regions, making it a valuable tool for neuromodulation and the management of chronic pain. Research in this area currently focuses heavily on the brain, with transcranial FUS (tFUS) representing an innovative neuromodulation technique characterized by its high spatial resolution and capacity to stimulate deep brain regions. In the current studies, we aim to investigate the analgesic effect triggered by LIFU through the sensory nerve system and explore the downstream mechanisms involved.

Using a rat model of neuroinflammatory pain induced by Complete Freund's Adjuvant (CFA) and neuropathic pain induced by chronic constriction injury (CCI), we assessed the analgesic effects of FUS stimulation on the lumbar DRGs. Subsequently, we measured the expression levels of the pain mediator, calcitonin gene-related peptide (CGRP). Additionally, the impact of FUS on capsaicin-triggered CGRP release was evaluated using primary DRG cells. Our results may open new avenues for developing therapeutic approaches to manage chronic pain conditions.

Disclosures: Y. Lin: None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.07/D33

Topic: D.01. Somatosensation – Pain and Itch

Title: Green light exposure causes analgesia via the vLGN in a model of chronic, widespread hyperalgesia

Authors: *A. RAMIREZ[1], A. WILKINSON[2], L. F. FERRARI[2], N. TAYLOR[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Anesthesiol., Univ. of Utah, Salt Lake City, UT

Abstract: Green light is a promising, non-invasive therapy shown to produce anti-nociceptive effects in patients with nociplastic pain. However, the mechanisms responsible for this effect are poorly understood. We recently identified the Dahl salt-sensitive (SS) rat as a model of inherited, widespread hyperalgesia. SS rats show several additional phenotypes consistent with nociplastic pain conditions, such as dysfunction in descending pain modulation and stress response systems, along with increased sensitivity to hyperalgesic mediators and fatigue-like behaviors. SS rats may have greater face and predictive validity than mice models of neuropathic pain in predicting responses in nociplastic patients because of rat retinal characteristics and the presence of widespread instead of focal pain. The efficacy of green light treatment was tested by exposing adult male and female SS rats to green light at 200 lux for 6 hours per day for 5 days. White light at 200 lux was used as a control, and the experimenter was blinded to the treatment conditions. We injected 200nL of a two-virus cocktail into the ventrolateral geniculate nucleus (vLGN); (AAV8-cfos-ERT2-Cre-ERT2-PEST and AAV8-hSyn-DIO-HA-hM3D(Gq)-IRES-mCitrine). After waiting two weeks to establish stable viral transfection, we exposed rats to either green light or white light for four hours daily for five days. Tamoxifen (100mg/kg) was injected i.p. in both groups of rats immediately after removal from the light to activate CreER and induce DREADDs incorporation into vLGN neurons of green light-exposed rats. Ten days later we delivered the DREADD agonist c21 (1mg/kg ip) twice daily for five days. Paw withdrawal thresholds were measured 45 min following the morning c21 dose. Immediately upon completing paw withdrawal threshold evaluation, the brains were removed for

immunohistochemical analysis. Green light exposure significantly increased paw withdrawal thresholds by days 3 in male ($p<0.0003$) and female SS rats ($p=0.004$). Exposure to white light did not result in changes in paw withdraw threshold. Rats with green light activated TRAPed neurons showed higher thresholds than white light exposed controls ($p \leq 0.01$). Rats were exposed to white or green light for 4 hours then immediately sacrificed, perfused and brains collected for immuno-histochemistry. hM3Dq DREADDs coexpressed with cFOS in animals exposed to green light, demonstrating successful tamoxifen TRAPing. Little cFOS was expressed in rats exposed to white light and consequently few cells incorporated hM3Dq DREADDs. Taken together, these findings demonstrate that green light causes analgesia in SS rats and is mediated through the vLGN.

Disclosures: A. Ramirez: None. **A. Wilkinson:** None. **L.F. Ferrari:** None. **N. Taylor:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.08/D34

Topic: D.01. Somatosensation – Pain and Itch

Title: Antinociceptive Effect of a novel conotoxin from Conasprella ximenes in Chronic Inflammatory and Neuropathic Pain Models in Rats

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Abstract: Chronic Pain affects 35-55% of the global population and presents a therapeutic challenge. Conotoxins found in cone snail venom offer promising avenues for studying nociceptive pathways and developing new treatments for Chronic Pain. We investigated the antinociceptive effect of a synthetic novel O1-conotoxin (s-xm6a) derived from the *Conasprella ximenes* transcriptome. We established two Chronic Pain models in Wistar Rats. In the first one, we induced Chronic Inflammatory Pain (CIP) by intraplantar injection of Complete Freund Adjuvant, eliciting thermal hyperalgesia, and assessed withdrawal latency using a Hargreaves apparatus. In the second model, we induced Neuropathic Pain by tight ligating the L5 and L6 nerves (SNL) and measured the mechanical allodynia using Von Frey filaments (2.36 - 6.65). s-xm6a was tested at three doses via intravenous administration (i.v. : 0.5, 0.05, and 0.005 mg/kg) and at three concentrations via intrathecal (i.t.: 5, 0.5, and 0.05 µg/10µl) and intracerebroventricular (i.c.v.: 5, 0.5, and 0.05 µg/5µl) administration. We established various control groups for each model: i.v. (MVIIA at 0.5 mg/kg, Gabapentin at 100 mg/kg in SNL and

Dexamethasone at 4 mg/kg, in CIP), i.t. (MVIIA at 0.3 µg/10µl), and i.c.v. (MVIIA at 0.3 µg/5µl). We assessed motor-level side effects using a rotarod test in all s-xm6a and MVIIA doses. Statistical differences were analyzed using one-way ANOVA and Tukey or Dunnet as *post hoc* tests. s-xm6a demonstrated a dose-dependent reduction in nociception. In the CIP model, the highest dose (0.5 mg/kg, i.v.) resulted in an 86 ± 3.89% (n=6) reduction of nociception, with no significant difference compared to the normal rat group (NRG) ($p<0.05$). I.t and i.c.v. administration showed reductions of 85.42 ± 1.35% (n=6, $p<0.05$) and 60.09 ± 3.41% (n=3, $p<0.05$), respectively, compared to the NRG. In the SNL model, s-xm6a reduced nociceptive response by 73.33 ± 4.13 % (i.v., n=6, $p<0.05$) and 85.63 ± 1.05 % (i.t., n=6, $p<0.05$) at higher doses. Remarkably, s-xm6a displayed no motor-related side effects across all doses, contrasting with MVIIA, which induced motor impairments in the animals. To our understanding, s-xm6a is a pioneering conotoxin, showcasing antinociceptive effects through systemic administration (i.v.) and has a notable advantage over other conotoxins necessitating catheter implantation, positioning it as an excellent candidate for advancing novel therapeutic strategies in Chronic Pain management.

Disclosures: J. Lopez Carrillo: None. **N. Morales Cardona:** None. **A. Licea:** None. **N. Caram-Salas:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.09/D35

Topic: D.01. Somatosensation – Pain and Itch

Support: TWU Research Enhancement Program Grant
TWU Center for Student Research Grant

Title: Euphorbia bicolor phytochemicals reduce neural and inflammatory markers of burn pain in a rat model of full thickness thermal injury

Authors: *T. P. OLAOLUWA, D. L. HYNDS, C. MAIER, D. L. AVERITT;
Biol., Texas Woman's Univ., Denton, TX

Abstract: Pain associated with burn injury has been linked to nociceptor sensitization and can lead to chronic pain. Treatment for pain associated with burns include oral and intravenous analgesics such as non-steroidal anti-inflammatory drugs, opioids, and gabapentinoids; all linked to undesired negative side effects. As burn is a challenging type of injury to treat, novel strategies that mitigate burn pain and its chronification are needed. Our previous studies indicate that phytochemicals of the latex extract of *Euphorbia bicolor*, a plant native to the southern United States, provide long-lasting, non-opioid peripheral analgesia in multiple rodent models of inflammatory pain. Our current work extends our findings in a rat model of full thickness burn to

assess behavioral signs of affective pain and detect changes in the central afferents of sensory neurons innervating the dorsal horn of the spinal cord. We hypothesized that *E. bicolor* phytochemicals reduce pain signaling and nociceptive sensory afferent input at the dorsal horn corresponding to reduced pain behaviors in rat model of burn pain. Following baseline behavior testing, adult male Sprague-Dawley rats (250-350 g) received a full thickness thermal injury to the plantar surface of the right hind paw (100°C for 30 seconds). Escape latency from aversive light to nociceptive probes using the mechanical avoidance conflict system (MACS) was then recorded at 24 hours post-injury to confirm development of pain behaviors. *E. bicolor* extract (100 µg/mL, 500 µg/mL, or vehicle) was injected at the burn injury site and pain behaviors were re-examined at 48 hours post-injection. Rats were perfusion-fixed in 4% paraformaldehyde and dorsal root ganglia and spinal cords (L2-L5) were collected. Fluorescent immunohistochemistry was performed on 30 µm sections using primary antibodies against isolectin IB4 biotin conjugate, neurofilament heavy, peripherin, calcitonin gene-related peptide (CGRP), and transient receptor potential vanilloid 1 (TRPV1) ion channels. Inflammatory mediators and markers of oxidative stress were also quantified in the blood and sensory ganglia. We report reflexive and affective pain develop at 24 hours following thermal injury, which was significantly reduced by local injection of *E. bicolor* phytochemicals. Burn-injury evoked a significant increase in CGRP immunoreactivity at the nociceptive central afferent terminals in the dorsal horn, which was attenuated by *E. bicolor* treatment. Together, these data indicate that *E. bicolor* phytochemicals evoke peripheral analgesia via anti-inflammatory and analgesic mechanisms that contribute to pain chronification in a rat model of burn pain.

Disclosures: T.P. Olaoluwa: None. **D.L. Hynds:** None. **C. Maier:** None. **D.L. Averitt:** None.

Poster

PSTR218: Non-Opioid Treatments for Persistent Pain Including Non-Pharmacological Interventions

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR218.10/D36

Topic: D.01. Somatosensation – Pain and Itch

Support: NIH Grant R01NS045954
NIH Grant R01DA037621
NIH Grant R01NS062306

Title: Intranasal administration of a neuropeptide Y Y1 agonist for the treatment of postsurgical and neuropathic pain

Authors: *A. NIE[1], A. HELLMAN[1], T. S. NELSON[2], B. K. TAYLOR[1];
[1]Anesthesiol., Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Spinal cord and brain interneurons that express the neuropeptide Y (NPY) Y1 receptor (Y1-INs) are a promising target for the treatment of chronic postsurgical and neuropathic pain. We previously reported that intrathecal or intracranial administration of NPY or the Y1 agonist [Leu^{31}Pro^{34}]-NPY acted at Y1-INs to alleviate behavioral and molecular signs of persistent pain. However, these routes of administration risk injury and infection, making them problematic for translation to humans. Furthermore, neuropeptides do not readily cross the blood brain barrier, rendering systemic administration inefficacious for CNS delivery of [Leu^{31}Pro^{34}]-NPY. To overcome this barrier and thus target CNS Y1-INs for the treatment of postsurgical and neuropathic pain, we leveraged the intranasal route of administration, which has emerged as a promising approach for the brain delivery of neuropeptides to address psychiatric and neurological disease (Lochhead & Davis, 2019). We first completed a dose response curve using the Y1-selective agonist [Leu^{31}Pro^{34}]-NPY (0.3 - 5.0 mg/kg or saline control, 0.5ml/kg) in a plantar incision model of acute postsurgical pain in male and female C57BL/6 mice. 2 days after incision, we confirmed the development of mechanical hypersensitivity using an up-down method with von Frey filaments. [Leu^{31}Pro^{34}]-NPY at 1mg/kg and 5mg/kg, but neither 0.3mg/kg nor saline, reversed mechanical hypersensitivity ($p<0.05$, n=6-8 per group). In a latent sensitization model of chronic postsurgical pain, we allowed initial hypersensitivity to resolve (21 days), and then subcutaneously administered 3mg/kg naltrexone (NTX, an opioid receptor inverse agonist) to elicit pain reinstatement. 5mg/kg intranasal [Leu^{31}Pro^{34}]-NPY (n=8) but not saline (n=7) prevented NTX-induced reinstatement of mechanical hypersensitivity and did not change time spent on an accelerating rotarod (0.5 RPM increase every 5 seconds starting at 2 RPM). We also completed a dose response curve for [Leu^{31}Pro^{34}]-NPY in mouse spared nerve injury (SNI) and porcine common peroneal nerve injury (CPNI) models of neuropathic pain. In mouse, [Leu^{31}Pro^{34}]-NPY dose-dependently (1, 3, and 5mg/kg but not 0.1, 0.3mg/kg, or saline 0.5ml/kg) alleviated mechanical and cold hypersensitivity 14 days post-SNI (n=6 per group). In pig, [Leu^{31}Pro^{34}]-NPY again dose-dependently (0.008 and 0.08mg/kg but not 0.000008, 0.00008, 0.0008mg/kg or saline) alleviated mechanical hypersensitivity following CPNI (n=4). These results support intranasal [Leu^{31}Pro^{34}]-NPY and other Y1 agonists as a promising pharmacotherapeutic approach for the treatment of postsurgical and neuropathic pain.

Disclosures: A. Nie: None. **A. Hellman:** None. **T.S. Nelson:** None. **B.K. Taylor:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.01/D37

Topic: D.02. Somatosensation – Touch

Support: Taiwan NSTC Grant NMRPG3M0442
Taiwan CGMH Grant CMRPG3M1652

Title: Long-term map plasticity of non-columnar neurons in somatosensory cortex following reinnervation in infra-orbital nerve.

Authors: *J.-J. TSENG[1], J.-J. HUANG[1,2,3], Y.-C. PEI[1,2,3,4];
[1]Physical Med. and Rehabil., Chang Gung Mem. Hosp., Linkou, Taiwan; [2]Ctr. of Vascularized Tissue Allograft, Taoyuan, Taiwan; [3]Sch. of Medicine, Chang Gung Univ., Taoyuan, Taiwan; [4]Healthy Aging Res. Center, Chang Gung Univ., Taoyuan, Taiwan

Abstract: Neuroplasticity is considered to mediate the recovery of normal topography in the somatosensory cortex after peripheral nerve reconstruction. An electroneurophysiological study performed by our group indicated that, following infra-orbital nerve (ION) reconstruction, the topography in the barrel cortex (S1BF) can partly recovered to its physiological pattern. However, it remains unclear regarding the long-term changes of topography along the course of recovery that could span for several months. For this, we recorded calcium neural imaging by 2-photon microscopy to investigate long-term dynamics of whisker tuning and topography remapping in S1BF in awake GCaMP6 transgenic mice following ION reconstruction (n=5). The neural activities elicited by whisker stimulation was recorded for over 70 days following surgery. The results showed that stimulus-responsive neural activities disappeared approximately 7 days following the surgery, and the whisker tuning was restored as early as when the neural responsiveness appeared. Importantly, the whisker tuning had not yet stabilized by the end of the first month after surgery, as evidenced by consistent changes in the tuned whisker for 80% of the recorded pyramidal neurons. Four out of five animals showed recovery of the topography by showing that a population of neurons at the end of the 70-day follow-up had principle whiskers identical to their principle whiskers in physiological or pre-operative conditions. The present study developed a methodology for conducting long-term 2-photon calcium neural imaging in awake mice before and after ION reconstruction and demonstrated that majority of non-columnar neurons in S1BF remarkably restore their physiological whisker tuning and topography after surgery. This study offers an opportunity for characterizing the neural dynamics underlying the restoration of neurological function after neurological disorders or injuries.

Disclosures: J. Tseng: 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.; NMRPG3M0442, CMRPG3M1652.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.02/D38

Topic: D.02. Somatosensation – Touch

Support: Marcos Moshinsky 001
VIEP-BUAP
Comite de Internacionalizacion de la Investigacion BUAP

Title: Optogenetic Control of GABAergic Neurons Restores Inhibited Cortical Somatosensory Responses

Authors: J. GUTIERREZ[1], N. N. REQUEJO MENDOZA[2], R. GUTIERREZ[2], *E. MANJARREZ[1];
[1]Inst. of Physiol., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [2]Dept. of Pharmacol., CINVESTAV-IPN, Mexico City, Mexico

Abstract: Understanding how cortical inhibition is modulated holds significant implications for neuroscience. This study utilized VGAT-ChR2-YFP transgenic mice, where blue light activates GABAergic neurons expressing ChR2 under the control of the GABA transporter (VGAT). We aimed to demonstrate that optogenetic activation of these inhibitory neurons in the barrel cortex could modulate inhibited cortical somatosensory responses. Whisker-stimulation evoked potentials were measured before and after transcranial random noise electrical stimulation (tRNS) of the primary somatosensory cortex (S1). We found that high-frequency (100-640 Hz) tRNS for 2 minutes significantly reduced the evoked potential amplitude, lasting over 2 hours. Remarkably, subsequent optogenetic noise-photostimulation (ONP) applied intermittently (1 minute every 5 minutes) over S1 reversed this inhibition, restoring and even enhancing the evoked potential amplitude. These findings reveal that ONP-mediated optogenetic activation of GABAergic neurons has the potential to reverse the inhibition of cortical somatosensory responses. This opens exciting new avenues for research into the mechanisms of cortical disinhibition and its potential therapeutic applications.

Disclosures: J. Gutierrez: None. **N.N. Requejo mendoza:** None. **R. Gutierrez:** None. **E. Manjarrez:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.03/D39

Topic: D.02. Somatosensation – Touch

Support: ERC
DFG
NIH

Title: Unveiling the role of protein synthesis in medial-temporal lobe-gated memory consolidation: insights from a neocortical stimulation paradigm

Authors: *M. SCHUTTE[1], R. N. SACHDEV[1], M. DRÜKE[1], G. DORON[2], M. E. LARKUM[1];
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Abstract: The quest to pinpoint the precise locus of memory formation continues to challenge neuroscientists. While it may occur at a cellular or circuit level, involve structural alterations, synaptic modifications, or protein synthesis, the exact mechanisms remain elusive. In this study, we aimed to explore the role of local protein synthesis in dendritic memory formation, specifically within the medial-temporal lobe (MTL) projections to the apical tuft of Layer 5 (L5) cells in the somatosensory cortex's Layer 1 (L1). Building upon our previous findings (Doron et al., 2020) that demonstrated the necessity of the perirhinal cortex's pathway to L1 of the somatosensory cortex (S1) for memory formation, we employed a microstimulation paradigm to test the hypothesis that MTL-gated memory consolidation is contingent upon local protein synthesis. To investigate this, we injected mice with either non-specific (anisomycin, n=4) and specific (rapamycin, n=6) inhibitors of local protein synthesis in L1 dendrites. Our results revealed that non-specific inhibition of protein synthesis via anisomycin had no discernible impact on the mice's ability to behaviorally report microstimulation. In contrast, rapamycin, which specifically targets the mTORC1 pathway in L1, significantly impaired the animals' capacity to learn the task. This was evidenced by an approximate 80% decrease in stimulus sensitivity and a heightened variability in response times when compared to saline-injected control mice. Our work supports the hypothesis that protein synthesis is integral to both the learning and consolidation phases of memory formation. Yet, the timing (during learning or consolidation) and the specific cortical locations (such as in L1 at the apical tufts of pyramidal neurons or elsewhere) at which this protein synthesis happens remains unknown. To further decipher these intricacies of memory consolidation we anticipate extending our research using cell-type-specific and light-activated molecular inhibitors of protein synthesis. This approach will allow for a more nuanced understanding of the local processes underlying memory formation.

Disclosures: M. Schutte: A. Employment/Salary (full or part-time):; Institute for Biology, Humboldt University of Berlin, D-10117 Berlin, Germany. **R.N. Sachdev:** A. Employment/Salary (full or part-time):; Institute for Biology, Humboldt University of Berlin, D-10117 Berlin, Germany. **M. Drüke:** A. Employment/Salary (full or part-time):; Institute for Biology, Humboldt University of Berlin, D-10117 Berlin, Germany. **G. Doron:** A. Employment/Salary (full or part-time):; Data Sciences & AI, R&D, Pharmaceuticals, Bayer AG, Berlin, Germany. 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.; NeuroCure Cluster of Excellence, Charité – Universitätsmedizin, Berlin, Germany. **M.E. Larkum:** A. Employment/Salary (full or part-time):; Institute for Biology, Humboldt University of Berlin, D-10117 Berlin, Germany. 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.; NeuroCure Cluster of Excellence, Charité – Universitätsmedizin, Berlin, Germany.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.04/D40

Topic: D.02. Somatosensation – Touch

Support: Max Planck Society
DFG, German Research Foundation-HE 7321/1-1

Title: Comparison of connectomic traces of Hebbian plasticity in mouse and human cortex

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Abstract: The neuronal circuits undergo reorganization and the strength of the connections between neurons is modified through synaptic plasticity during learning, development and aging. Animals models, in particular rodents, have been extensively used to study mechanisms of synaptic plasticity. However, despite the recent progress in accessibility of human tissue for physiological studies on tissue slices, how the mechanisms of synaptic plasticity apply to the human brain is incompletely understood. In this study, we used 3-dimensional electron microscopy-based connectomic reconstructions in human, non-human primate and mouse supragranular cortical samples and investigated the structural correlates of Hebbian plasticity. We compared the rate of excitatory spine synapses undergoing synaptic weight adaptation consistent with Hebbian learning across species. Notably, the human cortex shows prevalence of spines with large weights, a feature that is absent in both mouse and non-human primate cortex. Furthermore, the weights of spine synapses consistent with Hebbian plasticity are strongly correlated in human cortex compared to mouse and non-human primate cortex. These results indicate that the principles of Hebbian plasticity may be quantitatively different between the human cortex and other species. Additionally, we used age-matched samples for inter-species comparison as a crucial control to study the dependence of age on our findings. Together, this paves the way for future investigations into the significance of these distinct human-specific plasticity features.

Disclosures: S. Loomba: None. **A. khalifa:** None. **A. Motta:** None. **J. Gempt:** None. **H.S. Meyer:** None. **M. Helmstaedter:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.05/D41

Topic: D.02. Somatosensation – Touch

Support: SNSF Grant 219343

Title: Cortical circuits for context-dependent sensory processing

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Abstract: In nature, humans and animals are constantly bombarded with sensory information, which needs to be promptly contextualized to elicit the appropriate motor sequences through a process known as sensorimotor transformation. While we begin to understand how sensory information is transformed into motor action, it is still unclear in what way contextual information can modify a given sensorimotor plan. In this project, we investigate this phenomenon by training water-restricted mice in a context-dependent multisensory detection task. We developed a behavioral paradigm in which mice are required to detect two sensory stimuli of different modalities. The first stimulus (whisker deflection) is rewarded upon licking in a context-dependent manner, while the second stimulus (auditory tone) is always rewarded upon licking, irrespective of context. In this task, the context is provided by two different auditory background textures that change every block of 20 trials. Upon learning, mice were able to promptly switch their behavior according to the contextual auditory textures and perform on average 10 switches per session. The probability of correctly responding to the first context-sensitive stimulus increased on the timescale of seconds after the context block switch, suggesting an evidence-accumulation strategy to integrate contextual information. This was further supported by our observation that mouse performance collapsed in the absence of the auditory contextual cues. To investigate the cortical regions required for the execution of this task, we are carrying out a random-access optogenetic inactivation screening of the dorsal cortex in VGAT-ChR2 mice, as well as pharmacological inactivation using muscimol. Our optogenetic and pharmacological manipulations so far have established the necessity of the whisker primary somatosensory cortex (wS1) in task execution. We are also investigating how different subpopulations of excitatory neurons in the cortex are involved in context-dependent sensory processing using widefield calcium imaging in mice expressing GCaMP6f selectively in L2/3 excitatory neurons and in mice expressing jRGECO1a across all cortical layers. With this approach, we hope to begin to understand how contextual and sensory information integrate to guide behavior in a flexible manner.

Disclosures: P. Bech Vilaseca: None. **R. Dard:** None. **S. Crochet:** None. **C.C. Petersen:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.06/D42

Topic: D.02. Somatosensation – Touch

Support: DST - CSRI - 2021/83
IITGN Internal funds

Title: Lack of visual experience leads to severe distortions in the hand representation of the body model

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Abstract: We investigate the impact of vision on the development and maintenance of hand representation in the implicit body model. We performed a hand landmark localization task on congenitally and late blind individuals who lacked visual experience of their bodies and compared their performance to normally sighted and blindfolded participants. A series of studies investigating the distorted body model of hand using localization task has consistently shown that the participants underestimate their finger lengths and overestimate their hand widths. Some evidence suggests that this body model is not attributed to proprioceptive inputs alone, as some aspects of these distortions appear in visual judgments of body-part size and visual memory for non-body objects. Through measurements of finger lengths, hand width, and shape index, we demonstrate that blind participants exhibit significantly greater distortions in their hand representation compared to sighted and blindfolded controls. Notably, blind individuals displayed a marked overestimation of hand width and an underestimation of finger lengths, particularly in digits D2, D3, and D4. Surprisingly, blind subjects with partial vision displayed more severe distortions than those with no residual vision. Furthermore, our findings reveal that late-blind participants exhibit similar levels of distortion as congenitally blind individuals, suggesting an extended period of susceptibility to the lack of visual input in shaping body representations. The Reverse Distortion hypothesis provides a plausible explanation for these distortions, suggesting that a compensatory mechanism occurs in the body model to counteract the anisotropic cortical representations. Our results support this hypothesis: blind individuals have expanded cortical representations processing tactile information, so this could lead to more pronounced distortions in their hand representation of the body model representations. Overall, our study highlights the malleability of body representations and the intricate interplay between sensory inputs and cortical processing in shaping the implicit body model.

Disclosures: S. Kottu: None. **L. Lazar:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

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Program #/Poster #: PSTR219.07/D43

Topic: D.02. Somatosensation – Touch

Support: JST ERATO Grant Number JPMJER1701 (Inami JIZAI Body Project)
JSPS KAKENHI Grant Number 15K12623

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JSPS KAKENHI Grant Number 23K17460

Title: Neural differences induced by the location of an embodied independent supernumerary finger

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Abstract: Body representation in the brain is plastic. A perception of embodiment towards artificial limbs and bodies can be induced by even relatively short multi-sensory (usually visuo-haptic) stimulations (Botvinick and Cohen 1998, Longo et al. 2001, Aymetrich Franch and Ganesh 2016), leading to changes in behavior as well as the brain (Kieliba et al., 2021, Ehrsson et al. 2005). However, previous studies have focused on the "substitution" of body part(s) with artificial limbs that are controlled by remapping the movements of innate limbs. In our recent study, cognitive and behavioral experiments suggest that humans can also embody an "independent" supernumerary limb (robotic "sixth finger") that was attached to the side of the left little finger (Umezawa et al., 2022). Here, we examine how these independent limbs and, specifically, their locations change the human brain after embodiment. A total of 25 healthy individuals (all right-handed) participated in our experiments after granting written informed consent. Experimental procedures were approved by the institutional review board of The University of Electro-Communications and National Institute for Physiological Sciences. In the experiments, the participants habituated to the robotic sixth finger fixed at two different locations on the left hand, once beyond the little finger and once between the index finger and thumb. We recorded the brain activity using a 7T functional magnetic resonance imaging scanner before and after the habituation task. The habituation task was either the embodiment condition (the robotic sixth finger was voluntarily controllable with somatosensory feedback) or the non-embodiment condition (the robotic sixth finger was randomly moved without somatosensory feedback). We analyzed the geometrical relationship between innate finger representations in brain activity patterns using cross-validated squared Mahalanobis distance. We compared these matrices between before and after the habituation task, further to the embodiment and non-embodiment conditions. Results showed that the distance between innate finger representations increased when the robotic sixth finger was worn near the little finger side in the embodiment condition compared to the non-embodiment condition, whereas it decreased when worn between the index finger and thumb. These results suggest that the location of the independent artificial finger has a different effect on the body representation in the brain.

Disclosures: R. Yasuda: None. **K. Arai:** None. **Y. Takahara:** None. **D. Ueda:** None. **M. Fukunaga:** None. **G. Ganesh:** None. **Y. Miyawaki:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.08/D44

Topic: D.02. Somatosensation – Touch

Support: PAPIIT, UNAM, IN214524
fellowship 1002049 from CONAHCYT

Title: Differences in brain microestructure, psychological functions and mobility in people with lower limb amputation due to diabetes or trauma.

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Abstract: The primary causes of amputation are vascular diseases, including diabetes and peripheral arterial disease, and trauma. However, the cerebral, psychological, and physical changes surrounding the process of using a prosthesis are understudied in the mexican population. The present study aims to ascertain the distinctions in psychological functions, mobility, and cerebral microstructural parameters between 4 different groups. 1) a group with unilateral lower limb amputation due to type 2 diabetes mellitus (ADM, n=28); 2) a group with unilateral lower limb amputation due to trauma (ATR, n=15); 3) a group of non-amputees with diabetes (CDM, n=21) and 4) a control subjects group without amputation or a diagnosis of chronic degenerative disease (C, n=21). A cross-sectional study with non-probability convenience sampling was conducted. The following tests were employed: the predictor of lower limb amputee mobility without prosthesis (AMPnoPRO) or with prosthesis (AMPPRO), the Montreal Cognitive Assessment in Spanish (MoCA-E), the Beck Anxiety and Depression Inventories (BAI, BDI-II), the physical and mental quality of life questionnaire Health SF-36, and the Psychopathological Symptom Inventory SCL-90-R. Furthermore, diffusion-weighted magnetic resonance images were acquired. Data was analyzed by the Kruskal-Wallis test and the Games-Howell post hoc test. The results indicated that both amputation groups exhibited lower MoCA-E total and language scores. ADM demonstrated lower total and language MoCA-E scores. The ADM group exhibited lower scores in the visuospatial, memory, and orientation domains than the C group. In the SF-36, the ADM and ATR groups demonstrated lower scores than the C group, while the ATR group exhibited higher scores than the CDM group in the physical role domain. No statistically significant differences were observed in the BAI or BDI-II. The ATR group exhibited a higher level of mobility than the ADM group in the AMPPRO, and a lower score in the SF-36 Body Pain domain. Diffusion parameters, such as the anisotropy fraction (AF) of the corticospinal fascicles, exhibited lower values in the ADM group (n=22) than in the C (n=20) and CDM (n=12) groups. Furthermore, AF in the CDM group's medial lemniscus fasciculus was lower than that of the C and ADM groups. The observed differences between the amputation and control groups in our study were found to vary according to the

etiology of amputation. Consequently, this information could be employed to tailor approaches to the specific implications of each amputation etiology.

Disclosures: S. Pedroza Ramírez: None. **D. Arreguín:** None. **D. Trejo:** None. **E. Pasaye:** None. **R.G. Paredes:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.09/D45

Topic: D.02. Somatosensation – Touch

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Title: Dynamic sensorimotor maps in phantom limb pain

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Abstract: Title: Dynamic sensorimotor maps in phantom limb pain
Authors: Shahrzad Damercheli[1,2], Jamila Andoh*[1,3]& Herta Flor*[1] and the PLP group
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Phantom limb pain (PLP) is characterized by the perception of painful sensations in a limb that has been amputated, affecting approximately 80% of amputees and significantly impacting their quality of life. Various scientific hypotheses have been proposed to explain PLP, including cortical reorganization, maladaptive plasticity, and alterations in peripheral and central nervous system signaling. Yet, the driving factors behind dynamics in somatosensory and motor maps post amputation are subject to an ongoing debate. We investigated the role of diverse task contexts (motor, sensory), regions of interest (motor, somatosensory), as well as factors such as telescoping, voluntary control, prosthesis use and assessed the impact on PLP. We recruited

upper limb amputees both with (n=15) and without phantom limb pain (n=30) alongside matched controls (n=23), including a total of six female participants, with no significant differences in sex ratio and age between groups. Functional magnetic resonance imaging (fMRI) was carried out during a sensory and a motor task involving the mouth to assess cortical reorganization in the primary somatosensory (S1) and in the primary motor cortices (M1). Cortical distances were assessed as the shorted path between a reference point to the peak of the activation cluster of mouth activation. In the sensory task, the magnitude of PLP (rho=-.40, p=.036) and the degree of telescoping (rho=-.45, p=-.017) were positively correlated to cortical reorganization of the deafferented mouth (i.e. medial shift in the mouth representation). Furthermore, between-group comparisons reveal a significant difference in cortical distances between the PLP group and both the non-PLP and matched control groups, with p-values of 0.0140 and 0.030, respectively. These data suggest that processes related to reorganization and preserved limb function interact and may suggest a common origin for PLP. The exact mechanisms of these processes require to be further investigated.

Disclosures: S. Damercheli: None. **J. Andoh:** None. **H. Flor:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

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Program #/Poster #: PSTR219.10/D46

Topic: D.02. Somatosensation – Touch

Support: AHA 18AIREA33960251 #959486
Barkley Trust (Barlow)

Title: Machine learning tools for predicting gait-like plantar stimulation from BOLD activity of the brain

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Abstract: Leveraging machine learning, prior research has shown promising predictive models for human sensorimotor control during walking. This advancement could offer insights into disease prognoses and gait rehabilitation options. Our previous study in healthy adults showed that the accuracies for classifying walking conditions based on gait metrics were 97% with support vector machines (SVM), 96% with random forest (RF) and >95% with convolutional

neural network (CNN). A follow up study in stroke survivors showed a drop in such predictive accuracy displaying a considerable overlap in their gait patterns across the different tasks. Our current study aims to extend this approach to anticipate brain activation changes from plantar stimulation that mimics the tactile sensation perceived during walking, detected through fMRI analysis, and potentially serve as a biomarker of changes in sensorimotor control post-stroke. Ten healthy subjects were recruited for imaging in a 3.0T fMRI with a 1000ms repetition time, while receiving tactile stimulation to the plantar surfaces of the feet. Stimulation was applied by 6 pneumatic cells on each foot. Three stimulation speeds (25cm/s, 40cm/s, 55cm/s) were applied on each foot that traversed from heel to toe along the lateral border of the foot. Stimulation was applied in a block design, where each speed was repeated four times in a 14sec block and was followed by 14sec of no stimulation. Each speed stimulation block occurred 7 times per scan. This protocol was repeated twice per foot, resulting in four 9.8min scans. Significant brain activation was determined by comparison of stimulation and no stimulation periods. For machine learning analyses, the condition variable was used to create class labels. Remaining variables, like activation location and magnitude, served as predictors. Analysis methods included SVM, K-Means Clustering, RF, and CNN. Subject data was divided into training (n = 7) and test (n = 3) data sets. Results are expected to follow a similar trend obtained for gait analysis with high accuracy in healthy adults and a significant drop after stroke. If the machine learning models prove to have substantial predictive accuracy with healthy MRI data, it could be implemented to quantify disease-related neurological variations in clinical populations. By using a healthy predictive model, positive changes in predictive accuracy of stroke survivors during rehabilitation could indicate the intervention's efficiency in restoring the neural pathways damaged by stroke.

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Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.11/D47

Topic: D.02. Somatosensation – Touch

Support: AHA 18AIREA33960251 #959486
Barkley Trust (Barlow)

Title: Plantar Tactile Stimulation of Gait Speeds: An fMRI Study

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Abstract: Sensory information from the plantar surfaces of the feet plays a crucial role in the control of standing and walking. However, the specific sensory information utilized by the brain for effective walking control remains unclear. Previous research has indicated that the brain exhibits increased blood-oxygen-level-dependent (BOLD) responses in both sensory and motor areas when presented with a saltatory tactile stimulus at a velocity of 25cm/s on the palm of the hand, compared to stationary stimuli or stimuli at other velocities. Our investigation aims to determine if a similar response is present for the plantar surfaces of the feet. Specifically, if the preferred velocity of tactile stimulation could be related to the velocity of the center of pressure (CoP) traversing along the foot during walking. If so, this would point out a key role of the plantar surfaces in regulating gait and balance control. Therefore, the purpose of this study was to determine if there exists a specific stimulation velocity on the feet that elicits maximal BOLD responses. Additionally, to determine if this preferential velocity closely corresponds to the velocity at which the CoP traverses the foot during walking. Ten healthy young adults (6 Males, 4 Females) received plantar stimulation at three different velocities (25cm/s, 40cm/s, and 55cm/s) while BOLD responses were recorded using 3.0T functional MRI (Siemens) with a repetition time (TR) of 1000ms. Each foot received a total of 56 stimulations at each velocity using an MRI-compatible pneumatic device (pTACS). BOLD activity within sensory and motor regions during the stimulation velocities was compared to periods of no stimulation using SPM12 in MATLAB. Significant brain activation ($p < 0.05$ with familywise error correction) was determined within the primary sensory cortex, premotor cortex, supplementary motor cortex, and supramarginal gyrus at each velocity. Additionally, greater BOLD responses were found during stimulation at a velocity of 55cm/s compared to the other velocities. Given that the preferred velocity on the foot exceeds that found for the hand and falls within the range of CoP traversal velocities for preferred human walking speeds, these results suggest a learned neural association between CoP movement patterns and walking control. Further investigations into how diseases such as stroke may influence this tactile velocity preference of the foot could provide insight into the neuromechanics for changes in preferred walking speeds after stroke and future neuromodulatory techniques for improving gait functionality in stroke survivors.

Disclosures: C.P. Engsberg: None. **S.M. Barlow:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Provisional US Patent application submitted through UNL Tech Transfer for pTACS (Greenwood & Barlow). **Y. Wang:** None. **M. Mukherjee:** None.

Poster

PSTR219: Plasticity and Reorganization in the Somatosensory System

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR219.12/D48

Topic: D.02. Somatosensation – Touch

Support: ERC/EPSRC EP/X040372/1
EPSRC EP/W004062/1
ERC 715022
Observatory for Human Machine Collaboration Award

Title: Integration of Sensory Feedback for Wearable Devices

Authors: *L. DOWDALL[1], E. DA SILVA[1], G. DOMINIJANNI[2], M. MOLINA-SANCHEZ[1], M. BIANCHI[3], D. CLODE[1], T. R. MAKIN[1];
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Abstract: Our motor system relies on somatosensory feedback to inform about the current state of our limbs and their interactions with the external environment. Accordingly, great resources have been devoted to creating artificial somatosensory feedback for wearable robotics. However, the natural tactile feedback received from where such devices are worn on the body has been crucially overlooked. This 'intrinsic' sensory feedback may inform about device 'somatosensation'.

We first wanted to directly compare natural feedback to its artificial counterparts. We created three sensory feedback systems for use with the 'Third Thumb' (Dani Clode Design), a supernumerary robotic finger worn on the hand for motor augmentation: (1) Utilising natural mechanical feedback received on the side of the hand during Thumb usage. (2) Vibrotactile feedback where impact movements on the Thumb's tip elicited vibration delivered to the user's ring finger. (3) Skin stretch feedback, where deformation on the Thumb's tip produced linear skin stretch on the user's wrist.

We considered a series of tasks informing us about whether meaningful perceptual information can be extracted from the feedback. Participants (N=20) performed a texture discrimination task (most suited to the vibrotactile system) and a material density discrimination task (suited to the skin stretch system) with the three systems. We found the natural feedback performed comparably to each of the artificial systems, suggesting artificial sensory feedback may not provide additional value.

Following our demonstration that interpretable sensory information can be extracted from this natural feedback, we examined how the human somatosensory system processes and integrates this information. We assessed changes to the tactile hand and Thumb representation in primary somatosensory cortex (S1) before and after a week of motor training using the Third Thumb (n=30). For control, we also looked at tactile representations before and after keyboard training (n=20), and no training (n=20).

At baseline we observed that S1 processes tactile input arising from the Third Thumb in a topographically appropriate alignment to the biological fingers, but distinctly from that of the skin where it is attached. Following Thumb training, the representational similarity between the Third Thumb and the biological fingers increased, hinting at an integrative adaptation to the augmentation device.

Overall, our findings demonstrate the versatility of natural sensory feedback arising from

wearable technologies. Successful training with such a wearable device appears to trigger adaptive changes to its S1 representation in relation to our body.

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Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR220.01/D49

Topic: D.05. Auditory and Vestibular Systems

Support: Academy of Medical Sciences SBF007\100031

Title: Neuronal mechanisms supporting the perceptual learning of degraded speech

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Abstract: Human speech perception has a remarkable capacity to cope with sub-optimal auditory stimuli. This ability to cope depends, in part, on perceptual learning, which is a relatively long-lasting improvement in understanding degraded speech as a result of past experience or training (Davis et al., 2005). Two divergent theoretical frameworks of perceptual learning have been proposed. Transformation mechanisms suggest listeners learn to reverse the effect of degradation by a process of compensation or inverse transformation (see Cooke et al., 2022). This contrasts with a cue-reweighting mechanism which suggests a reweighting of acoustic-phonetic cues, for example, upweighting of intact cues and downweighing of degraded cues (Goldstone, 1998; Sohoglu & Davis, 2016). In the present study, 30 normal hearing listeners (25 female; mean age 21.47) were trained over three days to understand spoken sentences in which fine spectral modulations or fast temporal modulations were filtered out (see Flinker et al., 2019; Elliott & Theunissen, 2009; Webb & Sohoglu, 2023). Participants also listened to a clear speech control condition whereby the acoustic signal was unchanged. From day one to three, participants' word report accuracy increased from ≈ 20% to ≈ 60%, showing robust perceptual learning. On days one and three, we used EEG and temporal response function (TRF) analysis (Crosse et al., 2016), to assess neural tracking of intact modulations i.e. those present in both clear and filtered speech. We also assessed neural tracking of degraded speech modulations i.e. those present in clear speech but attenuated in filtered speech. We found a significant speech type x day interaction effect on neural tracking (TRF model accuracies for intact modulations: $F(1, 29) = 4.490$, $p = 0.04$; for degraded modulations: $F(1, 29) = 5.352$, $p = 0.03$). Comparing day one to day three, for filtered speech, we found a significant increase in tracking of intact modulations ($F(1,29) = 6.549$, $p = 0.02$), and a significant increase in tracking of degraded modulations ($F(1,29) = 6.183$, $p = 0.02$). No significant change for either modulation type was apparent in the clear speech control condition ($p > .05$). These results provide evidence

in favour of a transformation-based account of perceptual learning. Our findings shed important insights into how the brain adapts to perceptually challenging stimuli, with possible future clinical implications for cochlear implant users (Niparko et al., 2010).

Disclosures: E.M. Ambridge: None. **E. Sohoglu:** None.

Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR220.02/D50

Topic: D.05. Auditory and Vestibular Systems

Support: Mowat Wilson-Syndrome Foundation Maci Whisner Research Grant

Title: Role of specialized Zeb2 protein expression in intracortical connections subserving vocal learning

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Abstract: In Mowat-Wilson Syndrome, mutations of the zinc finger E-box-binding homeobox 2 (ZEB2) transcription factor are responsible for severe deficits in speech and language ability. Despite this, little is known regarding the neural mechanisms of how Zeb2 affects language. In the zebra finch songbird, T. guttata, the premotor cortical nucleus dedicated to learned song, HVC (proper name), innervates the primary motor nucleus known as the robust nucleus of the arcopallium (RA). This connection is critical to song development from subsong to plastic song, analogous to reduplicative babbling in babies. Juvenile male zebra finches engage in socially-mediated vocal learning by interacting with adult male tutors, securing the zebra finch as a plausible model for vocal development. Prior work bioinformatically describes Zeb2 upregulation in the HVC and cites its established role in axon guidance, suggesting a relationship between Zeb2 and intracortical connections that subserve vocal learning. As male, but not female, zebra finches engage in song learning during developmental critical periods, we generated a timeline of expression across ages and sexes to establish where and when Zeb2 acts. Preliminary results suggest a male-specific upregulation of Zeb2 in HVC during the sensorimotor critical period for vocal learning. Six shRNA plasmid constructs were developed to target conserved regions of Zeb2 mRNA across humans, parrots, and songbirds. SH-SY5Y cells were transfected with these plasmids and immunocytologically assessed for efficacy. One targeting plasmid attenuated Zeb2 by ~30% in cells that were successfully transfected 48 hours prior. In preparation for the HSV and AAV packaging and delivery of this promising plasmid, an additional time course study found that peak expression of the reporter gene occurred at 72 hours following in vivo HSV injection. Ongoing experiments are aimed at testing behavior in adult birds experiencing Zeb2 overexpression or knockdown. This study is the foundation of future

work elucidating morphological and neurobehavioral variances acquired through overexpression or knockdown of Zeb2 in premotor cortex at critical stages of song development.

Disclosures: L. Atkinson: None. **A. Holder:** None. **D. Sarmiento:** None. **G. Gedman:** None. **S.A. White:** None.

Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR220.03/D51

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R01 DC016868

Title: Phospholipid PIP_2 is the key Mediator of Slow Adaptation in Auditory Hair-Cell Mechano-transduction Process

Authors: *Y.-R. KIM[1,2,3], G. A. CAPRARA[1], S. JUN[1,2,4], A. W. PENG[1];
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Abstract: Auditory stimuli are transmitted to the inner ear, eliciting vibrations within the cochlear partition, comprised of hair cells and supporting cells situated atop the basilar membrane and covered by a tectorial membrane. Hair cells serve as auditory mechanoreceptors utilizing apically located hair bundles to transform sound-induced mechanical vibrations into electrical activity, a phenomenon referred to as mechano-transduction. This process is pivotal for cochlear amplification, which underlies exceptional sound level sensitivity, wide dynamic range, and notable frequency discrimination. This mechano-transduction is similar in the vestibular hair cells within the inner ear, which are responsible for balance. With a sustained step-like displacement, the hair-cell receptor current peaks followed by a decrease in the receptor current, termed adaptation. Among these adaptation mechanisms, slow adaptation, characterized by time constants typically ranging from 10-100 milliseconds, has been previously hypothesized to operate via a motor model of adaptation. This form of adaptation relies on Ca^{2+} influx through MET channels modulating the attachment of myosin motors along the stereocilia core. Our recent investigations have challenged the motor model of slow adaptation, proposing instead a novel model reliant on the phosphoinositide PIP_2. Previous work by others and us have shown that when a myosin motor, *Myo1c*, is inhibited in vestibular hair cells, slow adaptation is reduced. Strikingly, we could rescue slow adaptation during *Myo1c* inhibition with exogenous PIP_2, indicating that the binding of PIP_2 to the MET channel is an essential in mediating slow adaptation, and that the myosin motor may only be required to help PIP_2 localize close to the MET channel. In further support of this hypothesis, a point mutation in the TMIE protein, which

is an essential subunit of the MET channel and is known to mediate binding to phospholipids, including PIP_2, that results in reduced PIP_2 binding exhibits reduced slow adaptation. In further experiments, we attempt to rescue slow adaptation in the TMIE mutant mice with exogenous PIP_2. Additionally, we use exogenous PIP2 to rescue of slow adaptation inhibited by myosin ATPase activity blockers in cochlear hair cells to determine whether myosin motors help position PIP_2 near channels in cochlear hair cells, as we observe in vestibular hair cells. Our results will provide the first data describing a new model of slow adaptation. Additionally, our results will provide mechanistic insight into slow adaptation.

Disclosures: Y. Kim: None. **G.A. Caprara:** None. **S. Jun:** None. **A.W. Peng:** None.

Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: PSTR220.04/D52

Topic: D.05. Auditory and Vestibular Systems

Support: NIDCD Grant R01DC017439

Title: Causal involvement of superior temporal gyrus in retention of speech-acoustical maps

Authors: *N. RAO[1], D. J. OSTRY[2,3];
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Abstract: Acquisition of novel speech-acoustical maps is central to speech motor learning. Our recent work showed that even a single session of speech motor learning leads to retention of these maps for at least ~24 hours following acquisition. We also showed that the retention is indifferent to gradual or abrupt acquisition of speech-acoustical maps and is dependent upon the availability of speech auditory feedback. Recent evidence indicates the sensitivity of neurons within the superior temporal gyrus (STG) to the formant and vowel generation properties of speech in humans. However, the causal involvement of STG in retention of speech-acoustical maps is unknown. We hypothesized that plasticity in STG is critical to the retention of newly acquired speech-acoustical maps. To test this hypothesis, we recruited healthy young adults who performed a speech motor adaptation task to probe motor learning and retention. We delivered continuous theta burst transcranial magnetic stimulation (cTBS) over STG following learning and assessed its effect on retention and relearning ~24 hours later. In our preliminary data (n=22), we observed that retention was impaired when cTBS was delivered over STG versus that observed in controls. These findings establish the essence of plasticity in STG, a predominantly sensory cortical area, in retention of newly acquired speech-acoustical maps. Our study has implications on the role of sensory cortical areas as a ‘backdoor’ to motor areas for rehabilitation research.

Disclosures: N. Rao: None. **D.J. Ostry:** None.

Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: PSTR220.05/D53

Topic: D.05. Auditory and Vestibular Systems

Support: Baseline Research Funding from KAUST

Title: Functional maturation of layer 1 interneurons in the auditory cortex

Authors: *T. REYES VALLEJO[1], L. A. IBRAHIM[2];
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Abstract: Adaptation is a fundamental feature of the auditory cortex, where excitatory neurons show a decrease in their responses to recurrent and predictable auditory stimuli. This feature facilitates the detection of sounds and sudden changes in the environment. It is likely that Inhibitory interneurons contribute to the adaptation of excitatory neurons by inhibiting them through different mechanisms. Top-down modulation plays a key role in the ability to pay attention to relevant stimuli, likely by activating specific inhibitory neuron types. Layer 1 (L1) interneurons, specifically Neurogliaform cells (NGFCs), have been implicated in the integration of top-down signals with bottom-up signals coming from our environment. However, it is still unknown whether the NGFCs are involved in the adaptation observed in the auditory cortex and if their involvement in this process changes across development. Using in vivo 2-photon calcium imaging, we investigated the responses of NGFCs in L1 and L2/3 excitatory cells in the auditory cortex (AC) during an oddball auditory paradigm where mice were subjected to the repetitive presentation of a sequence of tones followed by the introduction of a non-predictable, “oddball” tone. In order to see if L1 NGFCs activity corresponded to the changes in response in L2/3 excitatory cells, we imaged semi-simultaneously from L1 and L2/3. Recordings were performed at two time points: development (p13-15, N=6) and adult (6-8 weeks, N=4). Results showed that a proportion (~ 34%) of adult L1 NGFCs in the AC increase their responses to the presentation of repetitive stimuli. We also observed that L2/3 neurons in adult reduce their responses to the presentation of repetitive stimuli, showing adaptation (~ 31%). Responses in both L1 and L2/3 during development show more variability across the trials. The proportion of cells showing adaptation and the increased response to repetitive stimuli also appears to increase in the adult compared to development. We will perform a causality analysis to determine whether the increase in L1 activity with the repetitive tone presentation can explain the adaptation observed in L2/3 responses. Our results suggest that a subset of L1 NGFCs play a role in the modulation of the adaptation responses to recurrent stimuli observed in the AC by increasing the inhibition onto L2/3 excitatory cells.

Disclosures: T. Reyes Vallejo: None. **L.A. Ibrahim:** None.

Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R01-DC04290
NSF Grant 2342847

Title: Human neuronal network sensitivity to probabilistic sequence learning

Authors: *Y. A. AYALA[1], Y. KIKUCHI[2], R. M. CALMUS[1], J. I. BERGER[1], C. K. KOVACH[1], H. KAWASAKI[1], T. D. GRIFFITHS[3,4], M. A. HOWARD III[1], C. I. PETKOV[1]; [1]Dept. of Neurosurg., Univ. of Iowa, Iowa city, IA; [2]Lab. of Cognitive and Translational Neurosci., Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [3]Biosci. Inst., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [4]Wellcome Centre for Human Neuroimaging, University College London, London, United Kingdom

Abstract: A fundamental aspect of human communication and cognition is the capability to extract ordering relationships between sensory events in a sequence. Studies from our group identified segregable neural processes in human and monkey auditory cortex, differentially sensitive to both high- and low-probability transitions following incidental auditory statistical learning (Kikuchi et al, 2017 PLoS Biology). However, the prior single-neuron data were only possible to obtain in monkeys. Here, we report human single-unit activity from multiple brain areas including auditory cortex and hippocampus recorded across nine epilepsy patients during clinical intracranial monitoring. The patient participants were first exposed to the statistical learning paradigm, listening to regularities in the ordering relationships in the sequences of nonsense words. Afterwards in a testing phase, they listened either to sequences consistent with the ordering regularities heard during the exposure phase (high-probability transitions) or to those containing sequencing violations (low-probability transitions). Expectedly, strongly driven responses to each sound in the sequence were observed in Heschl's gyrus and to a lesser extent in the hippocampus and other areas. We analytically contrasted the response to acoustically matched elements within the sequences that only differed in their prior sequencing context (e.g., high or low probability transitions). Neuronal signals stronger to high-probability transitions were categorized as 'prediction weighted' (Pw) signals and those to low-probability transitions as 'prediction-error weighted' (PEw). We observed human single-unit sensitivity to both types of signals, not only in the auditory cortex where they were first found in monkeys, but also in the broader system. Most of the auditory cortical neurons exhibited PEw (40%) than Pw signals (18%), while these proportions were reversed in the hippocampus (PEw: 24%, Pw: 38%) and other brain areas. Further work is underway to disentangle these signals in neuronal population spaces and to study the inter-areal interconnectivity via spike-field coherence. The results indicate that incidental auditory statistical learning engages a broad neuronal network in sequencing prediction and prediction error processing.

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Poster

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Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R00-DC015543
NIH Grant R01-DC021067

Title: Dynamic gating of perceptual flexibility by non-classically responsive neurons

Authors: *J. M. K. TOTH[1], B. SIDLECK[1], O. LOMBARDI[1], T. HOU[1], A. ELDO[1], M. KERLIN[1], X. ZENG[1], D. SAEED[1], P. AGARWAL[1], D. LEONARD[1], L. ANDRINO[2], T. INBAR[1], M. MALINA[2], M. N. INSANALLY[1];
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Abstract: Flexible responses to sensory cues in dynamic environments are essential for adaptive auditory-guided behaviors such as navigation and communication. How do neural circuits flexibly gate sensory information to select appropriate behavioral strategies based on sensory input and context? Auditory neural responses during behavior are diverse, ranging from highly reliable ‘classical’ responses (i.e. robust, frequency-tuned cells) to irregular or seemingly random ‘non-classically responsive’ firing patterns (i.e., nominally non-responsive cells) that fail to demonstrate significant trial-averaged responses to sensory inputs or other behavioral factors. While classically responsive cells have been studied for decades, the contribution of non-classically responsive cells to behavior remains underexplored despite their prevalence. Our previous work has shown that non-classically responsive cells in auditory cortex (AC) and secondary motor cortex (M2) contain significant stimulus and choice information and encode flexible task rules. Both classically and non-classically responsive units are essential for asymptotic task performance, yet their role during learning is unknown. Here, we explore how diverse cortical responses emerge and evolve during flexible behavior. We recorded single-unit responses from AC while mice performed a reversal learning task. Cortical response profiles during learning were heterogeneous, spanning the continuum from classically to non-classically responsive. We found that the proportion of non-classically responsive neurons significantly increased during late learning when the greatest gains in behavioral performance occur. Surprisingly, single-cell Bayesian decoding performance was highest for classically responsive cells during early and expert learning phases but significantly decreased during late learning. Population-level decoding revealed that during late learning mixed ensembles comprised of both classically and non-classically responsive cells encode significantly more task information than

homogenous ensembles and emerge as a functional unit critical for learning. Optogenetically silencing inputs from M2 selectively modulated non-classically responsive cells in AC, prevented mixed ensemble recruitment, and impaired reversal learning. Our findings demonstrate that top-down inputs recruit non-classically responsive neurons into diverse ensembles in auditory cortex to enable behavioral flexibility.

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Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: PSTR220.08/D56

Topic: D.05. Auditory and Vestibular Systems

Title: Cortical circuit plasticity in an auditory reversal shuttle-box task in Mongolian gerbils

Authors: *E. ACUN[1], M. F. HAPPEL[1,2], M. M. ZEMPELTZI[1], F. W. OHL[1];
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Abstract: The primary auditory cortex (A1) plays a central role in processing auditory stimuli and guiding decision-making processes. Despite the recognized importance of A1 in flexible behavior, the specific contributions of its individual cortical layers remain unclear.
To address this gap, we conducted multi-electrode recordings in Mongolian gerbils (Meriones unguiculatus) to investigate the relationship between the activity of different layers within the A1 and cognitive flexibility during auditory decision-making with multiple reversals of choice-outcome contingencies in a Go/NoGo shuttle-box paradigm. We analyzed laminar local field potentials and current-source density (CSD) profiles alongside behavioral outcomes (hit, miss, false alarm, and correct rejection). Utilizing signal detection theory, we categorized the subject's learning curves into low, intermediate and high performance levels based on the sensitivity index d'-values.
Our results reveal that as performance levels increase, significant layer-specific differences for each behavioral outcome become more pronounced, as verified by ANOVA analysis across different performance outcomes. Time-resolved generalized linear mixed models (GLMM) predicted accumulative evidence over the trial time of behavioral choices revealing layer-specific dynamics. Particularly, infragranular layer VI showed distinct activity patterns depending on performance levels. Increased activity during both low and high performance levels suggests the importance of layer VI, especially during phases of higher behavioral uncertainty and with high perseverative error rates.
Furthermore, during the intermediate level of discriminability, we found more pronounced differences in activation across all cortical layers with respect to certain behavioral choices

compared to low and high performing states. Particularly activity in supragranular layers I/II emerged as the best predictor for respective behavioral choices, indicating their importance in re-evaluation and re-learning changes in task rules and enhancing choice accuracy. These findings underscore the coordinated function of layer-specific circuitry in adapting behavioral strategies in response to multiple reversals of contingency, aligning with estimated predictions and enhancing choice accuracy.

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Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: PSTR220.09/D57

Topic: D.05. Auditory and Vestibular Systems

Support: NIH, Grant #R01 DC 018166

Title: Comparing information processing speed and speech perception in a mild traumatic brain injury (mTBI) population

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Abstract: Patients with a history of mild traumatic brain injury (mTBI) often have hearing complaints, such as understanding speech in complex auditory environments, that go unaddressed clinically. Past research shows that age, attention and working memory are significant contributors to a person's ability to understand speech in competition. These studies have attributed the impact to central processes, and possibly cognitive decline. Because mTBI patients tend to have greater cognitive difficulty, this raises the question of the contribution of cognition to mTBI patients' ability to understand speech in difficult situations. Here we explored the relationship between written language information processing rate and the ability to understand speech in competition. The main questions included were: (1) whether history of mTBI affects information processing rate, (2) whether mTBI relates to the ability to understand speech in competition, and (3) whether the information processing rate predicts the ability to understand speech in competition. Speech in competition was measured using a virtual spatial array where three spoken sentences were presented simultaneously via headphones. The three sentences were either presented in the same spatial location or in different spatial locations. Written information processing rate has previously been measured using a self-paced reading speed (RS) task. This study used a RS task that contains three short stories, where three words at a time are presented on the screen and participants are instructed to progress through at their natural pace. Sixty-three adults, 31 with and 32 without a history of mTBI, completed the speech in competition and RS tasks. Participant ages ranged from 18 to 74 years old, and their hearing

ability ranged from normal hearing to moderate sensorineural hearing loss. Preliminary results showed that there was not a significant difference between control and mTBI groups' performance on the RS or competing speech tasks. Linear regression models showed that information processing speed on the RS task was not a significant predictor of understanding speech in competition. Understanding the root of the hearing difficulty that mTBI patients experience is crucial to providing effective diagnostic and treatment strategies. This work begins to shed light on the cognitive factors that may apply to speech understanding.

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Poster

PSTR220: Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.05. Auditory and Vestibular Systems

Support: Biotechnology and Biological Sciences Research Council

Title: Auditory cortex neurons anticipate repeated sounds

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Abstract: The brain's ability to anticipate future sensory inputs is vital for navigating and surviving in the environment. While some theories propose that predictions and their influence are confined to the higher-order brain areas, others suggest that predictions underlie the computations performed across the brain. Distinguishing these two and other possibilities for the emergence of predictions would help us understand how perceptions emerge and change due to prior expectations. Neuronal activity in the sensory cortex is modulated by the predictability of the sensory stimuli. To disentangle the predictive signals from sensory-evoked responses, we investigate the neuronal responses when expected sounds are omitted. We use single-unit recordings in awake mice to observe how the auditory cortex neurons respond when anticipated sounds are omitted from a predictable sequence. Our findings reveal that a subset of neurons responds when the expected sound is omitted. The omission-responsive neurons belong to two distinct groups: (1) cells whose activity mimics that during the played sound and (2) cells with error-like responses which fire during unexpected omission of a sound but not its presentation. Several lines of evidence support the argument that both types of responses are specific to predictions: omission responses are robust across different sound sequences; the omission responses are not explained by the sound offset or the animal moving at the time of the unexpected omission. Lastly, the cells adjust their response to changing expectations, ceasing when sound omission becomes expected. Our results underscore that predictive signals can drive

activity in the auditory cortex. The responses we observe diverge from the ones proposed by existing theoretical frameworks, suggesting the potential for novel insights into mechanisms of predictive processing.

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Poster

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Topic: D.05. Auditory and Vestibular Systems

Support: DFG 52061794
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VF 2021-618

Title: Rare tone suppression in inferior colliculus that depends on the relative predictability of sounds

Authors: Z. YANG, ***L. DE HOZ**;
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Abstract: To make sense of the acoustic environment, the auditory system must segregate, based on their history, streams of sounds arriving at the ear simultaneously. This requires sensitivity to both the probability and the predictability of the different sounds in the stream. That the auditory system is sensitive to the probability of appearance of a sound is well established. Here we presented complex sound protocols with varying predictability to anesthetized mice. Neuronal responses in the mouse inferior colliculus (IC) were recorded using with Cambridge NeuroTech and Neuropixels probes. Neurons exhibited suppression that was specific to unpredictable sounds and could not be explained through tuning, probability of appearance, or adaptation triggered by the immediately preceding sounds. Notably, the magnitude of the suppression was dependent on the tuning of the neurons relative to the predictable sounds. Furthermore, the effect was insensitive to temporal expansions in the sound sequence resulting from increasing the inter-tone interval up to 4 times. Therefore, in complex auditory environments, neurons reflect unsupervised learning of the relative predictability of various sounds, in a process that might be relevant for stream segregation.

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Poster

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Program #/Poster #: PSTR220.12/D60

Topic: D.05. Auditory and Vestibular Systems

Support: EVF 2021-618

Title: Cortical and subcortical adaptive sound representation depending on stimulus statistics

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Abstract: The auditory system's sensitivity to stimulus statistics is essential for the temporal binding of auditory objects, which is in turn critical for complex scene analysis. While it is believed that both feedforward and feedback interactions within the auditory pathway dynamically modulate these functions, the underlying circuit mechanisms that extract and represent stimulus statistics remain poorly understood.We investigate these circuits mechanisms by simultaneously recording from the inferior colliculus (IC) and auditory cortex (A1) in awake and anesthetized mice. Broadband noise stimuli with modulated amplitude are a good model for statistical learning, since neurons in the IC code the current intensity differently dependending on th wider distribution of sound intensities. How is the distribution detected and in which time-scale? We examine how IC and A1 neurons encode sound statistics by changing the intensity range, and the time-scales of amplitude modulation. We investigate how these parameters are represented in terms of the dynamics of neuronal adaptation and timescales of integration. In addition, we use optogenetics to dissect the specific roles of excitatory and inhibitory sub-types in mediating these neuronal dynamics. In summary, we characterize how cortical and subcortical neurons encode and support a reliable stimulus encoding across different sound contexts and noise levels. These findings are critical for better understanding the way the auditory system can represent a noisy and constantly changing sound environment, ultimately helping to comprehend the mechanisms underlying the stable sound representation.

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Poster

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Program #/Poster #: PSTR220.13/E1

Topic: H.03. Decision Making

Support: German Childhood Cancer Foundation
Cluster of Excellence Hearing4all

Title: Establishing an oddball paradigm to investigate neural processing of auditory stimuli in rats

Authors: *F. M. DECKER, K. SCHWABE, J. K. KRAUSS;
Neurosurg., Hannover Med. Sch., Hannover, Germany

Abstract: The oddball paradigm allows to investigate the processing of behaviorally relevant and irrelevant auditory stimuli. In this study, we recorded local field potentials (LFPs) within the medial prefrontal cortex (mPFC) in rats performing an auditory oddball paradigm to analyze sensory event-related potentials (ERPs), in particular the early and late component analyzed between a time range of 50 - 190ms and 200 - 450ms after auditory stimuli, respectively. Rats (n=9) were trained in a three-tone auditory oddball paradigm, where they had to respond by nose poke to a rare target tone (5000 Hz), being rewarded by a casein pellet, while ignoring a rare distractor (1500 Hz) and frequent standard tone (3000 Hz). After reaching a predefined success criterion of correct responses to the target tone and correct rejection of the standard and distractor tones (80%, each), electrodes were stereotaxically implanted into the mPFC under general anesthesia with postoperative pain control. The recording of the neuronal activity took place in the three-tone oddball paradigm as well as in a passive two-tone oddball paradigm with unfamiliar frequencies of 95% (standard; 8000 Hz) and 5% (distractor; 13300 Hz). We showed that correct responding to the target tone is accompanied by a higher amplitude in the early and late component of the ERP in comparison to the standard and distractor tones ($p<0.05$). The ERP amplitudes to incorrect responses to auditory stimuli did not differ between target, distractor and standard. We also found that the rats poke more precisely to the target tone, whereas the rats poke more randomly to the standard and distractor tone. In the two-tone oddball paradigm, the amplitude of the early and late component of the distractor ERP matches the distractor three-class ERP, and was larger than the ERP to the standard stimulus ($p<0.05$). Overall, this model provides an opportunity to investigate the processing of behaviorally important and irrelevant auditory stimuli in subcortical brain regions in more depths, also regarding the false processing. Future work will focus on modulating the frequency of both the target and distractor tones to avoid over-training of the rats.

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Poster

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Topic: D.05. Auditory and Vestibular Systems

Support: Individual Research Grants 1126/18 from the Israel Science Foundation to IN

Title: Neural and behavioral correlates of sound preferences in auditory cortex

Authors: *K. SEHRAWAT, I. NELKEN;
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Abstract: Sound preferences may be innate but are often learned. In humans, sound preferences are often studied using music, and preference for music was related to the release of dopamine in the striatal reward system. Moreover, there was an increase in functional connectivity between the auditory cortex and the reward system during pleasurable music listening. We studied sound preferences in mice. Studies have shown that mice can be trained to prefer human music. We exposed mice during critical periods of development to musical excerpts from the first movement of Beethoven Symphony 9, with free access to food and water. As control, we used 'sham' exposed mice (exposed to silence), mice exposed to chirp sounds instead of music, and naive mice, who were left in the animal house and didn't undergo any exposure. We measured their preference for exposed music in adulthood using a two-choice preference test. The test lasted 3 hours, and mice could choose between the music or silence zone without reinforcement. We found that the preference for the exposed music was sex-dependent. Male mice exposed to non-music environments avoided the music zone. However, both music-exposed and naive male mice spent more time in the music zone. Female mice did not show a clear preference due to frequent switching between the music and silence zones. We measured the neural responses of the same mice under anesthesia using wide-field calcium imaging. We observed an overall suppression in the neural activity of the exposed (to music and to silence) compared to the naive mice. There was a robust negative correlation between sound preference and auditory cortex activity in female mice. In contrast, the auditory cortex activity did not correlate with preference among males.

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Poster

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Program #/Poster #: PSTR220.15/E3

Topic: D.05. Auditory and Vestibular Systems

Support: ISF Grant 1126/18

Title: Early music exposure affects neural activity in the ventral tegmental area (VTA)

Authors: *S. SOUFFI, I. NELKEN;
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Abstract: Dopaminergic release from neurons of the ventral tegmental area (VTA) has been widely studied for its role in reward, prediction error encoding, behavioral reinforcement, motivational salience and learning processes. Dopamine release has been implicated in determining sound preferences as well, but this role is less well characterized. We exposed male and female mice in their homecage to human music (1st movement of Beethoven's 9th

symphony) or silence from P7 to P40, covering both early and late auditory critical periods. At early adulthood, we performed a free-choice behavioral test in which mice could choose to dwell in a music zone or in a silence zone of the test box. Following the test, we performed fiber photometry in the VTA of these mice, while they moved freely and heard simple (broadband noise and pure tones) and complex (exposed and unexposed music excerpts) sounds. On average, we found that exposed mice (music- or silence-exposed) spent a longer time in the music zone than in the silence zone compared to naive mice, with noticeable differences between males and females: in males, the music-exposed mice spent longer time in music than in silence compared to the silence-exposed mice and in contrary, in females, the music-exposed mice spent shorter time in music than in silence compared to the silence-exposed mice. All sounds were associated with both increasing and decreasing calcium transients. The music-exposed mice showed a large decrease in VTA activity to all sounds compared to naive mice; silence-exposed mice showed a smaller decrease in activity (males) or no significant change (females). Further experiments are currently conducted on naive TH-cre mice using a Cre-dependent GCaMP virus (AAV9-Syn-DIO-jGCaMP8m) allowing chronic recordings of the dopaminergic neurons only.

Disclosures: S. Souffi: None. **I. Nelken:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.01/E4

Topic: D.05. Auditory and Vestibular Systems

Title: Auditory spatial processing and cortical activity in a 5xFAD mouse model of Alzheimer's Disease

Authors: *C. BOWE[1], C. RODGERS[1], R. GARGIULLO[1], L. WILLIAMSON[1], K. COBB[1], J. MAI[2], V. ESHO[2];
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Abstract: Research in Alzheimer's disease (AD) has primarily focused on the presence of amyloid beta and tau neurofibrillary tangles in affected neurons with little focus on how the pathology alters neural network activity. Currently there is evidence of impaired communication within brain networks, described as changes in “functional connectivity”, resulting in improper coordination and integration of information between brain regions in networks such as the default mode network. However, the studies that describe these findings mostly use FMRI which lacks the temporal and spatial resolution to accurately measure the changes in neural activity that relate behavior to functional connectivity. Using an animal model with similar findings will allow for the use of more invasive and accurate methods of recording neural activity. To investigate how these changes in neural activity affect behavior, we train a mouse model of amyloidosis (5xFAD) to perform an auditory spatial processing task. This task is designed to test their ability to integrate auditory, navigational, and motor information. During this task, we use a

video tracking algorithm (SLEAP) to record their movements and use this data to compare the differences in motor behavior between the 5xFAD models and controls. Preliminary data currently shows 5xFAD models perform worse at the task in comparison to their control counterparts, although we have also observed strain differences between B6SJL and a hybrid of C57BL/6J with CBA/CaJ. The average speed and distance traveled does not significantly differ between 5xFAD and control, meaning that this performance difference cannot be explained by gross changes in locomotor ability. We are currently characterizing the behavioral patterns in both groups of mice using an unsupervised machine learning tool known as Keypoint MoSeq. In the future we plan to implant tetrodes into the auditory cortex, motor cortex and hippocampus as the mice perform their task and compare the neural activity and functional connectivity of 5xFAD mice and controls. Enhancing our understanding of how AD pathology translates to the aberrant neural activity that leads to AD symptoms will allow us to explore new therapeutic targets for neural modulation methods.

Disclosures: C. Bowe: None. **C. Rodgers:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

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Program #/Poster #: PSTR221.02/E5

Topic: D.05. Auditory and Vestibular Systems

Support: AARF-22-970734

Title: Uncovering hidden sensorimotor memories in mice with Alzheimer's disease relevant pathology

Authors: *A. SANTI[1], S. Y. MOORE CORONA[2], J. LAWLOR BLONDEL[2], A. WANG[2], K. FOGELSON[3], K. KUCHIBHOTLA[4,5,6];
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Abstract: Memories must be accessible for them to be useful. Alzheimer's disease (AD) is a progressive form of dementia in which cognitive capacities slowly deteriorate due to underlying neurodegeneration. Interestingly, anecdotal observations have demonstrated that AD patients can exhibit cognitive fluctuations during all stages of the disease, including the paradigmatic case of lucidity. It is thought that contextual factors are critical for unlocking these hidden cognitive abilities. Here, we used a modified auditory go/no-go task together with two-photon calcium imaging in the auditory cortex of behaving AD-relevant mice (APP/PS1+), to test whether amyloid accumulation impacts underlying sensorimotor memories, their contextual expression,

or both. We found that, while contextual expression was significantly impaired in APP/PS1+ mice, the sensorimotor memories were surprisingly preserved. This poor contextual expression was driven by overall network suppression, reduced stimulus selectivity, and aberrant behavioral encoding, but was surprisingly counteracted by compensatory disinhibition. These impairments were specific to contextual expression and concentrated near Aβ plaques. A reinforcement learning model pointed to deficits in contextual scaling of inhibitory processes as the primary determinant of poor expression, consistent with our neural data. Taken together, our results suggest that amyloid deposition impacts the integration of behavioral signals that enable contextual expression, rather than direct degradation of the underlying sensorimotor memories, suggesting that modulating these circuits may hold promise to reveal hidden memories in AD.

Disclosures: A. Santi: None. **S.Y. Moore Corona:** None. **J. Lawlor Blondel:** None. **A. Wang:** None. **K. Fogelson:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.03/E6

Topic: D.05. Auditory and Vestibular Systems

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NIH R00 DC015014
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Brain & Behavior Research Foundation NARSAD

Title: Rapid emergence of latent knowledge in the sensory cortex drives learning

Authors: *C. DRIEU[1], Z. ZHU[2], Z. WANG[1], A. WANG[1], S. ELNOZAHY[1], K. KUCHIBHOTLA[3];
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Abstract: Rapid learning confers significant advantages to animals in ecological environments. Despite the need for speed, animals appear to only slowly learn to associate rewarded actions with predictive cues. This slow learning is thought to be supported by a gradual expansion of predictive cue representation in the sensory cortex. However, evidence is growing that animals learn more rapidly than classical performance measures suggest, challenging the prevailing model of sensory cortical plasticity. Here, we investigated the relationship between learning and sensory cortical representations. We trained mice on an auditory go/no-go task that dissociated

the rapid acquisition of task contingencies (learning) from its slower expression (performance). Optogenetic silencing demonstrated that the auditory cortex (AC) drives both rapid learning and slower performance gains but becomes dispensable at expert. Rather than enhancement or expansion of cue representations, two-photon calcium imaging of AC excitatory neurons over weeks and unsupervised tensor decomposition revealed two higher-order signals that were causal to learning and performance. First, a reward prediction (RP) signal emerged within tens of trials, was present after action-related errors only early in training, and faded at expert levels. Strikingly, silencing at the time of the RP signal impaired rapid learning, suggesting it serves an associative and teaching role. Second, a distinct cell ensemble encoded and controlled licking suppression that drove the slower performance improvements. These two ensembles were spatially clustered but uncoupled from underlying sensory representations, indicating a higher-order functional segregation within AC. Our results provide a mechanistic dissociation between learning and performance and reshape our understanding of the fundamental role of the sensory cortex.

Disclosures: C. Drieu: None. **Z. Zhu:** None. **Z. Wang:** None. **A. Wang:** None. **S. Elnozahy:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.04/E7

Topic: D.05. Auditory and Vestibular Systems

Support: R01DC018650
R00DC015014
HHF973559

Title: The dynamics of auditory cortical astrocytes during sensorimotor learning

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Abstract: Astrocytes, the predominant glial cell type in the brain, have emerged as active participants in neural information processing and plasticity. However, their functional dynamics across learning remain poorly understood. Here, we used chronic two-photon calcium imaging to longitudinally track individual astrocyte Ca^{2+} dynamics in the auditory cortex of awake mice (expressing Aldh1l1-dependent GCaMP6s in an inducible manner) across the acquisition of an auditory discrimination task. We trained mice to lick to a tone for water reward (S+) and withhold from licking to another tone (S−) to avoid a timeout. Over several days of training, astrocytes exhibited learning-related modulation of their Ca^{2+} dynamics, with cells showing

enhancement of their evoked responses during rewarded trials. This increased activity was not due to licking as the same astrocytes exhibited suppressed activity on errors of action (incorrect licking to the S-, false alarm). Omitting reward on correct trials (hits, S+), however, led to biphasic responses where a transient increase in activity was followed by a profound suppression, suggesting that reward consumption may drive extended increases in astrocyte activity. Interestingly, reward-related astrocyte calcium signals extended beyond individual trials, potentially playing a role in maintaining a signature of reward and trial history for upcoming choices. Our data suggest that coordinated astrocyte ensembles may provide a scaffold for integrating reward signals with sensory processing to facilitate learning, potentially bridging trial-level and inter-trial computations. This study expands our understanding of astrocyte contributions to neural circuit dynamics underlying adaptive behavior.

Disclosures: S. Moore Corona: None. **Z. You:** None. **K.V. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: PSTR221.05/E8

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01DC018650
NIH R00DC015014

Title: Revealing abrupt transitions from goal-directed to habitual behavior

Authors: *Z. WANG[1,2], S. MOORE CORONA[3], Z. ZHU[4], R. SUN[5], A. LEE[3], A. CHARLES[5], K. V. KUCHIBHOTLA[3,6,7];
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Abstract: A fundamental tenet of animal behavior is that decision-making involves multiple 'controllers.' Initially, behavior is goal-directed, driven by desired outcomes, shifting later to habitual control, where cues trigger actions independent of the motivational state. Clark Hull's question from 1943 still resonates today: "Is this transition [to habit] abrupt, or is it gradual and progressive?" Despite a century-long belief in gradual transitions, this question remains unanswered as current methods cannot disambiguate goal-directed versus habitual control in real time. Here, we introduce a novel 'volitional engagement' approach, motivating animals by palatability rather than biological need. Providing less palatable water in the home cage reduced motivation to 'work' for plain water in an auditory discrimination task compared to water-restricted animals. Using quantitative behavior and computational modeling, we found that

palatability-driven animals learned to discriminate as quickly as water-restricted animals but exhibited state-like fluctuations when responding to the reward-predicting cue-reflecting goal-directed behavior. After thousands of trials, these fluctuations spontaneously and abruptly ceased, with animals always responding to the reward-predicting cue. In line with habitual control, post-transition behavior displayed motor automaticity, decreased error sensitivity (assessed via pupillary responses), and insensitivity to sensory-specific outcome devaluation. Bilateral lesions of the habit-related dorsolateral striatum (DLS) blocked transitions to habitual behavior. Finally, we used bilateral fiber photometry in the putative controllers of goal-directed (dorsomedial striatum, DMS) and habitual (DLS) behavior to monitor the evolution of neural activity across learning. Both the DMS and DLS exhibited learning-related plasticity in cue, lick, and outcome-related signaling at similar timescales in parallel. Immediately after transitioning to habitual behavior, outcome-related signaling was suppressed in the DLS and, to a lesser extent, in the DMS, while cue-evoked responses further sharpened. This abrupt shift (reduction in outcome signaling and sharpening of cue-evoked responses) indicated that sensory cues rather than outcomes drive habitual responding. Our results demonstrate that both controllers (DMS and DLS) exhibit learning-related plasticity in parallel but that the behavioral manifestation of habits emerges spontaneously and abruptly in a DLS-dependent manner, suggesting the involvement of a higher-level process that arbitrates between the two.

Disclosures: Z. Wang: None. **S. Moore Corona:** None. **Z. Zhu:** None. **R. Sun:** None. **A. Lee:** None. **A. Charles:** None. **K.V. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: PSTR221.06/E9

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01DC018650
R00DC015014
JHU Kavli NDI Distinguished Graduate Fellowship

Title: Multi-area cortical mechanisms underlying continual learning

Authors: *Z. ZHU[1], A. CHARLES[2], K. KUCHIBHOTLA[3];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Univ. - Homewood Campus, Lutherville-Timonium, MD; [3]Psychological and Brain Sci., Neurosci. and Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Humans and other animals can learn and execute many different tasks throughout their lifespan, a process known as continual learning. However, this biological ability challenges many artificial neural networks that suffer from catastrophic forgetting, unless these networks are regularized or expanded. Specifically, unique information about new tasks can be encoded

through expansion (adding ‘neurons’ into the network for a new task), while shared information between old and new tasks can be integrated into shared representations. Here, we aimed to test how the biological brain naturally solves this problem. We trained mice on a class of tasks involving the learning of multiple, related, sensorimotor associations, specifically multiple distinct auditory two-choice tasks using a moveable wheel. We exploited two training curricula where mice learned these tasks either sequentially or simultaneously. In both configurations, mice expertly performed both tasks in a block-based manner at the final stage of training. We tracked neural activity of L2/3 pyramidal cells in the auditory cortex (AC) and the posterior parietal cortex (PPC) using multi-area two-photon mesoscopic calcium imaging. This recording method allowed us to longitudinally track expansion and integration of neural representation at single-cell resolution throughout different stages of multi-task learning. Surprisingly, a sub-area in PPC showed both reliable auditory responses even in naive animals and dynamic response patterns during learning, indicating its importance in learning of multiple auditory tasks. Together, our behavioral and neural approach promises to help us better understand the precise computations used by biological neural networks for continual learning and how this depends on the learning curriculum.

Disclosures: Z. Zhu: None. **A. Charles:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: PSTR221.07/E10

Topic: D.05. Auditory and Vestibular Systems

Support: NSF CAREER Award
JHU Discovery Award

Title: Investigating rule learning in mice with an olfactory-cued auditory two-choice serial reversal task

Authors: *F. ZHU[1], X. LE[1], K. KUCHIBHOTLA[1,2,3];
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Abstract: Animals can flexibly adapt their responses to the same sensory stimuli in different contexts to maximize their reward rate. In situations where two distinct contexts reliably predict opposite action-outcome associations across multiple pairs of sensory stimuli, animals can solve the task more efficiently by applying a context-dependent reversal rule. To investigate the extent to which mice could learn such a rule, we trained mice to perform an auditory two-choice (licking left versus licking right) serial reversal task in which odor cues provided at the start of each trial to indicate the context for discriminative action-outcome associations (standard or

reversed). Mice (n=11) learned both the standard and reversed contexts in ~2 weeks and a subset of the mice stably performed multiple serial reversals to an accuracy of >90% over subsequent training sessions. We confirmed that animals used the odor to differentiate the two contexts as behavioral performance was significantly degraded on 'catch' trials in which the odor was omitted or changed. Importantly, mice rapidly learned new stimulus sets in both the standard and reversed task. Mice adopted two distinct strategies when learning the task initially - one group of animals displayed a static bias that corrected over time while another showed a dynamic bias that switched between the two sides. Expert animals continued to display a response bias at the start of each reversal. We further performed two-photon imaging with a micro-periscope in the medial prefrontal cortex to quantify neural responses to different task variables (context, stimuli, response, reward). These findings lay the groundwork for future optogenetics probing of the causal role of 'rule-related' neuronal activity.

Disclosures: F. Zhu: None. **X. Le:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

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Program #/Poster #: PSTR221.08/E11

Topic: D.05. Auditory and Vestibular Systems

Support: JHU Discovery Award

Title: Compositional learning in the multi-task mouse playground

Authors: *A. BAL[1,2], A. SANTI[3,4], S. SOTO[3,4], P. H. JANAK[3,4,5,6], K. KUCHIBHOTLA[3,4,5,6,7]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Psychological & Brain Sciences, Johns Hopkins University, Baltimore, MD; [3]Psychological & Brain Sci., Johns Hopkins Univ., Baltimore, MD; [4]Johns Hopkins University, Baltimore, MD; [5]Johns Hopkins Kavli Neuroscience Discovery Institute, Johns Hopkins University, Baltimore, MD; [6]Solomon Snyder Department of Neuroscience, Johns Hopkins University, Baltimore, MD; [7]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD

Abstract: Humans exhibit remarkable abilities in multi-task learning, utilizing compositionality to rapidly excel at novel tasks by building upon simpler primitives. However, the behavioral and neural mechanisms underlying compositional learning remain unknown. Studying the neurobiological basis of such learning presents unique challenges, including the need to obtain large-scale neural recordings across many unique tasks. Mice emerge as a promising candidate organism, given their amenability to longitudinal neural recordings. However, there is a common belief that mice may have limited cognitive capacity for more complex learning paradigms. To overcome these obstacles, we created the Multi-Task Mouse Playground, an innovative fully-automated behavioral training system that provides mice with a naturalistic environment and full volitional control over their learning process. Using the Mouse Playground, we first trained mice

(n=7) on two primitive Go-NoGo tasks sequentially. Mice learned an auditory sweeps direction task (T1), where the S+ was a frequency upsweep, and the S- was a frequency downsweep. We subsequently trained these mice on a second Go-NoGo task, in which a short pure tone served as the S+ (40ms 6kHz) and a long pure tone was the S- (160ms 6kHz). Finally, in the compositional task (T3), mice were required to discriminate between four stimuli: short upsweep (coherent, S+), short downsweep (S-), long upsweep (S-) and long downsweep (coherent, S-). Across these task phases, we demonstrate that mice a) exhibit the ability to learn two primitive tasks (T1, T2), b) exhibit high proficiency in learning a compositional task (T3), and c) display remarkably rapid learning in the compositional task on coherent stimulus features derived from the primitive tasks. Furthermore, mice retained high performance on both primitive sub-tasks following exposure to the compositional task, indicating that the compositional task does not interfere with prior learning. These results provide evidence that mice may combine knowledge from primitive tasks to solve a compositional task. Additionally, these results provide evidence that the Mouse Playground is a valuable platform for the automated training of multiple tasks, which we can use for future studies into multi-task learning more broadly.

Disclosures: A. Bal: None. **A. Santi:** None. **S. Soto:** None. **P.H. Janak:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Program #/Poster #: PSTR221.09/E12

Topic: D.05. Auditory and Vestibular Systems

Support: Kavli Postdsoctoral Fellowship to JL
NIH Brain Initiative Grant R34 NS118462-01

Title: Spatially clustered neurons encode vocalization categories in the bat midbrain

Authors: *J. LAWLOR BLONDEL[1,2], M. J. WOHLGEMUTH[3], C. F. MOSS[1,2,4,5], K. KUCHIBHOTLA[1,2,4,6];
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Abstract: Categorical perception of sensory inputs, including human speech, enables adaptive behavior and is thought to emerge in the sensory cortex. There would be significant computational advantages, however, to functional specialization before cortical processing. To what extent do categorical representations emerge early in the auditory hierarchy? To address this, we conducted two-photon imaging experiments in an animal species that depends heavily

on acoustic signaling processing and exhibits a rich repertoire of communication calls, akin to human listening and speech. The big brown bat, *Eptesicus fuscus*, stands out in the animal kingdom for its reliance on sound processing for navigation (through echolocation) and for social interactions (through acoustic communication with conspecifics). *Eptesicus fuscus* move in three dimensions through their environment and must rapidly distinguish between acoustic signals used for navigation and those used for social interaction. Here, we used two-photon calcium imaging in the awake big brown bat, to enable large-scale (9,446 neurons in five bats), spatially resolved recordings in the inferior colliculus (IC) of animals listening to auditory playbacks. We discovered a novel, superficial tonotopy in the IC that was orthogonal to spatially clustered representations of social and navigation vocalizations. Population decoding revealed sharp boundaries across, but not within, these categories. Temporally reversing the vocalizations degraded the categorical boundary, suggesting that IC neurons are sensitive to the ethological relevance of the stimuli. To examine the categorical nature of single neurons, we created four acoustic morphing continua that transition between social and navigation exemplars. We found a substantial fraction of recorded neurons responded in a categorical manner to one of those continua. Auditory models for perceptual categorization rely on the idea that the periphery and midbrain possess mostly a feedforward and filter-bank role. Our data support a revised view of neural categorical representation in which ethologically relevant sensory streams are spatially segregated early in the auditory hierarchy and provide parallel channels of categorical 'primitives' to downstream regions.

Disclosures: J. Lawlor Blondel: None. **M.J. Wohlgemuth:** None. **C.F. Moss:** None. **K. Kuchibhotla:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.10/E13

Topic: D.05. Auditory and Vestibular Systems

Title: Contextual Modulation of Sound in the Central Auditory System of Bats

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Abstract: Socio-emotional context of sound is essential to how we communicate throughout our day-to-day lives. However, there is still a gap in the literature regarding how exactly we as humans contextually modulate social communication. A tractable mammalian model that can be used to investigate this gap in knowledge is the bat, Carollia perpicillata. Carollia perpicillata, like most bats, are auditory specialists that can emit echolocation calls as well as social communication calls. These bats live in dynamic social groups where they communicate through a wide and well-categorized vocal repertoire. Using behavioral and electrophysiological

methods, we investigate the distinct neuronal mechanisms used to perceive and emit echolocation calls and social communication calls. Behaviorally, we performed two different paradigms- the social preference task and the playback test. In the social preference task, a focal bat is presented with the opportunity to choose between an empty cage and a known conspecific. We analyzed the bat's preferences and found that the bats spend more time exploring the known individual than the empty cage. Furthermore, we found that the bats emit a variety of social calls before approaching the conspecific. The speaker test consists of a focal bat in an acoustic chamber with a speaker that emits echolocation calls, and two types of social calls: aggressive down sweeps and distress calls. In this way, we can evaluate the behavioral responses to these calls as they are presented via loudspeaker and thus evaluate to which extent these compare to the behavior of the bat when it hears a live conspecific (as in the social preference test). Lastly, we took extracellular recordings from the inferior colliculus (IC) in wake bats passively listening to playbacks of the same types of calls as in the speaker test. We evaluated the neural responses in the IC to different spectral properties of the acoustic stimuli (i.e. duration, intensity, and bandwidth of the calls). Our results show that there is differential selectivity to different call categories in the IC of Carollia perspicillata bats. With this research, we continue to build on previous work from our and other labs that aim to understand the mammalian neural mechanisms underlying the auditory processing of socially relevant sounds.

Disclosures: Z. George: None. **I. Marin:** None. **A. Salles:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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HHF Emerging Research Grant

Title: Deep-layer projection neurons develop representations of perceptual categories and behavioral choice

Authors: *M. MALINA[1], N. A. SCHNEIDER[2], R. S. WILLIAMSON[2];
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Abstract: Auditory-guided behavior is a fundamental aspect of our daily lives, whenever auditory information guides our decisions and actions. Nestled amongst several populations, extratelencephalic (ET) neurons reside in the deep layers of auditory cortex (ACtx) and provide a

primary means of routing auditory information to sub-cortical targets associated with decision-making and action. To investigate the behavioral role of L5 ET neurons, we trained head-fixed mice to categorize the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either slow or fast to receive a water reward. We then used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of L5 ET, as well as layer (L)2/3 and L5 intratelencephalic (IT) populations.
L5 ET neurons significantly changed their stimulus tuning across learning. Longitudinal recordings revealed that these neurons dynamically shifted their responses to selectively encode the slow and fast categories of the trained stimuli. This categorical selectivity correlated with performance and was completely absent in untrained mice. In trained mice, these L5 ET neurons showed notably weaker selectivity during passive listening, suggestive of top-down modulatory input. Furthermore, decoding analyses revealed a robust representation of category identity in the L5 ET population which grew with learning. Categorical information was weaker and stayed relatively constant in both L2/3 and L5 IT populations, implicating L5 ET cells specifically in the acquisition of auditory categories.
Behavioral choice could also be robustly predicted from L5 ET activity. Choice activity grew with learning and preceded motion onset, emphasizing that these signals were separate from motor activity. Choice signals were only weakly present in L2/3 and L5 IT populations and did not change across learning. However, while L5 IT neurons did not show categorical selectivity at stimulus onset, they did display categorical selectivity following the animal's choice. This effect was only present in the earliest days of learning, hinting at a role for these neurons in early association learning of auditory stimuli. Together, these results suggest that ACtx projection neuron sub-types differentially encode behaviorally relevant stimuli throughout learning, emphasizing the divergent pathways from ACtx and their contributions to auditory-guided behavior.

Disclosures: M. Malina: None. **N.A. Schneider:** None. **R.S. Williamson:** None.

Poster

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Klingenstein-Simons Fellowship Award in Neuroscience
T32 Training Grant in Auditory and Vestibular Neuroscience

Title: Arousal-dependent modulation of distinct excitatory cell-types within the auditory cortex

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Abstract: The neural basis of perception involves the interplay between external inputs and internal states. For example, an animal's arousal level exerts continuous influence on the neocortex, impacting membrane potentials, cortical state, neuronal gain, and sensory processing. Stimulus encoding recruits an array of distinct excitatory cell-types that span the cortical lamina (intratelencephalic (IT), extratelencephalic (ET), and corticothalamic (CT) cells), each with unique genetic profiles, morphology, and connectivity. We hypothesized that arousal, indexed by pupil diameter, would differentially modulate the sensory coding of these subclasses in mouse auditory cortex. We combined *in vivo* two-photon calcium imaging and pupillometry to investigate state-dependent changes in L2/3 (n=1796), L5 IT (n=2886), ET (n=2263), and CT (n=3432) cells. We first used a multivariate regression model to discern whether a cell was modulated by sound and/or pupil. There was heterogeneity across all groups, including suppression and enhancement effects, with about 22% of cells modulating their firing rate with arousal. We next analyzed the response strength to pure tones and found that neural activity increased with arousal in all cell types, except CT. We next examined shared trial-to-trial response variability (noise correlations) and found that higher states coincided with a decrease in shared variability in all groups. Correlating tuning curves (signal correlations) provides a comprehensive measure of receptive field similarity between a pair of cells. We found that signal correlations decreased in L5 IT/ET cells but increased in L2/3 and CT cells at higher states. This suggests that wakefulness alters correlated neural activity, highlighting the role of arousal in shaping functional connectivity. State-dependent changes in tuning and reliability could influence the precision of sound encoding. We tested this by training a support vector machine classifier to decode stimulus identity at each arousal state using population activity. At higher arousal states, decoding accuracies increased for L5 IT/ET cells but were uniform in L2/3 and were highest at intermediate states for CT cells (inverted-U pattern). We found an identical trend when analyzing the response reliability in the population across states. Consistent with other research, this suggests that greater stability in a population's encoding of an event (higher reliability) significantly impacts decoding accuracy. These insights illuminate the complex relationship between arousal and sensory coding, providing crucial groundwork for understanding neural dynamics in auditory perception.

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Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

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Title: Stimulus-specific suppression distinguishes layer 5 from layer 6b extratelencephalic neurons.

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Abstract: Extratelencephalic (ET) neurons of the auditory cortex (ACtx) receive both ascending and intra/inter-cortical inputs, forming an intricate feedback circuit to higher-order subcortical targets. Located in both layer (L) 5, and L6b of ACtx, they play important roles in learning-induced plasticity. L5 and L6b ET neurons are morphologically and physiologically distinct. Unlike L5 ET neurons that have pyramidal-shaped cell bodies with a single prominent apical dendrite, L6b ET neurons have radially oriented soma with profusely branched dendrites extending over a millimeter, allowing them to integrate information across longer distances. *In vitro* studies have found that a significant fraction of L5 ET neurons are burst spiking while L6b ET neurons are largely regular spiking. The *in vivo* ramifications and functional significance of such ET diversity remains poorly understood. To address this, our current study focused on characterizing single-cell and population-level sound processing by both L5 and L6b ET neurons. Using an intersectional viral strategy, we expressed GCaMP8s in both L5 and L6b ET neurons and used two-photon microscopy to record calcium activity in response to pure tones and sinusoidally amplitude modulated (sAM) noise. We then used hierarchical clustering to identify and characterize distinct neural response motifs in an unsupervised manner. Pure tones elicited a response in over 33% of the L5 ET neurons (24% enhanced, 9% suppressed), while only 23% of L6 ET neurons showed a response (10% enhanced, 13% suppressed). In contrast, sAM responses demonstrated greater diversity, with clusters representing distinct patterns of both enhanced and suppressed firing. Most L5 ET neurons exhibited excitatory responses (30% enhanced, 8% suppressed), while the dominant response motif in L6b ET neurons was suppression (19% enhanced, 25% suppressed). These results demonstrate a dichotomy in stimulus processing between L5 and L6b ET neurons, potentially leading to differential impacts on downstream targets that modulate auditory-guided behavior.

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Poster

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Title: Frontal and sensory cortical dynamics during auditory reversal learning

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Abstract: The mammalian auditory system is remarkably adaptable, allowing animals to flexibly respond to sensory information in dynamic environments. Cortical responses from behaving animals are highly variable, ranging from cells that are highly modulated to sensory stimuli (i.e. classically responsive) to those that appear to fire randomly (non-classically responsive). While classically responsive cells have been extensively studied for decades, the contribution of non-classically responsive cells to behavior has remained underexplored despite their prevalence. Flexible behaviors such as perceptual learning involves the engagement of various neural circuits including top-down regions such as frontal areas. To determine how frontal and sensory cortical areas interact to enable flexible behavior, we used silicon probes to record single-unit responses simultaneously from auditory cortex (AC, n=1,365 cells) and a downstream region in frontal cortex called secondary motor cortex (M2, n= 1,343 cells) while mice performed a go/no-go auditory reversal learning task (N=12 mice, d'=1.8±0.18). Mice first were trained to respond to a target tone for water reward, and to withhold from responding to a non-target tone (i.e. pre-reversal). Once animals reached expert criteria a rule switch was introduced, and the rewarded tone was reversed (i.e post-reversal). Both chemogenetic and optogenetic silencing experiments indicated that both regions are required for reversal learning (chemogenetics, d'=0.29±0.46, N=9 animals; optogenetics, d'= -0.08±0.10, N=7 animals). Single-unit responses in both regions were highly diverse, spanning the range from classically to non-classically responsive. However, there were notable differences between AC and M2. While sensory-responsive AC neurons mainly exhibited onset responses, the vast majority of sensory-responsive neurons in M2 were offset responses that had shorter latencies as animals learned post-reversal. While evoked responses to the target tone were suppressed in AC during post-reversal, they were enhanced in M2. Using a previously published spike-timing dependent Bayesian decoder we found that the number of task-encoding cells increased during both pre and post-reversal learning in both AC and M2. While task-encoding non-classically responsive cells were preferentially recruited in AC during periods of rapid learning, classically-responsive neurons in M2 were recruited when animals achieved expert performance levels suggesting a dissociable role for these neural response types. These results indicate that distinct neural dynamics in both regions drive flexible auditory behavior.

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Poster

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Title: Sleep evokes distinct ensemble activity in auditory thalamus

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Abstract: Auditory thalamus (medial geniculate body, MGB) serves an important role as an active computational unit processing sensory information during complex behaviors. To this extend, we recently demonstrated that learning-dependent changes in large-scale neuronal dynamics and plasticity of single cell responses in MGB encode sensory as well as task-related information (Hasegawa, et al., 2023, bioRxiv), highlighting MGB's function in encoding relevant information for adaptive behaviors.
In addition, previous studies have shown that neuronal activity in MGB undergoes plastic changes during different stages of sleep after auditory conditioning paradigms (Maho, Hennevin, 2002, Behav. Neurosci.; Hennevin, et al. 1998, Behav. Neurosci.), suggesting a functional role in memory formation that is not understood in detail yet. Here we investigated the neuronal dynamics of MGB during a frequency discrimination decision-making task followed by an open exploration and sleep session using longitudinal calcium imaging with miniature microscopes. During open field sessions, mice explored the arena unrestrained and showed a variety of complex behaviors including episodes of sleep. To dissect the behavioral repertoire within the freely moving session, we adapted a sleep stage classification algorithm using single channel EEG recordings (Tezuka, et al. 2021, Sci. Rep.) to identify episodes of non-rapid-eye-movement (NREM) and rapid-eye-movement (REM) sleep. Strikingly, selected ensembles of MGB neurons exhibit distinct activity patterns that are phase-locked to various cortical EEG stages, while multimodal embeddings (Schneider, et al. 2023, Nature) of population Ca^{2+} activity and behavioral labels revealed latents that resembles separate sleep phases.
This new approach will help to identify dynamic representations of neuronal ensemble activity and their representations during memory consolidation in sleep, which can be used for future causality testing via interventions with closed-loop all-optical manipulations and serve as a neuronal network biomarker to read-out out memory formation/loss and disease state in animal models of cognitive decline in early stages of neurodegenerative disease.

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Poster

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Title: Micro-circuitry and beneficial effects of auditory perceptual learning

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Abstract: Age-related impairments of auditory and cognitive functions have emerged as a great health concern in the worldwide aging population. Studies in humans and rodents have found that auditory perceptual learning can restore the ability to process information through intensive training in sound detection and discrimination. However, the underlying mechanism of auditory learning in improving cognitive functions and memory remains unknown. The parvalbumin-positive GABAergic interneurons (PVINs) have long been suggested to be major regulators of the excitatory and inhibitory (E/I) balance in the neural circuits, which play crucial roles in cortical plasticity, neuronal connectivity, and memory formation. We hypothesize that perceptual learning induces cortical plasticity to enhance cognitive functions via the regulation of E/I balance. A GO/NOGO training system was designed for mice to learn to discriminate different sound frequencies. Our data showed that most animals achieved great and consistent performance in differentiating two different frequency tones after discrimination training. Compared to the controls, the trained animals showed improved performance in a memory-related test, novel object recognition test. Based on the animals' performance in this preliminary experiment, we will further investigate the structural and functional changes of excitatory pyramidal neurons and inhibitory PVINs using in-vivo imaging in parallel with training. We may further explore the beneficial effects of auditory perceptual learning on cognitive functions and memory in aged animals or disease models.

Disclosures: L. Xiao: None. **C.S. Lai:** None.

Poster

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Title: Cortical surface plasticity promotes map remodeling and alleviates tinnitus in adult mice

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Abstract: Cortical surface plasticity promotes map remodeling and alleviates tinnitus in adult miceAuthors*H. ZHAO[1], S. PAK[1], M. LEE[1, 2], S. LEE[2, 3], S. YANG[2, 3, 4], S. YANG[1];
[1]Neurosci., City Univ. of Hong Kong, Hong Kong SAR; [2]Nano-bioengineering, Incheon Natl. Univ., Incheon, Korea, Republic of; [3]gBrain Inc., Incheon, Korea, Republic of; [4]Ctr. for Brain-Machine Interface, Incheon, Korea, Republic of**DisclosuresH. Zhao:** None. **S. Pak:** None. **M. Lee:** None. **S. Lee:** None. **E. Baeg:** None. **S. Yang:** None. **S. Yang:** None.**Abstract**Tinnitus induced by hearing loss is caused primarily by irreversible damage to the peripheral auditory system, which results in abnormal neural responses and frequency map disruption in the central auditory system. It remains unclear whether and how electrical stimulation of the auditory cortex can alleviate tinnitus. We hypothesized that stimulation of the cortical surface can alleviate hearing loss-induced tinnitus by enhancing neural responses and promoting auditory map reorganization. We first assessed and activated auditory maps using our newly designed graphene-based electrode array on Sham control mice and a noise-induced hearing loss (NIHL) mouse model. Then, we applied theta-burst stimulation (TBS) to the surface of the primary auditory cortex to examine the map plasticity. To test the effect of TBS on tinnitus-like behavior, an active-avoidance test for observing tinnitus behavior was performed on an NIHL-induced tinnitus animal model. Auditory representation was sparse in the cortical map of the NIHL mouse model compared to that of the control mice. TBS effectively increased the cortical activity and reshaped the auditory map by increasing the cortical gain and map size. Moreover, TBS successfully alleviated the behavioral symptoms of NIHL-induced tinnitus in adult mice. These findings suggest that cortical surface activation can be used to facilitate functional recovery from phantom percepts induced by sensory deprivation. They also provide a working principle for various treatment methods that involve electrical rehabilitation of the cortex.

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Poster

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Title: Impaired top-down behavioral regulation of the primary auditory cortex in an Angelman Syndrome mouse model

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Abstract: Atypical behavioral responses to sensory inputs are observed in 60-96% of individuals with autism spectrum disorder (ASD). A dominant theory, informed by human functional imaging, suggests that sensory symptoms in ASD result from local hyperconnectivity and long-range hypoconnectivity within the neocortical network. This reduced long-range connectivity is considered to impair top-down modulation of sensory cortices, leading to inflexible sensory processing. However, the neuronal circuit-level consequences of this long-range hypoconnectivity remain poorly understood. In this study, we address this gap in knowledge using a mouse model for Angelman Syndrome (AS), which exhibits high ASD comorbidity. We conducted in-vivo extracellular recordings from the primary auditory cortex (A1) in AS and control mice during a sound offset-lick detection task that requires sustained attention. During the learning phase, AS mice demonstrated impaired performance, characterized by slower learning curves and prolonged lick latencies than their control littermates. On the recording day, local field potential (LFP) analysis of spontaneous activity in A1 showed significantly higher low-frequency (delta and theta) power in AS mice. During behavioral engagement, wild-type mice exhibited suppression of low-frequency oscillation power, indicative of effective top-down regulation of A1 sensory processing. In contrast, AS mice showed diminished behavior-dependent modulation. Auditory brainstem response (ABR) and current source density (CSD) analyses indicated no significant differences in peripheral sensory inputs between AS and control groups, further supporting the impairment in top-down control. Our ongoing research aims to link these electrophysiological findings with poor behavioral performance in AS mice. Together, these results bridge our understanding of ASD between the anatomical and perceptual levels, potentially guiding the identification of therapeutic targets to normalize altered sensory processing.

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Poster

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Title: Auditory cortical mechanisms of self-generated sound-guided navigation

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Abstract: The ability to process sounds rapidly and efficiently during locomotion is critical for survival and adaptive behaviour. During locomotion, some incoming sounds originate from external sources (such as the sound of a passing car) while others are self-generated (such as the sound of our own footsteps). Many self-generated sounds are predictable based on prior experience, and previous studies have shown that their neural representations are suppressed via mechanisms of corollary discharge. However, in some situations, such as during navigation in novel and/or dark environments, self-generated sounds carry rich and potentially behaviourally relevant information, such as about the substrate being walked on and about the subject's location within their environment. Indeed, human studies show that self-generated sounds can influence behaviour in an ongoing manner. However, the neural mechanisms underlying the encoding and usage of self-generated sounds during navigation are poorly understood. To address this gap, we designed a novel experimental setup for freely behaving rats, consisting of a track with different substrates sandwiched under a uniform surface, such that running on different sections of the track generated different footstep sounds. Rats were trained to navigate this track to find a fixed reward location. To test if well-trained animals do indeed use their own footstep sounds to guide their navigation, or if they use distal visual cues instead, the track was spatially rotated, resulting in an inconsistency between the visual and auditory information. We found that when the lights were on, rats followed the distal visual cues to navigate, while in the dark they used their own footstep sounds to spatially orient and navigate. As rats are nocturnal, this form of self-generated sound-guided navigation may reflect a critical ability for their survival, raising the question of its underlying neural mechanism. A key candidate region that could encode behaviourally relevant footstep sounds and facilitate sound-guided navigation is the auditory cortex. Indeed, inactivation of the auditory cortex of rats being tested in the dark impaired their performance on this task. Finally, we recorded neural activity from the auditory cortex during learning which showed robust and selective responses to footstep sounds. Together, our findings suggest that self-generated footstep sounds encoded by the auditory cortex are used for navigation in the dark.

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Poster

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Title: Hippocampal responses to sound in naive mice

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Abstract: Studies of auditory processing in hippocampus (HC) have mostly focused on sounds with learned behavioral meaning (e.g., tones predicting reward). Recent insights into the relationship between hearing and cognitive health have generated interest in clarifying the nature and extent of auditory processing in HC, including passive responses to sound without behavioral training. For instance, hearing loss (HL) can cause spatial memory impairment and HC abnormalities (e.g., dendritic simplification) in rodents. HL is also a risk factor for Alzheimer's disease (AD), which appears first in entorhinal cortex and HC. Finally, sensory stimulation protocols in which tone pips and/or light flashes are delivered at 40 Hz have recently attracted attention as therapeutic interventions for restoring AD-related deficiencies in HC gamma oscillations. In naïve AD mice, these tone pips evoked responses in some HC units, reduced amyloid-β plaques, and rescued spatial memory impairment. Thus, HC is affected by hearing manipulations and may respond to sounds without behavior training. Little else is known about the auditory properties of HC. How many HC neurons respond to sound? Which sound features do they prefer? Do they interact with other auditory pathway neurons? Answering these questions could clarify the relationship between hearing and cognition and provide insights relevant to hearing-based cognitive therapeutics. In the present study, we recorded single-unit activity in awake mice using Neuropixels probes spanning HC, auditory cortex (ACtx), and medial geniculate body (MGB). We presented diverse sounds including pure tones, broadband noise bursts, and dynamic moving ripples (DMRs). Roughly 1/25 HC units responded robustly to tones (FDR < 0.001), which had longer response latencies and narrower bandwidths than ACtx and MGB units. Responses were much more common for noise bursts (~1/10 units). Although we observed responses to DMR onsets in ~1/20 HC units, none showed consistent preference for spectrotemporal features in continuous DMRs using spike-triggered averaging techniques. Ensemble analysis during spontaneous recordings revealed that over 1/3 of all HC units showed synchronous activity patterns with AC and/or MGB units. Of these, most tone-responsive HC units were synchronized with both structures, whereas most others were synchronized with ACtx only. Our results suggest responses to passively presented sounds are not uncommon in HC (>1/10 units), that sound onsets (especially broadband noise) most effectively drive these units,

and that they are more likely synchronized with auditory pathway structures including cortex and thalamus.

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Poster

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Topic: D.05. Auditory and Vestibular Systems

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Title: Can ferrets extract abstract rules in sound sequences?

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Abstract: The ability to extract rule-like structures from items or tokens in sound sequences facilitates the acquisition of language as has been well documented with an artificial language task in adults and infants. Some studies have claimed that such an ability exists in non-human species, although an alternative explanation might be that the acquisition of patterns relies on the memory of sensory features (***Corballis 2009***; ***Becker et al, 2016***). In a previous study (***Yin et al 2010***), we found that ferrets were able to categorize 2-tone sequences into two abstract groups of *rising* vs *falling* sequences based on their frequency-contours. In this study we assess the ability of ferrets to learn patterns and rules of sound sequences with spectrally more complex sound elements, such as musical notes, through extensive behavioral training. We selected 4 musical notes (***violin***) and placed them into two different classes based on their pitches: low pitch (***class A***) and high pitch (***class B***). 3-element sequences then were constructed out of these elements to form two types: **ABA** vs **ABB**. Two ferrets were trained to discriminate **ABA** sequences from **ABB** sequences in an appetitive Go/No-Go paradigm: licking the spout after an **ABA** sequence to receive a water reward (~0.2 cc) or refraining from licking after an **ABB** sequence to avoid a 3-5 s timeout. Both ferrets succeeded in reaching the behavioral criterion and were able to transfer the performance to new sequences which were comprised of novel notes from the same training instrument (***Violin***) with notes within the same classes (**A** and **B**). The animals could not perform the task if the notes were from an untrained instrument (e.g., ***piano***) or from different classes. For example, animals failed to perform the task when the notes were selected from the wrong classes (e.g. **A** and **B** are switched to form **BAB** and **BAA**). But the animals performed well when we replaced **any repeating note** by notes from the same class (e.g., ***ABA' vs ABB'***) where **A'** and **B'** are in classes **A** and **B,** respectively. Therefore, to solve the task, ferrets seem to

abstract the 'repetition rule' and apply it to adjacent (***_BB***) or to non-adjacent (***A_A***) classes regardless of the specific notes within them. These results demonstrate that ferrets can extract rule patterns among sequences comprised of spectrally more complex sound elements and apply it to new sequences in a grammar rule-like manner.

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Poster

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NIDCD T32 DC000038

Title: Inhibiting distracting sounds: Developing neurophysiological and behavioral assays to distinguish between benign and bothersome tinnitus

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Abstract: Individuals with chronic tinnitus either hear an indefatigable and irrepressible phantom sound every hour of their waking day or a phantom sound that benignly fades into the background when unattended. The difference between benign and burdensome tinnitus may be explained by a combination of sensory insults (e.g. excess cortical gain), limbic arousal, or executive ability to suppress the sound. Previous results from our lab show that the degree of central gain does not correlate with tinnitus burden, but measures of affective processing are associated with increased burden. To test the hypothesis that individuals with burdensome tinnitus exhibit broader deficits in inhibitory control over external auditory sounds, we developed a paradigm to probe the neural and behavioral effects of auditory distraction. Subjects were presented with a target stimulus organized along four nested timescales, including temporal fine structure (~500 Hz), envelope (~25-80 Hz), envelope changes (~5 Hz), and embedded context (~0.5 Hz). EEG was recorded to capture following responses as participants reported perceptual judgments about the embedded context. The target was paired with two sets of competitor stimuli—melodies and matched noise—which share low-level features but differ in their level of distraction. Results in participants with normal hearing (n=15) showed that synchronization to rapid features was insensitive to the difference in distraction, whereas synchronization to the slower envelope changes was reduced when the target was accompanied by the more distracting melodies. In participants with tinnitus (n=11) and matched low-frequency hearing, behavioral

analysis confirmed no gross difference in distraction performance. Our ongoing work will focus on how each distractor degrades neural synchronization to envelope changes in individuals with high tinnitus burden against individuals with low tinnitus burden. Our results build on work that shows that individuals with tinnitus perform as well as individuals with normal hearing on listening tasks in noisy environments and expand the work into the neural representation of sounds in distracting environments. We also demonstrate that our novel paradigm for measuring auditory distraction can be applied in clinical populations.

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Poster

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Title: Auditory motor integration dynamics in the tail of the striatum

Authors: S. JULIANI[1], ***C. GARCÍA**[2], D. J. MARGOLIS[3];
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Abstract: The process of choosing an appropriate action based on sensory information is critical for survival. However, the mechanism to interpret initially irrelevant auditory frequencies to make a decision is not well understood. The tail of the striatum in the basal ganglia plays a significant role in combining sensory information from the auditory cortex and thalamus to determine an action. The striatum contains two spiny projection neuron subpopulations (SPNs): direct and indirect pathways, which are involved in selecting actions and understanding sensory values. The interactions of these neural populations while learning in an auditory-motor integration task are not well defined. Here, a novel auditory-discrimination paradigm is created, in which mice learn to associate a high frequency tone with the action of pushing a joystick, and a low frequency tone with the action of pulling the joystick. Two-photon calcium imaging is utilized to record neural activity of SPNs during the learning process. To quantify movement in these stages, we develop an algorithm to quantify how mice interact with the joystick by using vector field analysis and spatial directional variability. The following metrics were computed: total area visited in the workspace during trials, the angle of the mean vector for each division of the workspace, and angular deviation, the equivalent of standard deviation in circular statistics. Through these metrics, we aim to characterize the differences in behavior from naivety to

expertise to investigate how learning in the tail of the striatum impacts motor output. Using controlled human data and experimental animal data, we confirmed our hypothesis that the area visited and the mean vector for each division in the workspace accurately demonstrate optimized movements as the number of sessions increases. However, contrary to our hypothesis that the angular deviations of the trajectories would decrease over time, we observed that they remained constant.

Disclosures: S. Juliani: None. **C. García:** None. **D.J. Margolis:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.24/E27

Topic: D.05. Auditory and Vestibular Systems

Support: NIDCD Grant R01DC013073-01

Title: Neural activity in the dorsal inferior colliculus of mice is modulated by behavioral task

Authors: *G. XIAO[1,2], D. LLANO[1,2], B. LI[1,2], K. NANDANI[1,2];
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Abstract: Sensory perception can vary depending on different higher-level contexts, such as behavioral contexts. It has been shown that task engagement can modulate the activity of neurons throughout the mammalian brain in many sensory modalities, including the auditory system. The midbrain integration hub of the auditory pathway, the inferior colliculus (IC), was found to have task-engagement-related modulation. The dorsal nucleus of IC received enormous top-down corticocollicular projections from the auditory cortex and these axons and their target neurons might be involved in the top-down processing of auditory response, including the behavioral modulation. In this study, we used 2-photon microscopy to study the neural activity in the dorsal IC while the mice either performing a sound discrimination task or listening to the sound passively. We found that the neural activity of neurons in IC was modulated by the task-engagement with more than 50% of them in an increasing manner while there was no spatial pattern observed. The population neural activity in dorsal IC could also indicate the task status of the mouse.

Disclosures: G. Xiao: None. **D. Llano:** None. **B. Li:** None. **K. Nandani:** None.

Poster

PSTR221: Auditory Processing: Perception, Cognition, and Action

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR221.25/E28

Topic: D.05. Auditory and Vestibular Systems

Support: ISRAEL SCIENCE FOUNDATION

Title: Chronic stress exerts a selective influence on sound processing and perception in an intensity and timing-specific manner

Authors: G. BISHARAT, ***J. RESNIK**;
Ben-Gurion Univ., Beer Sheva, Israel

Abstract: Chronic stress, a prevalent experience in modern society, is a major risk factor for many psychiatric and sensory disorders. Despite the prevalence of perceptual abnormalities in these disorders, little is known about how stress affects sensory processing and perception. In this study, we combined chronic stress, longitudinal measurement of cortical activity, and auditory-guided behaviors to test if sound processing and perception of neutral sounds in adults are modulated by chronic stress. We found that chronic stress induces changes in sound processing, reducing sound-evoked activity in an intensity-dependent manner. These changes in sound processing led to specific changes in perception, assessed through behavior, modulating loudness perception while leaving tone in noise detection unaffected. Additionally, our work reveals that the impact of stress on perception evolves gradually as the stressor persists over time, emphasizing the dynamic and evolving nature of this mechanism. Our findings challenge the notion that chronic stress primarily manipulates stimuli with existing emotional valences, shedding light on a novel mechanism through which chronic stress influences physiology and behavior.

Disclosures: G. Bisharat: None. **J. Resnik:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.01/E29

Topic: D.05. Auditory and Vestibular Systems

Support: NIH Grant R01-DC018287

Title: Cognitive biases cause an overestimation of vestibular direction-recognition perceptual thresholds

Authors: E. LOPEZ-CONTRERAS[1], T. K. CLARK[2], ***F. KARMALI**[3];
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Abstract: There has been tremendous interest in the use of perceptual thresholds to understand the physiology and pathophysiology of the vestibular system, and as a potential clinical tool. For example, these thresholds vary with pathology and age, and are highly correlated with postural performance. These thresholds are typically assayed by a sequence of trials, each consisting of a stimulus (i.e., chair motion) and a perceptual response (e.g., left or right judgment). As with other sensory systems, these vestibular perceptual judgments exhibit serial dependence. That is, the response for each trial is affected by the stimulus of the current trial and responses in preceding trials. Prior work has shown that this serial dependence can broadly affect the psychometric curve fit. More specifically, we recently showed that this serial dependence affects the direction-recognition threshold estimate. We found that it caused an overestimation of threshold in a small group of subjects experiencing yaw motion (Gonzalez, EL, King SA, and Karmali F. "Your Vestibular Thresholds May Be Lower Than You Think: Cognitive Biases in Vestibular Psychophysics." American Journal of Audiology 32.3S (2023): 730-738.) We now report new results about the hypothesis that cognitive biases cause thresholds to be overestimated. We determined the theoretical predictions for overestimation as a function of dependence on preceding responses, finding that overestimation occurs whether subjects respond in the same or opposite direction as the preceding response. We also analyzed additional experimental data from subjects experiencing roll tilt, pitch tilt, interaural (lateral) translation and yaw rotation from multiple open-source datasets. We also analyzed visual and auditory datasets. These included data collected using both adaptive and non-adaptive stimulus selection. We found that experimental results did not differ from theoretical predictions for all motion directions (Chi-squared test, $p>0.05$). Thresholds were overestimated by up to 40%. Since the results indicate that the magnitude of cognitive bias varies across subjects, this enhanced model can reduce measurement variability and potentially improve the efficiency of data collection.

Disclosures: E. Lopez-Contreras: None. **T.K. Clark:** None. **F. Karmali:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.02/E30

Topic: D.05. Auditory and Vestibular Systems

Title: Causal roles of visual posterior sylvian(VPS) area in selfmotion perception

Authors: *Y. XU[1], Y. GU[2];
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Abstract: The functional role of visual posterior sylvian (VPS) area in self-motion perception Yue Xu, Yong GuInstitute of Neuroscience, Chinese Academy of Sciences, Shanghai, China
Precise self-motion perception relies on integration of multiple sensory inputs including visual

(optic flow) and vestibular cues. Previous studies have revealed that many areas contain a large proportion of “congruent neuron”. These neurons prefer similar heading preference defined by either vestibular or visual input, and thus allow facilitating cue integration when both cues are available. In contrast, the visual posterior sylvian (VPS), an area close to the posterior insular cortex, predominantly contains “conflict neuron” that exhibits opposite heading visual and vestibular heading preference, making it a puzzle about whether VPS really contributes to self-motion perception. To address this question, here we used microstimulation technique to artificially activate VPS neurons and examined its effect on the monkey’s heading judgment under vestibular-only and visual-only conditions. We first found that VPS neurons are well clustered in terms of vestibular or visual signals, allowing subsequent application of microstimulation. Microstimulation frequently biased the animals’ heading judgment under the vestibular condition by shifting the psychometric functions and the point of subjective equality (PSE). Interestingly, the PSE shift did not depend on the tuning preference of the activated neurons, but rather depend on which hemisphere the microstimuation was applied. In particular, microstimulation caused an ipsilateral effect by biasing the PSE to the same hemisphere direction. In contrast, microstimulation did not evoke very significant PSE shift in the visual condition.Our results indicate that vestibular signals in VPS causally contribute to heading perception. But unlike the tuning-dependent microstimulation effect that is typically seen in the visual system, vestibular signals contribute to perception with a hemisphere effect. This effect is ipsilateral, which is exactly same to the noninvasive galvanic stimulation (GVS) effect applied in human psychophysical experiments. Indeed, we found that GVS also biased the monkeys’ heading perception with an ipsilateral effect, and induced significant stronger activity in ipsilateral than contralateral hemisphere, supporting our hypothesis.

Disclosures: Y. Xu: None. **Y. Gu:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.03/E31

Topic: D.05. Auditory and Vestibular Systems

Support: T32-DC000023
1UF1-NS111695

Title: Neural population dynamics in vestibular nuclei during locomotion in primates

Authors: *R. WEI[1], O. R. STANLEY[2], K. E. CULLEN[3];
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Abstract: The vestibular system detects the motion of the head in space and transmits this self-motion information to the brain in order to generate stabilizing reflexes. In particular,

vestibulospinal reflex pathways play a critical role in maintaining head and body posture by stabilizing the head and body relative to space during locomotion. These pathways receive input from vestibular-only (VO) neurons, a population within the vestibular nuclei which are insensitive to eye movements. To investigate the signals encoded by vestibular pathways in nonhuman primates during locomotion, we recorded from the vestibular nuclei of both normal and bilateral vestibular loss monkeys using 128-channel electrodes. We tracked head and trunk positions with a 6D motion sensor and marker-based systems, and captured limb motion using high-speed, markerless cameras. Our study began by assessing responses to passive movements to characterize vestibular responses. We then examined head stabilization and neuron sensitivity to multisensory inputs during 1) treadmill locomotion with fixed and free head positions at various speeds, and 2) during free overground movement. We also analyzed the population dynamics of VO neurons across these locomotion settings. In normal animals, VO neurons differentially encoded passive vestibular stimulation versus head movements during locomotion. A significant fraction of VO neurons modulated with gait cycle, and our findings reveal that at the population level, VO neurons exhibit a low-tangling geometry, indicative of robustness to noise. Furthermore, these neurons exhibit context-specific geometry that coherently changes across different treadmill speeds and demonstrating distinct differences between treadmill and overground walking. In contrast, in BVL animals, VO neurons did not exhibit phasic modulation with gait, and the resulting population activity structure demonstrated significantly more tangling. Finally, by using innovative dynamical systems analysis tools to distinguish the contributions of multisensory and motor signals, we find that VO neurons increasingly incorporate non-vestibular information in the absence of vestibular input. This research underscores the critical role of neuron-specific modulation at the level of the vestibular nuclei in coordinating voluntary movement and maintaining posture during locomotion. Furthermore, it sheds light on the brain's strategies for balance control and gait adjustment, enhancing our understanding of how posture is maintained and adjusted during daily activities.

Disclosures: R. Wei: None. **O.R. Stanley:** None. **K.E. Cullen:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.04/E32

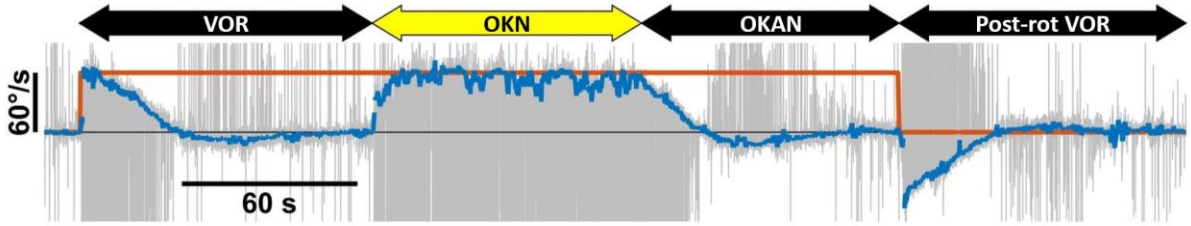
Topic: D.05. Auditory and Vestibular Systems

Title: The common marmoset as a novel model for visuo-vestibular self-motion sensing

Authors: D. SURENDRAN, F. LANZARINI, ***J. LAURENS**;
Ernst Strüngmann Inst., Frankfurt am Main, Germany

Abstract: Spatial orientation and Navigation require precise self-motion sensing through integration of visual, vestibular and motor efference copies. To date, empirical and theoretical evidences into self-motion processing in non-human primates (NHP) stems from macaques. In

the recent years, common marmosets (Callithrix jacchus) are emerging as a new NHP model in neuroscience. Their small size, active and agile nature makes them ideal to investigate navigation and self-motion in laboratory settings. The first step towards this goal is to characterize their vestibular function.
We have built a marmoset rotator in which the animal and/or the visual surround can be rotated independently, and performed a series of experiments to evaluate the vestibular functions and the visual-vestibular interaction in common marmosets. Our results indicate that Marmosets have visuo-vestibular responses similar to other primates, including a velocity storage time constant of ~14s, and robust OKN and OKAN. Furthermore, we successfully adapted the mathematical models of the vestibular system in humans and macaques to marmosets.
These findings will be the basis of further investigations of the vestibular system in marmosets, and of model-based studies of how the vestibular sense contributes to spatial orientation.



Eye velocity (grey) and slow phase velocity (blue) during a series of visuo-vestibular paradigms at 60°/s.

Disclosures: D. Surendran: None. **F. Lanzarini:** None. **J. Laurens:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.05/E33

Topic: D.05. Auditory and Vestibular Systems

Title: Spatio-temporal dynamics of otolithic information processing in healthy participants

Authors: *P. KOBLISKA, C. LOPEZ, L. SEROPIAN;
Neurosci., Ctr. for Res. in Psychology and Neurosci., Marseille, France

Abstract: The vestibular system processes sensory signals that inform our body posture andorientation, maintain balance, and orient us in our environment. Despite its importance, themechanisms by which the brain processes signals from otolithic sensors remain poorlyunderstood. Otolithic sensors in the vestibular apparatus respond to short acoustic burstsabove vestibular threshold: at 105 dB and 500 Hz and elicit a vestibulo-colic reflex response(Todd et al., 2014). We refer to this acoustic burst as the "activating sound.". This reflex istraditionnaly measured on the neck muscles to assess otolithic function. This study aims toidentify and describe cerebral markers of otolithic information processing in healthyparticipants (n=18) in response to acoustic stimuli. Using EEG and EMG recordings,

weexamined the cerebral and vestibulo-colic responses elicited by otolithic sensors. Werecorded electrophysiological responses from 64 active electrodes in two bodyorientations—sitting upright and lying supine—to investigate the effects of gravitationalorientation on otolithic processing. To minimize auditory influence and isolate vestibularresponses, a "masked" acoustic stimulus was used (the activating sound enveloped by whitenoise). Intensity and frequency controls for acoustic stimuli, set below the vestibularthreshold, ensured the specificity of vestibular responses. Our results revealed that theactivating sound elicited a vestibulo-colic response, indicating otolithic sensor activation. Theactivating sound significantly modulated the amplitude of early latency (Na/Pa: 20-30 ms)and middle latency (N*/P*: 40-50 ms) components of vestibular evoked potentials (vEPs)recorded at the FCz electrode. Additionally, we observed significant variations in theamplitude of the Na/Pa and N*/P* components between seated and supine positions,confirming our hypothesis that these two complex components are cortical markersreflecting otolithic processing. By identifying specific cerebral markers modulated by bodyorientation, this study provides significant insights into how vestibular inputs are integrated,advancing our understanding of sensory integration within the vestibular system.

Disclosures: P. Kobliska: None. **C. Lopez:** None. **L. Seropian:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.07/E34

Topic: D.05. Auditory and Vestibular Systems

Support: NIH AI129198

Title: Balance function analysis in Stat1 knockout mice

Authors: *M. KIM[1], B. TORO FIGUEIRA[1], M. H. SAITO[2], R. COOK[2], T. MAKISHIMA[3]; [1]The Univ. of Texas Med. Br., Galveston, TX; [2]Univ. of Texas Med. Br., Galveston, TX, ; [3]Otolaryngology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Background and objective: Signal transducer and activator of transcription 1 (Stat1) knockout (KO) mice with mild immunodeficiency have been used frequently in viral infectious disease models. Our goal was to determine the baseline balance function in Stat1 KO mice to assess whether they are suitable for use in future vestibular function studies. **Methods:** Male and female wild type (WT) 129Sv/Ev mice (n=5 each) and male and female Stat1 KO mice (n=5 each) at 3-11 months of age were compared. The mice underwent the rotarod test starting at 4rpm accelerating 1 rpm every 8 seconds to 40 rpm. The motor coordination and balance were assessed with latency, distance traveled, and rotations per minute (RPM). The set of experiments lasted three days, with each mouse undergoing three trials per day, totaling nine trials per mouse at two different timepoints. **Results:** At three months of age, WT males, WT females and Stat1 KO females had significant improvements over the trials. However, there were no differences in

balance performance between the genotypes or sexes. In general, at this younger age, Stat1 KO females performed the best and WT females the worst, but there was no statistical difference between them. At 11 months of age, significant improvements over the trials were observed only for the WT females and Stat1 KO females across all parameters. Despite differences in genotype, female mice displayed a significant improvement over the course of the study in all three indicators, suggesting females have better motor learning at older age. However, at this older age, Stat1 KO males were the best performers, while Stat1 KO females the worst. Only Stat1 KO males improved their performance between 3 and 11 months, while all other groups maintained their performance. There was no difference in rotarod performance between WT males and females, and their performances were intermediate between those of Stat1 KO males and Stat1 KO females. In conclusion, our analysis suggests that both genotype and sex play a role in motor coordination and balance. Improved performance after repetitive trials suggests motor learning occurred in all groups. At young age there is no difference between rotarod performance in Stat1 KO males and Stat1 KO females, whereas at old age the Stat1 KO males perform significantly better than Stat1 KO females. We conclude that the Stat1 KO mice are suitable for vestibular function studies, and that both sexes should be included and compared as individual groups.

Disclosures: M. Kim: None. **B. Toro Figueira:** None. **T. Makishima:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.08/E35

Topic: D.05. Auditory and Vestibular Systems

Support: US Department of Veteran Affairs 1I01 RX001986-01
DOD Grant W81XWH-17-1-0172

Title: Biochemical diversity amongst calyx only afferents in the vestibular nuclear complex

Authors: *D. NAQVI[1], R. D. BRAUN[1], S. KING[1], S. K. GUPTA[1], A. HOLT[1,2];
[1]Wayne State Univ. Sch. of Med., Detroit, MI; [2]John D Dingell VAMC, Detroit, MI

Abstract: The peripheral vestibular end-organs are innervated by regular and irregular afferent fibers that slowly and rapidly adapt to stimuli, respectively. The range of rapid vestibular stimulation to which irregular fibers can adapt is relatively broad, suggesting that this functional heterogeneity among irregular afferents may result from biochemical heterogeneity, but this interpretation has not been substantiated. To begin to address this gap, calyx-only afferents were assessed in the vestibular nuclear complex (VNC). Calcium buffering proteins (CBPs) have been used to distinguish amongst vestibular ganglion neurons. The only vestibular ganglion neurons that produce the CBP calretinin are the calyx-only cells. Interestingly, the only source of calretinin-positive terminals in the VNC appears to be from calyx-only neurons. Calyx-only neurons are excitatory. Since vGluT1, vGluT2, and vGluT3 have been used to define subsets of

excitatory neurons, colocalization of calretinin with vGluT in the VNC may be useful in discriminating subsets of calyx-only afferents. Following transcardial perfusion (4% paraformaldehyde), Sprague-Dawley rat brains were collected, post-fixed, cryoprotected, and serially sectioned using a freezing sliding microtome. Immunohistochemistry was performed for calretinin and either vGluT1, vGluT2, or vGluT3 in free-floating sections of rostral and caudal VNC subdivisions. Punctae that were immunolabeled for vGluT1-3, but not calretinin were found throughout the VNC. As previously reported, calretinin labeling was punctate and was also observed throughout the VNC. Similarly, the distribution of vGluT1-3 was localized to terminals and had a distribution that correlated well with previous reports. Among rostral and caudal VNC subdivisions, less than 15% colocalization of calretinin with vGluT1-2 was observed. However the percent colocalization increased to 42 - 50% in mid and caudal VNC regions for vGluT3. Our data indicate that subpopulations of irregular afferent fibers exist and project to the VNC. The predominant glutamate transporter for these afferents appears to be vGluT3. These subpopulations may have different functional properties, such as firing rate, which may be attributed to the subtype of vGluT being utilized. The rate of adaptation to stimuli may be end-organ specific (e.g., canal vs otolith). Given that vGluT isoforms differentially signify synaptic plasticity and fidelity, future studies should use the biochemical signature of vGluTs to evaluate the origin and contributions of these irregular afferents to vestibular function centrally.

Disclosures: D. Naqvi: None. **R.D. Braun:** None. **S. King:** None. **S.K. Gupta:** None. **A. Holt:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.09/E36

Topic: D.05. Auditory and Vestibular Systems

Support: NIH NIDCD R01-DC002390

Title: Restoring dynamic posture with biomimetic prosthetic vestibular stimulation

Authors: *B. RAMADAN[1], O. M. LEAVITT BROWN[2], K. E. CULLEN[3];
[1]Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD; [2]Biomed. Engin., Johns Hopkins Med. Institutions, Baltimore, MD; [3]Dept. of Biomed. Engin., The Johns Hopkins Univ., Baltimore, MD

Abstract: In daily life, we must compensate for sudden and unexpected movements as well as sustained support surface motion. To maintain balance during sustained motion, individuals with bilateral vestibular loss (BVL) must rely on other sensory modalities to drive appropriate postural commands. However, even with alternative sources of sensory feedback, vestibular loss patients still suffer from postural imbalance and falls. Work in the past several decades offers an

innovative solution: restoration of vestibular signaling with vestibular prostheses.
A major hurdle of vestibular prosthesis implementation is in its suboptimal efficacy, leading to only partial rescue of behavior. One potential avenue beyond this barrier is in the replacement of linear stimulation mappings with more naturalistic, biomimetic mappings which may respond to transient and sustained platform dynamics. Using a customized postural platform, we compare the compensatory stabilizing behaviors of bilateral vestibular loss monkeys in response to various platform movements in the baseline condition and with prosthetic stimulation.
Rhesus macaque with BVL were unilaterally implanted with a multichannel vestibular prosthesis in the semicircular canals. Monkeys were trained to position themselves in a neutral starting posture on force plates secured to a hexapod motion platform while wearing a head mounted IMU. First, we collected data in the unstimulated, baseline BVL condition, in which the platform delivered transient motion, sinusoidal motion (5 frequencies, 0.25-5 Hz), broadband motion (0-10 Hz), or pseudorandom ternary sequence (0-2.3 Hz) in a particular axis. There were substantial deficits in the compensation of BVL monkeys compared to normal.
Second, we repeated the experiments described above, examining postural responses in BVL monkeys with canal stimulation of various patterns. In addition to the static mapping currently used in clinical trials, we tested pulse rate transfer functions which emulate the dynamics of 1) regular afferents or 2) irregular afferents. While all three stimulation conditions partially improved balance, we found that utilizing a mapping mimicking irregular afferents offered the best restoration of postural responses particularly during pseudorandom and broadband platform motion.
This study offers a direct evaluation of the contribution of biomimetic stimulation patterns in the context of postural rescue. These biomimetic stimulation dynamics offer an important step to restoring vestibular function for patients with vestibular loss.

Disclosures: B. Ramadan: None. **O.M. Leavitt Brown:** None. **K.E. Cullen:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.10/E37

Topic: D.05. Auditory and Vestibular Systems

Support: Wellcome Trust Fellowship 222747/Z/21/Z

Title: The role of expectation in the cortical processing of vestibular input in humans

Authors: *T. ELLMERS, S. SRIVASTAVA, A. BRONSTEIN;
Imperial Col. London, London, United Kingdom

Abstract: It is important that humans are able to discriminate between unpredictable vestibular stimuli and that which is self-triggered and thus expected. Such discrimination is crucial for ensuring that appropriate vestibular reflexes are triggered to maintain postural stability (e.g.,

triggering a reflexive action when head movement occurs during slipping/tripping but not when looking up at the sky). Disruptions in such ability to discriminate have been hypothesised to underpin higher-level disorders of 'functional dizziness', whereby dizziness occurs without any apparent peripheral vestibular dysfunction. Despite this, little is known about how expectation affects higher-level cortical processing of vestibular input in humans, and if such discrimination (and thus disruption to) occurs at the level of the cortex. Here, we recorded EEG from 22 healthy young adults undergoing discrete whole body yaw rotations. During one condition ('Unexpected'), rotations were unexpected with respect to timing, direction and magnitude. During another condition ('Expected'), rotations were predictable in terms of direction and magnitude, and were self-triggered by the participants themselves. During 'Expected' rotations, there was a significant increase in pre-rotation oscillatory beta power (a marker of reduced sensory sensitivity). This increase was restricted to the parietal cortex: an area of the brain known to process vestibular input. Expectation also abolished the initial post-rotation theta activation (a likely sensory 'error signal'). We also observed significantly increased inter-site phase clustering between the pre-frontal and parietal cortex during Expected rotations. Overall, these findings reveal that humans can cortically down-weight the processing of expected (and self-triggered) vestibular input. Ongoing work is exploring how these results directly influence vestibular perception, and how these mechanisms may be disrupted in disorders of functional dizziness.

Disclosures: T. Ellmers: None. **S. Srivastava:** None. **A. Bronstein:** None.

Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.11/E38

Topic: D.05. Auditory and Vestibular Systems

Title: Concurrent headshake and postural training using virtual reality improves vestibulo-ocular reflex

Authors: *K. O. APPIAH-KUBI[1], A. MADRID[2], M. TOWNSEND[1], E. HARRIGAN[1], P. GUTIERREZ[1], C. SMITH[1], T. GOUGH[1], A. STONER[1];
[1]Physical therapy, [2]Biomolecular Sci., Clarkson Univ., Potsdam, NY

Abstract: Background: Rhythmic head movements is effective in improving vestibulo-ocular reflex (VOR), an essential component of postural balance. VOR enables an individual to stabilize an image on the retina during head movements to prevent blurriness. Gaze stabilization exercises (GSEs) are employed to improve VOR. The novel Concurrent headshake and weight shift training (Concurrent HS-WST) protocol incorporates limits of stability exercises with headshakes to improve various aspects of balance. **Purpose:** To assess changes in VOR gain after virtual reality Concurrent HS-WST. **Methods:** Twenty-four healthy young participants (age = 22.2±3.2years; height = 1.7±0.1m) were randomized into two groups in this crossover design

study. Group 1 received the Concurrent HS-WST intervention for six days while group 2 did not receive any training. Each group was given a four-day washout period, and the order of interventions was reversed. The training involved participants donning an Oculus headset in a standing position on the floor or Airex foam. They followed the virtual environment (UpRight VR, LLC, Temple University) which instructed them to shift their center of mass (COM) as quickly as possible to accurately reach a visual target that surrounded their COM (every four seconds) in eight possible directions. While participants performed the weight shift, in some of the exercises, they concurrently rotated the head in a horizontal rhythmic pattern ($\pm$30°) synchronously to a metronome (80-120 beats/min). Each day's session comprised 18 exercises, each lasting for one minute. Pre- and post-lateral and -vertical VOR gain assessments were performed during the training and no-training periods. Significance was set at $p < 0.050$. **Results:** There was a statistically significant difference in the lateral VOR gain after training in both groups ($p = 0.040$). Group 1 had no significant change (VOR gain = 0.004) but a statistically significant downweighting VOR gain was found in group 2 (VOR gain = 0.053). However, there was no statistically significant group X training interaction. Also, there were no statistical differences in the training or group X training interaction for both vertical VOR gains (Left anterior right posterior [treatment, $p = 0.234$, group X training, $p = 0.244$]; Right anterior left posterior [treatment, $p = 0.179$, group X training, $p = 0.953$]). **Conclusion:** The Concurrent HS-WST significantly reduces lateral VOR gain during short training sessions, indicating a dominant effect of vestibular recalibration through adaptation. This finding also suggests that clinicians should incorporate limits of stability exercises during GSEs to enhance therapeutic outcomes.

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Poster

PSTR222: Vestibular Processing and Perception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR222.12/E39

Topic: D.05. Auditory and Vestibular Systems

Support: NIH R01 DC2390

Title: Beneficial effects of alternative stimulation pulse shapes on vestibular prosthesis-evoked reflexes and neural activities in the vestibular nuclei

Authors: *K. WIBOONSAKSAKUL[1], C. C. DELLA SANTINA[2], K. E. CULLEN[1];
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Abstract: Bilateral vestibular loss can lead to debilitating visual and postural instability in addition to constant dizziness. One approach to improve the quality of life in this patient population is a vestibular prosthesis that senses head rotation and transforms it into vestibular

afferent stimulation, substituting for the damaged periphery. These prostheses typically utilized the classic charge-balanced, biphasic pulses to deliver electrical stimulation to vestibular afferents. Recent neurophysiological studies, however, have shown that these standard biphasic pulses do not always reliably evoke firings in the afferents, which would impede optimal restoration of vestibular inputs to the brain. In cochlear implants, the use of alternative pulse waveforms has shown improvement in performance. Specifically, delayed pseudomonophasic (DPM) asymmetric stimulus pulses—characterized by a brief, high-amplitude cathodic phase followed by a long, low-amplitude anodic phase—result in desirable lower auditory thresholds. Here, we directly tested whether the use of DPM pulses can improve the performance of vestibular prostheses. We recorded eye and head movements of monkeys during prosthetic stimulation using standard biphasic and DPM waveforms. Simultaneously, we also record responses of single-unit vestibular nuclei neurons using high-density silicon probe. In comparison to standard biphasic pulses, we found that DPM pulses evoke markedly stronger reflexive eye and head movements, which are mediated by the vestibular pathways. Correspondingly, our single unit recordings revealed that DPM pulses evoked significantly ($p<0.001$) higher firing rates in vestibular nuclei neurons compared to standard biphasic pulses. Further analyses focusing on the time course of the evoked neural responses revealed that, compared to the standard pulses, DPM pulses evoke action potentials at shorter latency and with larger timing variance (i.e., less synchrony). Taken together, our findings indicate that DPM pulses can increase stimulation efficacy and potentially reduce synchrony within the afferent population. These features are highly desirable from a prosthesis development point of view and implementation of DPM pulses can likely improve prosthesis performance in the clinics.

Disclosures: K. Wiboonsaksakul: None. **C.C. Della Santina:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Labyrinth Devices, LLC. **K.E. Cullen:** None.

Poster

PSTR222: Vestibular Processing and Perception

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Program #/Poster #: PSTR222.13/E40

Topic: D.05. Auditory and Vestibular Systems

Support: BRIDGE GRANT 40B2-0_20356

Title: Using eye movements as biomarkers to assess the functional impact of bilateral vestibulopathy in<close-to-reality>, standardized laboratory environments.

Authors: G. GROUVEL[1], S. YADNIK[1], J.-F. CUGNOT[2], A. BOUTABLA[1], S. CAVUSCENS[1], M. RANIERI[1], R. VAN DE BERG[3], S. ARMAND[4], N. GUINAND[1], A. PEREZ FORNOS[1], ***J. CORRE**[1];
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Abstract: Aims Eye movement characteristics have been used as biomarkers of diseases like Parkinson's and diseases or autism spectrum disorder but have not been examined as biomarkers of bilateral vestibulopathy (BV) in daily-life settings. We aimed at: 1) the characterization of eye movements patterns in BV patients in a close-to-reality laboratory setting; 2) the assessment of their validity as biomarkers, and 3) the assessment of correlations with perception of vestibular-induced handicap, assessed using the Dizziness Handicap Inventory (DHI) questionnaire. **Population** Twenty subjects diagnosed with BV, according to the Barany Society diagnostic criteria, were compared to a group of age and gender-matched Healthy Control (HC) subjects. **Methods** Participants were equipped with a wearable eye tracking device, the Tobii Pro Glasses 3, giving subjects the freedom to move naturally. Subjects had to complete 13 tasks representative of daily life and normalized by difficulty (easy, intermediate, difficult) in a semi-standardized environment within the premises of the Geneva's hospital. Number of fixations, saccades, total gaze distance, and time to complete each task were recorded and analyzed. **Results** We observed a higher number of fixations and saccades in BV compared to HC. Total gaze distance was significantly shorter in BV compared to HC. Moreover, BV subjects took a significantly longer time than HC to complete 13 of the 15 tasks. Finally, we did not find any correlation between any of the outcome measures and the DHI scores. **Interpretation** In BV patients most of the routine daily living tasks are performed slowly and carefully to ensure a safe execution. Moreover, as patients' compensation strategies (mainly the use of external support) were removed, most of the tasks became challenging to execute, reflecting the loss of autonomy of these patients. Difference of eye movement patterns between HC and BV highlights the impossibility for BV patients to pay attention to environmental landmarks, which will thus likely lead to impaired spatial navigational strategies. **Conclusion** The pattern of eye movements in BV patients is representative of their difficulty to navigate safely and efficiently in a close-to-reality environment. Eye movements appear to be good biomarkers of BV. Eyes movements are another objective metrics that do not correlate with patient's perceived symptoms and handicap, highlighting the necessity to combine both objective and subjective measures.

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Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.01/F1

Topic: D.06. Vision

Support: Herman Lab Startup
Hillman Foundation

Title: The Emergence of Visual Attention and Working Memory in a Computational Agent

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Abstract: Attention is a fundamental component of vision that selectively guides behavior based on portions of visual input. However, the computational mechanisms that enable attention's flexible linkage of stimuli to action and thought remain incompletely understood. Recent artificial intelligence (AI) advancements have been propelled by a computational mechanism termed "self-attention" (SA), in the "transformer" architecture. Another significant approach is "deep reinforcement learning" (deep-RL): a deep neural network processes visual input and passes the result to an RL agent that chooses actions and observes rewards; the reward is then used to update the synaptic weights in the neural network. Both transformer-based models with SA and deep-RL models can flexibly use visual input depending on task demands, much like biological attention. However, it is uncertain whether these artificial visual selection mechanisms can provide insights into biological attention systems. In this study, we evaluate the performance of a vision transformer linked to an RL agent on several tasks commonly used to assess biological attention in non-human primates (NHPs). In a cued change-detection task, the model exhibits both (1) sigmoidal dependence of correct performance on change magnitude comparable to traditional NHP psychometric curves, and (2) higher probability of correctly detecting cued changes compared to uncued changes. Recurrent connections in the model, allowing visual information presented at different timesteps to influence value estimates and action selection at the current timestep, appear to be vital for the model to produce NHP-like attention task performance. Accordingly, we found that the same model architecture was capable of performing a visual working memory task much as NHPs do: exhibiting weaker performance for increasing memory load or for the duration of the delay period. Our findings demonstrate that reward-driven learning can support the acquisition of visual cognitive processes including attention and working memory. Although further interrogation of these models is required, including comparing NHP neuronal responses to model “neurons”, we conclude that the computational visual selection mechanisms we have tested are promising candidates for explaining biological attention.

Disclosures: J.M. Morgan: None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

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Topic: D.06. Vision

Support: Revson Foundation 22-21
NINDS RM1NS132981
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Title: Neuronal offsembles: stimulus encoding by specific inactivation of neurons

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Abstract: Neuronal ensembles are groups of coactive neurons associated with motor, sensory, and behavioral functions. However, how ensembles encode information remains unclear. To explore this, we investigated the responses of layer 2/3 visual cortical neurons in awake mice using two-photon volumetric calcium imaging during visual stimulation. We identified neuronal ensembles employing an unsupervised model-free algorithm. In response to visual stimuli, ensembles exhibited small trial-to-trial variability and high orientation selectivity. During ensemble occurrence, besides neurons that were significantly activated by the stimulus, we also found neurons whose activity was significantly decreased. To distinguish between these two groups of neurons, we introduce the terms “onsemble” for the significantly active neurons and “offsemble” for the inactive ones. Ensembles predicted visual stimulus orientation selectivity and tuning more accurately than summing the activity of individual onsemble or offsemble neurons. Interestingly, calcium decay kinetics in offsemble neurons became faster during stimulus presentation, as if offsemble neurons were selectively inhibited. We conclude that the combined activation and inactivation of onsemble and offsemble neurons enhances visual encoding and hypothesize that the inactivation is due to inhibitory interneurons. Ensembles could represent functional units of information in cortical circuits, combining selectively neuronal activation and inactivation as an emergent and distributed neural code.

Disclosures: J. Perez-Ortega: None. **A. Akrouh:** None. **R. Yuste:** None.

Poster

PSTR223: Visual Adaptation and Processing

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Program #/Poster #: PSTR223.03/F3

Topic: D.06. Vision

Support: NIH R01 EY032999
NSF Career #2146369

Title: Population activity in sensory cortex informs confidence in a perceptual decision

Authors: *Z. M. BOUNDY-SINGER, C. M. ZIEMBA, R. L. T. GORIS;
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Abstract: When we feel confident in a perceptual decision, that decision is more likely to be correct. How does the brain assess the quality of perceptual decisions? Here, we test two hypotheses. First, that perceptual confidence is related to the structure of population activity in the sensory cortex. And second, that this relation differs from the one between sensory activity and perceptual decisions. To this end, we studied neural population activity in the primary visual cortex of macaque monkeys performing a task that reveals the animal's subjective sense of perceptual confidence. Specifically, the animal judged the orientation of ambiguous stimuli ("clockwise" vs "counter-clockwise") and simultaneously reported their confidence in this decision ("high" vs "low"). Choices were rewarded in such a way that the most profitable strategy required the animal to take into account the quality of perceptual decisions. Analysis of the choice behavior revealed that high confidence choices were more accurate than low confidence choices, both at high and low stimulus contrast. This pattern of choice behavior suggests that the animals were meaningfully introspecting about the quality of perceptual decisions. We developed explicit hypotheses about the nature of the relationship between sensory activity and perceptual confidence. Confidence should be informed by the factors which make perceptual decisions more or less reliable. These factors include the strength of evidence for a particular choice derived from a perceptual impression, and the uncertainty of this perceptual impression. Next, to gain insight into the neural computations that implement this introspection process, we recorded population activity in the primary visual cortex and used nonlinear decoders to expose the relationship between this activity and the choice behavior. We found that both perceptual decisions and confidence can be predicted from V1 population activity. Our analysis validated both hypotheses and suggests that perceptual decisions arise from a computation that evaluates the most likely interpretation of a stimulus, while confidence instead reflects a computation that evaluates the quality of the sensory evidence that informed the decision. Our work establishes a direct link between neural population activity in the sensory cortex and confidence in perceptually guided decisions.

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Poster

PSTR223: Visual Adaptation and Processing

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Topic: D.06. Vision

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Vanderbilt startup funds
International Human Frontier Science Program Organization (LT0001-2023/L)
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Title: Current-source density in higher visual areas reflects distinct signaling motifs for oddball processing

Authors: *E. SENNESH[1], H. NEJAT[2], J. A. WESTERBERG[4], S. DURAND[5], A. BAWANY[8], H. CABASCO[6], H. LOEFFLER[8], H. BELSKI[8], B. HARDCASTLE[8], S. R. OLSEN[7], J. LECOQ[6], A. BASTOS[3];
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Abstract: A wide variety of behavioral evidence suggests that the neocortex implements a probabilistic internal model of the sensorimotor environment. The cortex also appears, at a gross level, to consist of canonical microcircuits, suggesting that each six-layer column of the repeating circuit implements a canonical computation. Theorists have suggested that this canonical computation may take the form of Bayesian prediction, either in the form of predictive coding or predictive routing. However, previous experiments have sometimes failed to deconfound prediction error as such from other possible causes for neural response dynamics, such as stimulus specific adaptation. While the experimental literature contains abundant evidence for a temporally “local” form of predictive coding, in which the “prediction error” arises from a change in stimulus, this “local oddball” paradigm conflates the possible causes of such a neural signal. These can include stimulus-specific adaptation (SSA) of neurons performing a feedforward computation, detection of stimulus deviance (DD) from a repeated sequence, or a prediction error (PE). By contrasting stimulus blocks xxxx or xxxX, with X/x vs Y/y for stimulus identity, lowercase letters x for fully deterministic stimuli, and capital letters Y for randomized stimuli, our experiment sought to disentangle these possible sources of variation. We recorded both spiking and local field potentials in six (6) visual areas in nine (N=9) mice as they passively viewed visual sequences across contexts: local (xxxY) and global (xxxX) oddball sequences or control sequences. Prior to recording, mice were habituated for 5-10 sessions to a specific sequence of visual stimuli containing a local oddball. During the main recording block, mice viewed the habituated sequence, intermixed with global oddball sequences. Current-source density analysis in mouse visual area rostrolateral (RL) showed distinct patterns when oddball stimuli were dissociated into SSA, global oddball PE, and DD. Both SSA and DD showed a current sink spreading from L4 outward after stimulus onset, consistent with a feedforward pattern of canonical circuit activation, although this effect began later for DD. Global oddball PE showed a distinct pattern with enhanced current sinks in superficial and deep layers only after stimulus offset. This argues against a canonical computation for all types of prediction error and in favor of a specialized circuitry for different types of prediction error. Ongoing work is characterizing these computations in cortical and subcortical structures.

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Poster

PSTR223: Visual Adaptation and Processing

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Title: Inferotemporal neurons represent decision formation during face categorization

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Abstract: Visually-guided behavior requires strategic use of visual information by the brain to guide actions. While current theories suggest that the inferotemporal cortex (IT), at the apex of the ventral visual pathway, mainly represents object information and relays it to a network of frontoparietal areas for inference, decision-making, and action planning, an alternative but underexplored hypothesis proposes that the IT also contributes directly to these cognitive processes. To test this, we utilized a visual task that distinguishes between the representation of object information and its integration into decision-making processes. Our results reveal that the IT not only represents object information but also engages in evidence accumulation, decision termination, and task-dependent computations, paralleling the functions typically attributed to frontoparietal regions involved in decision making. We recorded from face-selective neural clusters in the IT as macaque monkeys categorized face stimuli by species (human or monkey) or expression. The stimuli were generated by morphing prototype faces, allowing us to precisely define informative features (eyes, nose, mouth) and stochastically manipulate them within and across trials to control stimulus difficulty and uncertainty. We used our precise control over the stimuli to quantitatively characterize neural activity during evidence accumulation for categorization. Our analysis indicated that, during the task, IT neurons initially showed typical visual responses; however, a closer look reveals complex dynamics. First, the precision of stimulus category encoding at both the single-cell and population levels improved progressively during the stimulus presentation. Second, IT neurons reflected the termination of evidence accumulation; they continued to incorporate stimulus frames when uncertain but ceased integrating stimulus information once enough evidence was accumulated in favor of a choice. Third, IT population responses to the same stimuli varied between species and expression categorization, representing task context. Finally, IT responses began reflecting evidence accumulation earlier or concurrently with responses of simultaneously recorded neurons in the lateral intraparietal area (LIP), a key node in the decision-making network, indicating that decision-related IT dynamics is not merely a result of feedback from frontoparietal regions. These findings suggest that the role of IT in visually-guided behavior transcends mere object representation, contributing to the transformation of visual information into decisions.

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Poster

PSTR223: Visual Adaptation and Processing

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Program #/Poster #: PSTR223.06/F6

Topic: D.06. Vision

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HFSPO LT001-2023/L
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Vanderbilt University Startup Funds

Title: Multi-area, high-Density, Laminar Neurophysiology (MaDeLaNe) recordings in mice and monkeys reveal late hierarchical emergence of global prediction errors in spiking activity

Authors: *J. A. WESTERBERG[1,2], Y. XIONG[3], H. NEJAT[3], S. DURAND[4], H. CABASCO[4], H. BELSKI[4], H. LOEFFLER[4], A. BAWANY[4], R. PEENE[4], W. HAN[4], K. S. NGUYEN[4], V. HA[4], C. GRASSO[4], B. HARDCASTLE[4], J. SWAPP[4], R. GILLIS[4], B. OUELLETTE[4], S. CALDEJON[4], A. WILLIFORD[4], P. A. GROBLEWSKI[4], S. R. OLSEN[4], C. KISELYCZNYK[4], J. LECOQ[4], A. V. MAIER[3], A. M. BASTOS[3];
[1]Vision and Cognition, NIN-KNAW, Amsterdam, Netherlands; [2]Psychology, [3]Psychology and Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN; [4]Allen Inst., Seattle, WA

Abstract: It is clear that the brain utilizes past events to inform sensory processing. Predictive Coding has been proposed as a framework to explain cortical responses to “unexpected” stimuli whose probability are low (oddballs). This phenomenon is most studied using repetitive (and thus predictive) stimulus sequences that get interrupted by a novel stimulus, violating a repeating pattern (e.g., xxxY). Sensory neurons at the earliest stages of cortical processing have been shown to spike to such oddball stimuli, thus signaling “prediction error”. The sensitivity to such first-order, or local, oddballs increases along the cortical processing hierarchy, suggesting that error signals and predictions are repeatedly exchanged and enhanced, as sensory responses progress up the cortical hierarchy. A potential challenge to this paradigm arises from second-order, or global, oddball stimuli which occur when repeated presentations of local oddballs get disrupted by a sequence lacking the local oddball (e.g., xxxY, xxxY, xxxX). The study of neuronal activity to global oddballs is critical to evaluate current Predictive Coding theories. However, the brain-wide responses to global oddballs remain unknown. To address this, we performed Multi-Area, high-Density, Laminar Neurophysiology (MaDeLaNe) recordings of neuronal spiking across seven visual areas in mice (V1, LM, RL, AL, PM, AM, LP, n=5,389 single-units) and 11 visual and higher-order areas in macaques (V1, V2, V3, V3A, V4, MT, MST, TEO, FEF, PFC, PMD, n=8,672 multi-units). Both local and global oddballs evoked significant responses along the visual hierarchy in both species. However, in contrast to local oddball responses, which emerged at the earliest stage of sensory processing and propagated throughout cortex, we found global oddball responses emerged later in hierarchical processing (higher-order cortical area LM, AM and PM in the mouse, and V3, FEF, and PFC in macaque

monkeys). While local oddball responses follow core predictions of the Predictive Coding framework, there are several challenges posed by our global oddball findings, such as (i) why the global oddball prediction did not propagate to the earliest stages of processing, (ii) why the global oddball signals are largely absent throughout most of the visual system, and (iii) why the global oddball signals are largely confined to more cognitive rather than primary sensory areas of the brain. We propose that a modified theory of Predictive Coding that allows for predictions to be limited to relevant neural structures, rather than ubiquitously shared, might best account for these observations.

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Poster

PSTR223: Visual Adaptation and Processing

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The Picower Institute for Learning and Memory
NIMH 1R01MH131715-01

Title: Propofol-mediated loss of consciousness disrupts predictive routing and local field phase modulation of neural activity

Authors: *Y. S. XIONG[1], J. A. DONOGHUE[2], M. LUNDQVIST[3], M. K. MAHNKE[4], A. J. MAJOR[5], E. N. BROWN[6], E. K. MILLER[5], A. BASTOS[7];
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Abstract: Predictive coding is a fundamental function of the cortex. The predictive routing model proposes a neurophysiological implementation for predictive coding. Predictions are fed back from deep-layer cortex via alpha/beta (8-30Hz) oscillations. They inhibit the gamma (40-

100Hz) and spiking that feed sensory inputs forward. Unpredicted inputs arrive in circuits unprepared by alpha/beta, resulting in enhanced gamma and spiking. To test the predictive routing model and its role in consciousness, we collected data from intracranial recordings of macaque monkeys during passive presentation of auditory oddballs before and after propofol-mediated loss of consciousness (LOC). In line with the predictive routing model, alpha/beta oscillations in the awake state served to inhibit the processing of predictable stimuli. Propofol-mediated LOC eliminated alpha/beta modulation by a predictable stimulus in sensory cortex and alpha/beta coherence between sensory and frontal areas. As a result, oddball stimuli evoked enhanced gamma power, late period (> 200 ms from stimulus onset) spiking, and superficial layer sinks in sensory cortex. LOC also resulted in diminished decodability of pattern-level prediction error signals in higher order cortex. Therefore, auditory cortex was in a disinhibited state during propofol-mediated LOC. However, despite these enhanced feedforward responses in auditory cortex, there was a loss of differential spiking to oddballs in higher order cortex. This may be a consequence of a loss of within-area and inter-areal spike-field coupling in the alpha/beta and gamma frequency bands. These results provide strong constraints for current theories of consciousness.

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Poster

PSTR223: Visual Adaptation and Processing

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Topic: H.02. Perception and Imagery

Support: NIH Grant: R01 MH 119099
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Title: Persisting thoughts and hippocampal-cortical ripple coupling in the human brain

Authors: *A. R. CARDENAS[1], F. HEIDARI[2], K. MENGUC[3], T. A. VALIANTE[4], B. BELLANA[2], C. J. HONEY[5];
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Abstract: The themes, moods, and specific episodic content of a life experience will sometimes linger in our minds for minutes or hours beyond the end of the event. This phenomenon of “persisting mental content” is spontaneous, in that it does not require an intent to retrieve from or focus on the completed event. Persisting thoughts are functionally significant, because they can be associated with psychopathology (as in anxiety or obsessive compulsive disorder, OCD) and can also be adaptive (when they contribute to creative problem solving). The neural correlates of

persisting mental content remain unknown.
Hippocampal sharp wave ripple (SWRs) have been reported in the human brain during volitional memory retrieval. Therefore, we hypothesized that spontaneous SWRs may also underlie the spontaneous re-entry of narrative contents into thought, after the narrative has ended.
Specifically, we predicted that SWR rate and duration would increase during narrative-induced mind lingering.
We collected intracranial recordings in 11 patients during a narrative-induced mental lingering task adapted from Bellana et al. (2022). The persistence of mental content was measured in two ways. First, participants performed a spoken free-association task ("word chain game") before and after hearing the narrative. Increases in story-related words from pre-story to post-story free-association epochs were deemed evidence of persisting story-related mental content. Second, participants reported their subjective experience of "lingering thoughts" from the story and associated volitional factors.
In hippocampal sites, we observed an increase in SWR rate in the first 60 seconds following the narrative. Additionally, we observed marked increases in coupling between hippocampal SWRs and ripple-like events in the temporal and frontal cerebral cortex. Although the sample size is limited, these changes in ripple hippocampal-cortical dynamics were most reliable in participants who reported the subjective experience of lingering story-related thoughts.
Our results suggest a neural mechanism by which recent experiences can spontaneously reverberate in our mental experience, and that SWRs can manifest in human subjective experience in the form of intrusive thoughts. Moreover, our findings may shed light on the neural basis of persistent negative thoughts, such as observed in clinical anxiety and OCD.

Disclosures: A.R. Cardenas: None. **F. Heidari:** None. **K. Menguc:** None. **T.A. Valiante:** None. **B. Bellana:** None. **C.J. Honey:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.09/F9

Topic: H.02. Perception and Imagery

Title: Thalamic Orchestration of Consciousness: Unveiling Cortico-Thalamic Dynamics in State Transitions

Authors: *J. P. SAVARRAJ[1], E. MURPHY[2], M. J. MCCARTY[3], J. LI[4], N. TANDON[5]; [1]Neurosurg., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Univ. of Texas Hlth. Sci. Ctr., Houston, TX; [3]Neurosci., MD Anderson UT Hlth. Grad. Sch., Houston, TX; [4]Rice University/ UT Hlth. Houston, Houston, TX; [5]Neurolog. Surgery, McGovern Med. Sch. at UT Hlth., Houston, TX

Abstract: The thalamic nuclei are crucial for modulating cortical sites during transitions between consciousness states. However, challenges arise in human studies due to the difficulty

of simultaneously obtaining local field potentials (LFP) from cortical and thalamic regions. We recorded from epilepsy patients (n=4) with multifocal seizures unsuitable for surgical resection, undergoing stereo EEG (sEEG) implantation to monitor epilepsy and identify epileptogenic foci, including thalamic involvement. After removing sEEG electrodes, patients received propofol and were monitored as they transitioned from awake to unconscious states, performing an auditory tone-detection task until loss of consciousness (LOC). Time-frequency spectrogram analysis revealed an 'awake-α' signature that broadened to 'anesthesia-α' about ~10 seconds post-LOC in pre-central and temporal regions. Thalamic nuclei displayed 'awake-α' just before LOC, transitioning to 'anesthesia-α', with a late-β profile emerging in the ANT nuclei around 80 seconds after LOC; a phenomenon noted in orbital and cingulate regions, indicating a broader presence. During unconsciousness, phase synchrony was preserved, but phase reversals and shifts in the δ and θ bands occurred both inter-cortically and in thalamo-cortical connections. Reversals were primarily observed between homologous contralateral frontal and orbital regions, and heterologous contralateral areas like left-cingulate/right-frontal and left-frontal/right-insula. Thalamo-cortical phase reversals occurred between the thalamus and cortical sites including the orbital, frontal, precentral, and rectus regions. Strong, transient θ-γ coupling corresponded with spectral changes around 10 seconds post-LOC across all profiles. However, while phase-amplitude coupling (PAC) was transient in some regions, it persisted sporadically in others, especially the thalamo-hippocampal connections indicating a presence of spatial and temporal thalamo-cortical dynamics. The transition from awake to unconscious is characterized by multiple spectral components. The shift from in-phase synchrony to phase reversals suggests the presence of large-scale cortical traveling waves, potentially phase-modulated during consciousness transitions. The transient and sustained nature of θ-γ coupling indicates a significant spatial and temporal relationship in thalamo-cortical regulations. These insights significantly enhance our understanding of thalamic regulation of cortical activity, highlighting its critical role in consciousness state transitions.

Disclosures: J.P. Savarraj: None. **E. Murphy:** None. **M.J. McCarty:** None. **J. Li:** None. **N. Tandon:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.10/F10

Topic: H.02. Perception and Imagery

Support: NEI Grant RO1EY032085

Title: Spontaneous slow cortical potentials and brain oscillations independently influence conscious visual perception

Authors: *L. KOENIG, B. J. HE;
Neurosci. Inst., NYU Langone Med. Ctr., New York, NY

Abstract: Perceptual awareness results from an intricate interaction between external sensory input and the brain's ongoing internal spontaneous activity. Pre-stimulus ongoing activity influencing conscious perception has been described in both brain oscillations in the alpha (7-14 Hz) and beta (14-30 Hz) frequency ranges and aperiodic activity in the slow cortical potential (SCP, <5 Hz) range. However, it is unknown whether these brain oscillations and SCPs independently influence conscious perception, or instead interact with each other to shape perceptual awareness, thus relying on shared mechanisms. To address this question, we relied on two independent magnetoencephalography (MEG) datasets involving near-threshold visual perception tasks using low-level (Gabor patches, n = 11, 6 females) and high-level (objects, faces, houses, animals, n = 24, 15 females) stimuli, respectively. Participants viewed images at their individual recognition threshold and were instructed to report the orientation (low-level stimuli) or the category (high-level stimuli) and their recognition experience after a short delay. We found clusters of sensors in which pre-stimulus alpha or beta oscillatory power (i.e., amplitude) significantly differed between perceived (i.e., detected in the low-level stimuli, recognized in the high-level stimuli) and unperceived trials. We also constructed a decoder to classify perceptual outcome based on the whole-brain activity pattern of SCPs. We found that oscillatory power and large-scale SCP activity influence conscious perception through independent mechanisms that do not have shared variance. Further, using mediation analysis, we show that pre-stimulus oscillatory power and SCP activity have different relations to pupil size—an index of arousal—in their influences on conscious perception. Specifically, while pupil-linked arousal mediated SCP's influence on conscious perception, pupil-linked arousal's perceptual influence was itself mediated by the rise and fall of alpha and beta oscillations. Together, these findings suggest that oscillatory power and SCPs independently contribute to perceptual awareness, with SCP's influence partly mediated by arousal-linked mechanisms. Importantly, these results were reproduced in two datasets involving threshold-level perception of low- and high-level visual features, suggesting the robustness and generalizability of our findings. These results point to the existence of multiple, non-interacting neural mechanisms that can influence the perceptual awareness of an upcoming stimulus.

Disclosures: L. Koenig: None. **B.J. He:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.11/F11

Topic: D.06. Vision

Support: NIH R00MH116100
Vanderbilt startup funds (AMB)

Title: Distinct Single Neuron Omission Responses Across the Visual Hierarchy

Authors: *H. NEJAT[1], Y. S. XIONG[1], K. M. GABHART[1], J. A. WESTERBERG[2], A. M. BASTOS[3];
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]Dept. of Vision and Cognition, NIN-KNAW, Amsterdam, Netherlands; [3]Psychology and Vanderbilt Brain Inst., Vanderbilt Univ., Nashville, TN

Abstract: The unpredictable omission of expected stimuli serves as a valuable tool in neuroscience for investigating the neural basis of predictive coding. Prior studies have demonstrated the presence of non-specific omission-sensitive neurons in the auditory cortex. However, it is not clear whether these omission responses originate from lower-order sensory processing areas or in higher order areas that underlie cognitive functions. Building upon this, we hypothesize that single neurons across visual-cortical areas exhibit specific responses upon the omission of an expected stimuli. In this study, we habituated two macaque monkeys over approximately 30 sessions to four sequential visual gratings presented in two main blocks (AAAB/BBBA, where A&B are 45&135 degree drifting gratings, respectively) and one random control block (RRRR, R = A or B). During recording sessions (n=13, across two macaque monkeys), we implemented an omission paradigm by randomly omitting the second, third, or fourth stimulus in each trial at a rate of 10% (30% total omission probability). Intracranial signals were recorded from multiple cortical-visual areas (V1, V2, V3, V3d, V3a, V4, MT, MST, TEO, FST, FEF, PFC) during the omission sessions using multiple 128-channel (Diagnostic BioChips) probes. We preprocessed these recordings to detect single neurons using Kilosort2. This approach, which we refer to as Multi-area Dense LaminarNeurophysiology (MaDeLaNe), enabled us to analyze single neuron responses during omission, non-omission, and random control conditions across the visual hierarchy and cortical layers. Our analysis, based on ~2000 single neurons, revealed specific and non-specific spiking responses to visual stimulation across all areas. However, both stimulus specific and non-specific omission responses were observed solely in single neurons from higher order areas (~5% of ~600 neurons in FEF/PFC). These findings suggest that omission responses originate in higher order cortex and imply that prediction error coding of omissions is more of a cognitive rather than a sensory process.

Disclosures: H. Nejat: None. **Y.S. Xiong:** None. **K.M. Gabhart:** None. **J.A. Westerberg:** None. **A.M. Bastos:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.12/F12

Topic: H.02. Perception and Imagery

Support: NSF Grant 2050833

Title: Traveling waves link sensory and motor cortex during sensory readout.

Authors: *T. HOUGHTON[1], C. LUO[2], E. F. ESTER[3];
[1]Univ. of Nevada, Reno, Reno, NV; [2]Shenzhen Univ., Shenzhen, China; [3]Psychology, Univ. of Nevada, Reno Integrative Neurosci. Grad. Program, Reno, NV

Abstract: Flexible behaviors require rapid communication between sensory systems that process inputs and motor systems that respond to those inputs. Here, we report novel evidence suggesting that this communication is mediated by cortical traveling waves, or spatial coherent oscillations that propagate across the cortex. We recorded EEG while human observers (N = 27) performed a perceptual comparison task. Participants were required to adjust the orientation of a central probe to match one of two laterally presented bars. Importantly, the location of the probed bar (i.e., left vs. right visual field) and the hand required to respond (i.e., left vs. right) were independently varied across trials. This manipulation, coupled with the contralateral mapping of human sensorimotor systems, allowed us to make trial-wise predictions about the cerebral hemisphere responsible for processing task-relevant sensory information (i.e., contralateral to the target bar) and the cerebral hemisphere responsible for generating the appropriate response (i.e., contralateral to the appropriate response hand). We used image-based analyses to extract and analyze traveling waves of frequency-specific activity measured along planar axes linking electrode sites over occipital and motor cortex. This analysis revealed three unique signals: a feedforward (posterior-to-anterior) theta-band (2-4 Hz) wave that emerged over the task-relevant cerebral hemisphere shortly before response onset and whose peak latency predicted intra-individual differences in response speed, a feedback (anterior-to-posterior) beta-band (14-30 Hz) wave that emerged over the task-relevant cerebral hemisphere after participants ended their response, and a feedback alpha-band (8-12 Hz) wave that emerged over the task-irrelevant cerebral hemisphere around the time of response initiation. Our findings complement recent work implicating planar traveling waves in the role of working memory-guided behaviors (Luo & Ester, 2024) and suggest that similar waves coordinate the read-out of perceptual information for precise behavior.

Disclosures: T. Houghton: None. **C. Luo:** None. **E.F. Ester:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.13/F13

Topic: D.06. Vision

Support: NIMH R01MH127375
Simons Foundation
Pew Foundation

Title: Mediodorsal and ventrolateral thalamic nuclei represent the formation of perceptual decisions and associated feedback

Authors: *B. SIEVERITZ, R. KIANI;
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Past studies on the neural mechanisms of decision-making focused mainly on the frontoparietal cortex and basal ganglia. For example, in a direction discrimination task with random dots, where monkeys report the dominant motion direction with a saccadic eye movement to one of two peripheral targets, neural activity in the lateral intraparietal cortex (LIP), dorsolateral prefrontal cortex (dlPFC), and striatum encodes gradual accumulation of motion information to inform saccade direction. These observations have given rise to models in which cortical and basal ganglia circuitry play prominent roles in decision-making, ignoring the mediodorsal and ventrolateral thalamic nuclei; higher-order nuclei that are crucial nodes in the cortico-basal ganglia-thalamocortical loops and have extensive direct connections with the frontoparietal regions implicated in decision-making. Here, we test the role of these nuclei in the decision-making process.
We trained two monkeys to perform a reaction time version of the direction discrimination task, while we recorded neural activity in the mediodorsal and ventrolateral thalamus. On the single neuron level, mediodorsal and ventrolateral thalamic neurons show response properties distinct from their cortical counterparts. Whereas neurons in LIP and dlPFC monotonically change their activity with stronger stimulus support for their preferred saccade, most thalamic neurons change their activity with the absolute stimulus strength for both motion directions. Further, thalamic neurons have transient responses, unlike cortical neurons that tend to have persistent responses with consistent selectivity throughout the stimulus viewing epoch. However, population activity of the mediodorsal and ventrolateral thalamus systematically represents both stimulus strength and accumulation of sensory evidence during decision making. Mediodorsal and ventrolateral thalamus also encode feedback, and their response is modulated with motion strength in a manner compatible with the representation of reward prediction errors. Although these thalamic nuclei continue to encode stimulus strength at the beginning of the feedback epoch, they dominantly encode feedback at the end of it.
Given the difference in task variable encoding between cortex and thalamus on a single neuron level, and the diversity of task variables encoded across task epochs, we suggest that mediodorsal and ventrolateral thalamus have a distributed representation of task variables that remixes cortical neural signals. It remains to be investigated if such remixing plays a crucial role for the integration of stimulus information during decision-making.

Disclosures: B. Sieveritz: None. **R. Kiani:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.14/F14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY030747

Title: Stratification of visual impairment as a novel variable to understanding sex-specific degenerative changes in retinal structure and function of middle-aged C57Bl/6J mice

Authors: *G. EDWARDS[1], S. M. RIORDAN[1], C. BUCHHOLZ[1], M. MARDELLI[1], C. EURITT[1], R. PEREZ-MAGNELLI[1], A. RAFIQ[1], A. ENGELMEYER[1], P. KOULEN[2]; [1]Ophthalmology, Univ. of Missouri-Kansas City, Kansas City, MO; [2]Biomed. Sci. and Ophthalmology, Univ. of Missouri - Kansas City, Kansas City, MO

Abstract: The C57Bl/6J mouse model is widely used in pre-clinical research and allows the consideration of age and sex as critical biological variables. The goal of the present study was to determine age- and sex-specific differences in visual function and retinal structure in C57Bl/6J mice as potential underlying differential aging effects leading to visual loss. Empirical stratification of visual impairment based on behavioral measurements of visual performance identified age and sex as biological variables and reproducible parameters in the absence of disease. Visual acuity (VA) and contrast sensitivity (CS) were determined longitudinally as optomotor reflex (OMR) responses in male (n = 19) and female (n = 29) mice, from 5 to 12 months of age. Eyes were categorized into four groups: normal vision, low, moderate, and severe vision loss. Retinal function was measured by electroretinography (ERG) while retinal structure was imaged with spectral domain optical coherence tomography (SD-OCT), standard histology, and image analysis via ImageJ software. Significant differences were observed in visual function between male and female mice. Mean CS values indicate less sensitivity to fine detail and moving stimuli in female mice measured at 12 months of age ($p < 0.001$). In-vivo imaging showed a notable decrease in the thickness of the outer nuclear layer (ONL) of male mice suggesting that ONL thickness decreases differentially over time between sexes ($p < 0.0001$), but no significant differences were observed based on grouping animals due to their visual impairment. Retinal function, assessed by ERG, showed an increase in mean scotopic b-wave implicit time in male mice compared to female mice ($p < 0.01$). Mean photopic responses for low, moderate, and severe vision loss displayed differences between sexes with increased visual impairment. Histological examination indicated a significant sex-specific difference in the mean thickness of the inner plexiform layer (IPL) in mice, with males showing a greater decrease in IPL thickness ($p < 0.001$) implying that the aging process has a more pronounced effect on the IPL of males than of females, with the most significant decrease in IPL thickness in male mice with severe vision impairment ($p < 0.0001$). Thinning of the photoreceptor inner/outer segment (IS/OS) layer was noticeably more pronounced in female mice compared to males with normal vision ($p < 0.01$), suggesting a causal relationship with behavioral assessed CS. We identified sex-specific differences in aging C57BL/6J mice with visual function as well as retinal structure and function differentially affecting mice categorically based on the degree of vision loss.

Disclosures: G. Edwards: None. **S.M. Riordan:** None. **C. Buchholz:** None. **M. Mardelli:** None. **C. Euritt:** None. **R. Perez-Magnelli:** None. **A. Rafiq:** None. **A. Engelmeyer:** None. **P. Koulen:** None.

Poster

PSTR223: Visual Adaptation and Processing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR223.15/F15

Topic: I.04. Physiological Methods

Support: NIGMS R01-GM056398
the Department of Anesthesiology, University of Michigan

Title: Anesthesia Alters Complexity of Spontaneous and Stimulus-induced Neuronal Firing Patterns in Rat Visual Cortex

Authors: *D. LI, A. G. HUDETZ;
Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction: Complexity of neuronal firing patterns may index sensory information at different states of consciousness. General anesthesia disrupts visual stimulus-related neuron firing and decreases the complexity of the early (<200 ms) post-stimulus response. Its effect on the late (200-1000 ms) response component, which likely reflects recurrent processing necessary for conscious vision, is unclear. In addition, brain states may spontaneously change at constant anesthetic concentration, which may also influence neuronal complexity and the state of consciousness. Here we investigate the effect of spontaneous state transitions on the complexity of stimulus-related neuron firing at different anesthetic depths.
Methods: Extracellular unit activity was measured with chronically implanted 64-site silicon microelectrode arrays in cortical layers 5/6 of primary visual cortex of six freely moving male rats during stepwise decrease of desflurane concentration at 6, 4, 2, and 0%. Discrete light flashes of 10 ms duration were delivered to the retina at random interstimulus intervals (2-4 s; 100 trials per anesthetic level) by transcranial illumination. Population states were identified by applying principal component analysis to spontaneous single-unit activity, followed by a density-based clustering on the mean firing rate and permutation entropy of first 2 principal components. State membership of pre-stimulus spike data in each trial was assigned based on a Gaussian mixture model fitted to the spontaneous states. **Results:** Five states were distinguished of which S1-4 approximately followed the anesthetic condition. S5 showed increased population spike rate and permutation entropy, occurred mostly at 4% and 6% desflurane, and was identified as a paradoxical state. During wakefulness, visual stimulation elicited complex increase in firing consisting of early (<250 ms) and late oscillatory components (250-1000 ms). Anesthesia gradually suppressed the amplitude of the late response and split the spike timing of the early response into 2 peaks. At deeper levels of anesthesia, there were fewer active neurons and they spiked more synchronously. In paradoxical S5, more neurons fired and less synchronously; however, both early and late post-stimulus neuronal complexity remained comparable to that in deep anesthesia (S4).**Conclusions:** Multiple neuronal states with distinct visual stimulus-related spiking patterns occur at constant anesthetic concentrations. Neuronal complexity of stimulus-related patterns (<1000 ms) decreases in states associated with deep anesthesia, which may reflect the disruption of sensory information processing.

Disclosures: D. Li: None. **A.G. Hudetz:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.01/F16

Topic: D.08. Multisensory Integration

Support: The National Center for Artificial Intelligence CENIA, Chile FB210017
ANID-Subdirección de Capital Humano/Doctorado Nacional/2022-folio 21221549

Title: Tympanic oscillations associated with saccades occur in the absence of visual input

Authors: *M. LEON[1], P. E. MALDONADO[1,2];
[1]Neurosci., Univ. de Chile, Santiago, Chile; [2]Natl. Ctr. for Natl. Intelligence (CENIA), Santiago, Chile

Abstract: During active sensing, the changes of activity in the sensory organs result from self-initiated motor actions. This motor activity precisely modulates early sensory cortices such as V1, A1, and S1. It has been observed that active sensing is a multi-modal phenomenon. Recently, it was reported in humans and monkeys that the eardrums move when saccades are performed, with the phase and amplitude of tympanic oscillation being dependent on the direction and amplitude of the saccades. For instance, when performing a saccade to the right, the ipsilateral eardrum bulges while the other eardrum contracts. Is this phenomenon dependent on visual activity? Because direct connection from motor to early sensory areas has been reported, we hypothesized that the tympanic movement associated with movement would not depend on retinal visual activity. We recruited subjects to perform voluntary saccades while tympanic movements were measured through intra-aural microphones, and eye movements were measured through electrooculography. We observed the expected tympanic movement associated with the saccades, which were also observed during voluntary saccades performed with the eyes closed and covered. Our results indicate that during active sensors, tympanic oscillations associated with saccades do occur in the absence of visual input, demonstrating a central role for motor action in multimodal sensory sampling.

Disclosures: M. Leon: None. **P.E. Maldonado:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.02/F17

Topic: D.08. Multisensory Integration

Title: Synesthetes and Nonsynesthetes Differentially Process Grapheme-Color Information as Measured by Cortical Functional Near-Infrared Spectroscopy (fNIRS)

Authors: *A. G. BRISTER[1], E. A. BOURASSA[2];
[1]Mississippi Col., Clinton, MS; [2]Biol. Sci., Mississippi Col., Clinton, MS

Abstract: Grapheme-Color Synesthesia (GCS) is a neurological trait characterized by unique, intrinsic couplings between graphemes (letters and numbers) and colors. Upon stimulation with a grapheme, a person with GCS will experience a color, either projected onto their three-dimensional visual field or constructed within their "mind's eye." GCS is the most well documented and thoroughly studied form of synesthesia. However, the brain activity patterns in GCS in response to standardized synesthesia diagnostics (such as a modified, synesthetic Stroop task) remains poorly characterized, particularly because of an inconsistent definition of GCS as well as differences in methodology. In this study, a sample of 15 students ages 18-25 underwent a two-part GCS screening process that involved a baseline assessment of their associations followed by a modified synesthetic Stroop task. Analysis of the data obtained from these assessments yielded a synesthetic score for each participant that distinguished synesthetes from nonsynesthetes. Blood Oxygen Level Dependent (BOLD) responses were measured predominately from the left frontal, temporal, parietal, and occipital cortices using functional near-infrared spectroscopy (fNIRS) at baseline and for each trial type of the Stroop task. Analysis of synesthetic scores showed that synesthetes had much more consistent associations with better accuracy and faster response times than nonsynesthetes on the Stroop task. Analysis of the fNIRS data showed that synesthetes had an increased BOLD response in the left supramarginal gyrus (Brodmann Area 40) at baseline and an increased BOLD response in the left inferior frontal gyrus (Brodmann Area 45) during both congruent and incongruent trials of the Stroop task. When data from the congruent and incongruent trials were combined, synesthetes also had an increased BOLD response in the fusiform gyrus (Brodmann Area 37). Interestingly, synesthetes had a *decreased* BOLD response in the associative visual cortex (V3/V4) compared to nonsynesthetes during the Stroop task. These data suggest that visual processing of grapheme-color information in GCS is more heavily dependent on multimodal sensory processing areas such as the inferior frontal and fusiform gyri rather than the visual cortex.

Disclosures: A.G. Brister: None. **E.A. Bourassa:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.03/F18

Topic: D.08. Multisensory Integration

Support: NHMRC Grant N213455
Australian Government Research Training Program scholarship

Title: The causal effect of proprioception on upper limb function across the lifespan

Authors: *L. ROBERTSON[1,4], J. DIONG[5], A. A. BUTLER[2], S. GANDEVIA[2], M. E. HEROUX[3];
[1]Neurosci. Res. Australia, Sydney, Australia; [2]Neurosci. Res. Australia, Randwick, Australia; [3]Neurosci. Res. Australia, Sydney - Randwick, Australia; [4]Sch. of Clin. Med., Univ. of New South Wales, Sydney, Australia; [5]Univ. of Sydney, Sydney, Australia

Abstract: Proprioception, our awareness of the mechanical and spatial state of the body, may impair physical function. The causal effect of proprioceptive ability on upper limb function across the lifespan is unknown. We aimed to estimate (1) the causal effect of proprioceptive ability on upper limb function across the lifespan, and (2) the extent to which age modifies the effect of proprioceptive ability on upper limb function. A causal directed acyclic graph was developed with subject-matter expertise to specify the causal relationships between proprioceptive ability, upper limb function, and their common causes. We assessed the performance of 158 healthy individuals (18-90 years) on a battery of upper limb tests, a test of cognitive function, and a test of hand proprioceptive ability. Proprioceptive ability was assessed using a test of "low-level" proprioception which involved proprioceptive judgements made within a single frame of reference (i.e., proprioception to proprioception), and a test of "high-level" proprioception which involved proprioceptive judgements made between frames of reference (i.e., vision to proprioception). The causal graph was used to identify the minimal set of variables needed for adjustment to remove bias. Linear regression incorporating the minimal adjustment set was used to estimate the causal effect of proprioceptive ability on function, using log-transformed data as required. Proprioceptive ability was measured as variability (R^2, range 0 to 1) and error (mean absolute error in cm). Under the causal graph, for each increase of 0.1 in the explained variance of high-level proprioception variability, performance in the shirt task was faster by 3.1 s [95% CI 0.8 to 4.9 s]. Age modified the effect of high-level proprioception error on performance of the shirt task (interaction between age and proprioception: 0.003 years.cm [95% CI 0.000 to 0.006 years.cm], p = 0.04. For example, for individuals with mean absolute proprioception error identical to the group average (1.04 cm), a 70-year-old would take 7.0 s longer on the shirt task compared to a 30-year-old. Age modified the effect of low-level proprioception variability on performance of the shirt task (interaction between age and proprioception: -0.03 years [95% CI -0.037 to -0.002 to years]. For example, for individuals with R^2 identical to the group average (0.76), a 70-year-old would take 5.8 s longer on the shirt task compared to a 30-year-old. High-level proprioceptive ability contributes to upper limb function, and its contribution is modified by age. As this is a causal relationship, interventions which improve high-level proprioceptive ability should improve upper limb function.

Disclosures: L. Robertson: None. **J. Diong:** None. **A.A. Butler:** None. **S. Gandevia:** None. **M.E. Heroux:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.04/F19

Topic: D.08. Multisensory Integration

Support: JST, CREST JPMJCR23P, Japan

Title: Investigating the development process of the rubber hand illusion by EEG measurement and phenomenological interview

Authors: *F. SUZUKI, M. KIMURO, M. ISHIZU, S. SHIMADA;
Meiji Univ., Kawasaki, Japan

Abstract: Elucidating the mechanisms of the sense of ownership leads to a better understanding of the sense of self, and the rubber hand illusion (RHI) has widely been used to investigate it. Most previous studies have focused on behavioral indices, such as proprioceptive drift, and neural activities indicating the occurrence of RHI. On the other hand, a few studies have postulated that the qualitative data obtained through phenomenological interviews can be used to estimate subjective state changes more precisely during the illusion. In this study, we simultaneously utilized EEG measurement and phenomenological interview to investigate the RHI development process. Sixteen healthy adults participated in the experiment (eight females; 21.3 ± 0.56 years). Two experimental conditions were employed: the synchronous condition and the asynchronous condition. Each condition lasted up to thirteen minutes. We measured 30 electrodes of EEG according to the international 10-20 system, with a sampling rate of 512 Hz, during the RHI experience while simultaneously interviewed the participants. The present study employed the elicitation interview methodology, which has been employed in previous studies on the RHI to collect the semi-structured interview data. Based on the descriptions obtained from the interviews, we generated a state transition model of RHI. In the synchronous condition, we found that the there are three subphases before the state of "illusion (phase 2)": "perception of tactile stimulation (phase 1-1)", "association between visual and tactile stimulations (phase 1-2)", and "beginning of the incorporation of the rubber hand (phase 1-3)", similarly to the previous study (Valenzuela-Moguillansky et al., 2013). In the asynchronous condition, we found five subphases before the state of “illusion (phase2)” or “non-illusion (phase2’)”. The Friedman’s test was conducted using EEG data from the six seconds preceding each subphase, during which the participants were not making utterances. The EEG frequency analysis revealed that the power value of the β band in the premotor cortex was greater in the phase1-2 than in the phase1-1 in the synchronous condition (FC3: p=.04, r=0.53). Also, the γ band power in the parietal lobe was significantly greater in the phase1-3 than in the phase1-2 (TP7: p=0.04, r=0.51, CP3: p=0.04, r=0.51, P7: p=0.008, r=0.67, P3: p=0.04, r=0.51). There were no significant changes in power values between subphases in the asynchronous condition. These results suggest that not only subjective states, but also brain activity changes during the development of the sense of ownership for the rubber hand.

Disclosures: F. Suzuki: None. **M. Kimuro:** None. **M. Ishizu:** None. **S. Shimada:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.05/F20

Topic: D.08. Multisensory Integration

Support: The Scientific and Technological Research Council of Turkiye (BIDEB 2211 Program)

Title: Functional networks involved in audiovisual processing: a graph theory approach to identify functional connectivity across frequency bands

Authors: *I. AKDOGAN[1], S. AYDIN[2], H. KAFALIGONUL[3];
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Abstract: Multisensory perception involves the integration of sensory information from different modalities, crossmodal interactions over different cortical regions, and dynamic shifts of activity across different areas (Talsma et al., 2010; Murray et al., 2016). Multisensory processing is expected to be more complicated when the sensory stimulation is dynamic, as is the case in our daily lives. Despite the significance of these phenomena, our understanding of the cortical networks involved in multisensory processing at different stages is limited. Examining network organization using the mathematical framework of graph theory across frequency bands can be a promising approach to shed light on cortical networks underlying multisensory processing and crossmodal interactions. In this study, we used an EEG dataset of a dynamic multisensory paradigm in which audiovisual interactions in the temporal domain alter visual motion perception (Kaya & Kafaligonul, 2019). We employed graph theoretical modelling and network-based statistics (NBS) for each frequency band to examine the whole-brain functional connectivity with the directed transfer function (DTF). DTF was utilized as a model-based multichannel estimator, robust to volume conduction effects, to examine the intensity of information flow between cortical regions across different frequency bands. The analyses revealed distinctive functional connectivity patterns such that audiovisual interactions exhibited long-range connectivities between parietal and frontal regions in theta and alpha frequency bands. On the other hand, densely connected neighboring subgroups were observed over occipital and parietal areas when only visual input was provided. The analyses of global topological properties also revealed significant alterations. When only visual stimulus was presented, there was a higher clustering coefficient, local efficiency, and modularity, indicative of local segregation and local interconnectivity during relatively low cognitive and perceptual demand. Conversely, the audiovisual interactions engaged higher global efficiency across all frequency bands, suggesting the emergence of an integrated but metabolically costly workspace configuration during high processing demands (Sporns, 2011). These findings emphasize the adaptive nature of cortical networks in optimizing functional configurations for efficient processing of audiovisual information, with oscillatory activity selectively distributed across specific frequency bands.

Disclosures: I. Akdogan: None. **S. Aydin:** None. **H. Kafaligonul:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.06/F22

Topic: D.08. Multisensory Integration

Support: Kakenhi 21H04860

Title: Catching a slowly approaching object feels heavier: internal model-based predictions influence impact force perception

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Abstract: During mechanical interactions with objects in daily life, the brain predicts the force exerted by the object based on prior knowledge and visual information such as the size of the object. This predictive model not only generates appropriate motor commands but also influences the perception of the interaction force. For example, when lifting an object, the brain's predictive model (size-weight model) predicts that a larger object will be heavier, so a larger object is perceived as lighter than it would be if it weighed the same, a phenomenon known as the size-weight illusion (Charpentier, 1891). In this study, we show that the phenomenon of predictive models creating illusions is also observed in the catching a moving object task. We created the task using the robot manipulandum, in which participants attempted to catch a virtual moving ball with a paddle. When the ball collided with the paddle, the robot generated the impact force. Participants had to counteract the force by gripping their index and thumb fingers and the gripping force was measured by force sensors placed on the handle surface. Since the impact force is determined by its momentum (weight x velocity), the brain needs a model that predicts the impact force according to the momentum (momentum model) in order to generate the appropriate grip force. We investigated how the perceived impact force was influenced by the ball's approach speed, and the relationship between the perceived impact force and the grip force. Twelve subjects participated in the experiment and judged the strength of the perceived impact force compared to the reference for a combination of ball velocity and impact force. As a result, the probability of answering "strong" was higher in the slow ball speed condition than in the fast ball speed condition, and a two-way ANOVA with ball speed and impact force as two factors revealed a significant main effect of ball speed ($F(1,11)=16.5$, $p<0.01$). In other words, for the same impact force, it was perceived as weaker when the ball velocity was faster and stronger when it was slower. This counterintuitive result can be explained by the same mechanism as the size-weight illusion. In fact, the rate of change of grip force at impact was greater in the fast ball condition than in the slow ball condition, indicating that the brain predicted the steeper increase in impact force for the faster ball. Therefore, the brain predicts the impact force based on the model for predicting momentum, and the difference between the prediction and the actual force produces the illusory perception.

Disclosures: S. Okada: None. **D. Nozaki:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.07/F23

Topic: D.08. Multisensory Integration

Support: NSFGRFP

Title: Navigating the near: virtual reality investigations of peripersonal space in autism

Authors: *H. SRINIVASAN[1], C. J. CASCIO[2], M. T. WALLACE[3];
[1]Neurosci., Vanderbilt Univ., Nashville, TN; [2]Psychiatry, Vanderbilt Univ. Med. Ctr., NASHVILLE, TN; [3]Psychology, Neurosci., Vanderbilt Univ., Nashville, TN

Abstract: Our research investigates the dynamic interplay between sensory sensitivity and peripersonal space (PPS) in autistic and non-autistic individuals using a virtual reality (VR) bubble-pop task integrated with EEG analysis. Our study aims to explore how sensory hypersensitivity influences the boundaries of PPS and the associated physiological responses, providing insights into the relationship between sensory processing and spatial awareness. Participants, comprising 20 autistic and 20 non-autistic controls, will engage in a VR task designed to simulate PPS intrusions. The task is personalized through arm-length measurements to accurately assess each participant's PPS. Independent variables include bubble speed and distance, treated as continuous variables to derive reaction time (RT) curves. Dependent variables encompass RT gradients, attempt distance, localization errors, hits versus misses, and the physiological measure of heart rate, offering a comprehensive understanding of sensory-motor integration and autonomic responses. Additionally, our study incorporates a control task to benchmark motor abilities, addressing variations in sensory-motor integration accuracy. A whole-brain EEG analysis technique, with global field power and topographic similarity or dissimilarity, to observe overarching neural patterns during the VR task will potentially help reveal global neural dynamics associated with sensory sensitivity and PPS engagement in autism vs control group, offering novel insights into the neurophysiological mechanisms underpinning these interactions. By integrating VR, EEG, and control tasks, our study aims to contribute insights into the complex sensory and motor interactions characterizing autism, with potential implications for developing personalized therapeutic strategies.

Disclosures: H. Srinivasan: None. **C.J. Cascio:** None. **M.T. Wallace:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.08/F24

Topic: D.08. Multisensory Integration

Support: Tateishi Science and Technology Foundation, Research grant(A), 2221009
JSPS KAKENHI Grant Number 22H00526
JSPS KAKENHI Grant Number 22K18418
JSPS KAKENHI Grant Number

Title: Eeg responses to visuo-tactile stimulation around body in high-resolution vr scene

Authors: *N. KANAYAMA[1], N. MORISHITA[2], Y. SOBUE[2], M. HARA[3];
[1]Natl. Inst. of Advanced Industrial Sci., Tsukuba, Japan; [2]Natl. Inst. of Advanced Industrial Sci. and Technol. (AIST)/ Saitama Univ., Tsukuba, Japan; [3]Saitama Univ., Saitama, Japan

Abstract: Virtual Reality (VR) has been familiarized in our lives, and human activities in virtual space vary and are needed except for our entertainment. However, we know little about the alternation of human perception and cognition caused by the usage of VR space. In particular, immersive VR using a Head-Mounted Display (HMD) requires us to continuously see the scenes projected by two (right and left) lenses as binocular stereopsis. Under such an unusual condition, human perception and recognition of one's body could be altered. Our previous study has revealed that the EEG responses related to our visuotactile integration process during the induction of rubber hand illusion (RHI) can be observed also in the VR scene (Kanayama et al., 2021). In this study, we have modulated the resolution of the VR visual scene and seen the EEG response to the visuotactile stimulation inducing RHI. In the VR scene, a 3D model of a hand wearing a light yellow glove was placed in front of the participant as a rubber hand (VR rubber hand), with spheres attached to the tip of the index finger and ring finger. The emission of the sphere was used as visual stimulation. In addition, we have also adopted the condition in which participants see the real rubber hand in a real environment through HMD. LEDs were attached to the identical place of a real rubber hand in the same manner as a VR rubber hand. A tactile stimulation was the vibration of bone-conducting earphones in both VR and real (passthrough) environments. We recorded EEG waveforms from 63 electrodes placed over the whole head. Pre-processed waveforms were decomposed using independent component analysis (ICA) and the dipole locations of the independent components (ICs) were estimated. ICs for further analyses were selected by the ICLabel plugin and residual variance of the dipole, as a brain-related IC. Scalp topography and dipole location were used for the clustering analysis of all remaining ICs. The clustering revealed a cluster related to the cortical response around the parietal area, in which the gamma band oscillation was observed during 200-400 ms in time and 30-60 Hz in frequency. As a result, only in the passthrough environment, the more intense gamma band oscillation in visuotactile spatially congruent stimulation was observed compared to the incongruent condition. The gamma band oscillation has been considered as a visuotactile integration process inducing an illusory sense of body ownership. Our results suggested that the visuotactile integration process varies based on the quality of the visual scene projected onto the HMD.

Disclosures: N. Kanayama: None. **N. Morishita:** None. **Y. Sobue:** None. **M. Hara:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.09/F25

Topic: D.08. Multisensory Integration

Support: R01 DC-013543

Title: Tinnitus severity as a function of multisensory input

Authors: *A. SHAHIN[1], M. GONZALES[2], A. DIMITRIJEVIC[3];
[1]Univ. of California, Merced, CA; [2]Cognitive and Information Sci., Univ. of California, Merced, Merced, CA; [3]Dept. of Otolaryngology - Head and Neck Surgery, Univ. of Toronto, Toronto, ON, Canada

Abstract: 16.00 Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Aptos",sans-serif; mso-ascii-font-family:Aptos; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Aptos; mso-hansi-theme-font:minor-latin; mso-font-kerning:1.0pt; mso-ligatures:standardcontextual;} Tinnitus is a phantom sound sensation produced by deafferented neurons in the auditory cortex. A prevailing theory posits that tinnitus develops with a loss of high-frequency input from the ear, due to hearing-loss. This in turn causes corresponding neurons that code for the lost input to synchronize and produce the phantom sound. Neurophysiologically, tinnitus severity is reflected by larger auditory evoked potentials (AEPs) for lower-frequency pure tones in tinnitus versus non-tinnitus individuals. Research concerning tinnitus remediation have largely focused on testing inhibitory mechanisms affecting the deafferented neural populations. Incidentally, a well-established account of auditory inhibition occurs during audiovisual (AV) listening. The visual modality's inhibitory influence on the auditory cortex is indexed by the suppression of AEPs for AV versus auditory-only (A-only) listening. Taken together, we hypothesized that one factor influencing tinnitus severity is the lack of visual-to-auditory inhibition during AV listening situations. In the current study, we examined AEPs to 500-Hz tones and speech sounds during AV and A-only listening situations. Preliminary data indicate that compared to age matched healthy-hearing individuals with no tinnitus, individuals with tinnitus exhibited a lack of visual-to-auditory inhibition, during AV versus A-only listening tasks. This influence was more pronounced when speech was the auditory stimulus as opposed to tone. Our findings suggest that reduced evidence of multisensory modulation of auditory representations may contribute to tinnitus severity. Moreover, our findings also imply that electrophysiological tinnitus diagnosis may be more informative when using ecologically valid auditory stimuli, e.g., speech, as opposed to the more widely used pure tones.

Disclosures: A. Shahin: None. **M. Gonzales:** None. **A. Dimitrijevic:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.10/F26

Topic: D.08. Multisensory Integration

Support: NSERC

Title: Attentional effects on visual-tactile crossmodal enhancement at early cortical somatosensory processing.

Authors: *E. SALAZAR[1], S. MUGHAL[1], S. K. MEEHAN[2], R. STAINES[1];
[1]Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada; [2]Dept. of Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Sensory processing can be facilitated through bimodal interactions between relevant visual-tactile sensory inputs in order to achieve goal-oriented behaviours. While the specific neural mechanisms contributing to this modulation remain unclear, the dorsolateral prefrontal cortex (DLPFC) may have a role in regulating the observed processing facilitation seen in the somatosensory cortex (S1), though the extent is not yet clear. We used electroencephalography (EEG) to observe the temporal contributions of visual priming to the enhancement of S1 responses. We hypothesized that inhibiting DLPFC cortical activity would result in a diminished facilitation of tactile processing in S1 (represented by the P50), observed by a visual-tactile stimuli onset with a 200-300 ms time delay. Somatosensory modulation was inferred through amplitude and latency shifts in tactile event-related potentials (ERPs) recorded while participants performed a sensory integration task that required scaled motor responses dependent on the amplitudes of tactile and visual stimuli. Tactile stimuli were discrete vibrations (25 Hz) presented to the left index finger, visual stimuli were presented as a central horizontal bar on a computer screen at varying heights, and graded motor responses were made by squeezing a pressure-sensitive rubber bulb. Healthy adults completed a training session to become familiar with the stimulus-response relationships for both visual and tactile stimuli prior to completing a task where pairs of discrete stimuli with random amplitudes were presented: Tactile-tactile (TT, 500 ms each, 30 ms ISI), visual-tactile with a 200-300 ms delay (vTd 200-300 ms), and visual-tactile with a 300-400 ms delay (vTd 300-400 ms). Stimuli pairs were administered in a block setting, where each block contained 60 trials, with 20 trials for each of the discrete stimuli presented in a randomized order. The study design consisted of 10 blocks, with a short transcranial magnetic stimulation (TMS) intervention at the halfway mark. Participants were randomly assigned to either an intervention group (n=16) or control group (n=16) where TMS modalities of theta burst stimulation (TBS); continuous TBS (cTBS) and intermittent TBS (iTBS), were respectively applied to the right DLPFC. Preliminary analysis reveals that P50 upregulation observed in condition vTd 200-300 ms is significantly lower following cTBS on the right DLPFC but still greater then unimodal TT stimulation. These findings improve our

understanding of the role right DLPFC plays regarding crossmodal facilitation observed in visual-tactile processing.

Disclosures: E. Salazar: None. **S. Mughal:** None. **S.K. Meehan:** None. **R. Staines:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.11/F27

Topic: D.08. Multisensory Integration

Support: KAKENHI (21H03304)
KAKENHI (21K19798)

Title: Modulation of Thermal Perception via Visual Stimulation Using Virtual Reality

Authors: *H. SUGATA[1], Y. TAKEO[2,3], M. HARA[4];
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Abstract: Our perception of the environment and our body involves integrating multiple sensory modalities, both external and internal, within our brains. Conversely, perception and recognition can be altered by modifying sensory inputs. For instance, the rubber hand illusion (RHI), a prominent phenomenon in body illusion research, induces a perceptual illusion by synchronously stroking a participant's visually obscured real hand and a visible fake hand (rubber hand). Recent studies have extended this concept to virtual reality (VR), demonstrating that synchronized tactile stimulation on a participant's back can induce a perceptual illusion akin to RHI. Perception within a virtual hand, generated by body illusions, can be influenced by additional visual stimulation. For example, virtual hands synchronized with real hand movements have shown reduced thermal sensation compared to desynchronized virtual hands. Furthermore, studies have explored modulating sensation by applying heat pain alongside visual stimulation in various color tones to an embodied virtual hand. However, the neurophysiological mechanisms underlying these effects remain unclear. Given that sensory perception within an embodied virtual hand can be modulated by visual stimulation, we hypothesized that such modulation involves changes in brain activity induced by VR-based visual stimuli. Therefore, this study investigated whether accessory visual stimuli can alter thermal perception via virtual reality, examining both behavioral and neurophysiological aspects. Fifteen healthy participants were exposed to three accessory visual conditions (fire, water, and neutral) alongside pain stimulation (thermal grill stimulation). Oscillatory neural activities during accessory visual stimulation were recorded using electroencephalography (EEG). The association between accessory visual stimulation and the illusory body perception induced by virtual reality, particularly in relation to thermal perception, was analyzed using EEG data. Lasso regression analysis was employed to identify brain regions contributing to the modulation of thermal perception via accessory stimuli.

Results indicated that visual stimulation applied to the virtual hand modulated thermal perception under fire and water conditions. Additionally, the insula was consistently implicated in thermal perception across both fire and water conditions. As a sensory gatekeeper, the insula likely plays a critical role in regulating sensory information, while also serving as a hub for attention and cognition, thereby influencing overall cognitive and sensory processes.

Disclosures: H. Sugata: None. **Y. Takeo:** None. **M. Hara:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.12/F28

Topic: D.08. Multisensory Integration

Support: ERC advanced grant: MakingSense 101096659

Title: Audiovisual salience guides search in complex dynamic environments

Authors: *X. WENG, U. NOPPENEY;
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Abstract: Imagine searching for a parrot that makes a specific call in a dense forest crowded with many birds. While leaves rustle and other birds chirp and move, only the parrot moves in synchrony with its song. How do observers detect and locate the parrot amidst the cacophony of foliage and birds? To address this question, the present study investigated audiovisual (AV) search in parametrically-controlled AV multisource scenarios that combined visual dynamic search displays with auditory stochastic figure-ground stimuli (SFG). The visual search displays included six or twelve line segments that dynamically changed their colour approximately every second. The auditory stimulus were brief foreground chords (duration: 100ms) presented in synchrony with the colour changes. Across trials, we presented the foreground chords alone or embedded in randomly varying background tones. Further, we manipulated the foreground chord's salience by changing the number of temporally coherent tones that it comprised (i.e. four vs. eight tones). In the synchrony task, observers (N=12) located the visual line segment changing in synchrony with the foreground chord. In the orientation task, participants located a specifically oriented line segment either in a purely visual or audiovisual search display. In the synchrony task, the response times (RT) were unaffected by the number of search items, decreased for more salient foreground chords and were tightly locked to the onsets of the target colour changes synchronized with the foreground chords. In the orientation task, RTs increased with the number of distractors and were not strictly locked to the onsets of colour changes, when foreground chords were absent or less salient. Only with highly salient foreground chords did the visual target pop out, and the RTs were no longer influenced by the number of line segments. The RT distribution then turned into a mixture of responses that were aligned and not aligned with the onsets of colour changes and foreground chords. Collectively, our results suggest that

observers locate dynamic visual targets synchronized with foreground chords primarily via parallel search mechanisms with efficiency depending on the chord's salience. When targets are specified by a visual feature requiring serial search, synchronized salient foreground chords can induce the visual target to pop out. In these cases, observers employ mixed search strategies that combine serial visual search with parallel audiovisual search mechanisms based on audiovisual synchrony cues. Future MEG research will explore the neural mechanisms underlying these audiovisual search processes.

Disclosures: X. Weng: None. **U. Noppeney:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.13/F29

Topic: D.08. Multisensory Integration

Support: Craig Neilsen SCIRTS Pilot Research Grants (No. 882102)
NRF Korea Excellent Young Research Award (No. 00209864)

Title: Cross-modal reference frame conversion with distance offset increases the accuracy of Bayesian multisensory integration

Authors: *J. LEE[1], H. PARK[2];
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Abstract: Multisensory integration has been explained with the Bayesian framework under the condition of causal inference. However, this Bayesian model of multisensory integration is under question when each sensory modality uses different frames of reference to represent the same physical property. For example, vision and proprioception use intrinsic and extrinsic frames of reference, respectively, and their integration necessarily includes translation process between the two reference frames. Prior works reported the mismatch between the different sensory information, which should be considered at multisensory integration. However, the optimal way of integration between the differently referenced information is still under question.
The objective of this study was to test the effect of cross-modal reference frame conversion with distance offset on the accuracy of Bayesian multisensory integration. Seven healthy young subjects participated in the experiment and expressed proprioceptively and/or visually received distance information in two different ways: verbal expression and proprioceptive expression. Finger aperture control machine was used to control finger aperture to proprioceptively deliver distance information to subjects and black line on the monitor was used to visually deliver distance information to subjects. Subjects were asked to report the distance either verbally based on mm-scale distance or proprioceptively by controlling the finger aperture of the other hand. Experimental data showed that cross-modal reference frame conversion with distance offset increases the accuracy of Bayesian multisensory integration, both in the case of verbal

expression and proprioceptive expression. In case of verbal expression, distance offset needs to be applied to the proprioceptively received distance information to maximize the accuracy of Bayesian multisensory integration. On the other hand, in case of proprioceptive expression, distance offset needs to be applied to the visually received distance information to maximize the accuracy of Bayesian multisensory integration. Interestingly, distance offset was applied to the sensory modality in needs of reference frame conversion, considering verbal expression uses extrinsic reference frame and proprioceptive expression uses intrinsic reference frame. This result suggests that cross-modal reference frame conversion with distance offset, if applied properly to the sensory modality in needs, increases the accuracy of Bayesian multisensory integration.

Disclosures: J. Lee: None. **H. Park:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.14/F30

Topic: D.08. Multisensory Integration

Support: Craig Neilsen SCIRTS Pilot Research Grants (No. 882102)
NRF Korea Excellent Young Research Award (No. 00209864)

Title: Motion cue increases or decreases the level of simulator sickness by virtual-reality experience

Authors: *M. OH[1], B. YOO[2], H. PARK[3];
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Abstract: Simulator sickness is a subset of motion sickness, described as a complicated feeling of discomfort caused by emulated sensory stimuli during virtual experience. As the emulated condition often leads to the mismatch between the anticipated sensory feedback based on the encountered situation and accumulated experience and the present sensory feedback based on sensory cue, simulator sickness is usually explained by the sensory conflict theory like the case of motion sickness. However, it is hard to explain the effect of simulator fidelity on simulator sickness, because it affects both the anticipated sensory feedback and the present sensory feedback. Prior studies also reported that increased simulator fidelity may or may not increase the level of simulator sickness.
The objective of this study was to investigate the effect of motion cue (*i.e.*, motion stimuli corresponding to the anticipated sensory feedback), increasing the simulator fidelity, on the level of simulator sickness. Six healthy young subjects participated in the study with the 129-second session that provided simulated roller coaster experience. During the session, subjects wore the

virtual reality (VR) headset and rode the 3-axis motion platform, which provide the visual cue and the motion cue of the roller coaster experience, respectively. The session was repeated for four times, for each subject to experience four kinds of feedback scenarios (visual cue on/off and motion cue on/off). Experiment was conducted as crossover study design and all subjects experienced four feedback scenarios at random order with sufficient interval in between to minimize the aftereffect. Simulator sickness questionnaire (SSQ), realism questionnaire (RQ), and functional near-infrared spectroscopy (fNIRS) data were collected to evaluate the simulation sickness and investigate its mechanism.
Based on the SSQ data, 50% of the subjects reported increased level of simulator sickness when both motion and visual cue were provided compared to the case when only visual cue was provided, while the other 50% of the subjects reported the opposite result (*i.e.,* decreased level of simulator sickness). This result suggests that the motion cue can either increase or decrease the level of sensory conflict between anticipated and present sensory information. The mechanism of simulator fidelity affecting simulator sickness needs additional explanation on top of the sensory conflict theory, and RQ and fNIRS data would play an important role in finding the difference between the subject groups showing opposite results.

Disclosures: M. oh: None. **B. Yoo:** None. **H. Park:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.15/F31

Topic: D.08. Multisensory Integration

Support: Craig Neilsen SCIRTS Pilot Research Grants (No. 882102)
NRF Korea Excellent Young Research Award (No. 00209864)

Title: Location-based electrotactile feedback enhances training effectiveness of visual feedback on increasing hitting accuracy of the golf club head

Authors: *K. YOUNGDEOK[1], C. PARK[2], H. PARK[2];
[1]Intelligent Precision Healthcare Convergence, Sungkyunkwan Univ., Suwon-si, Korea, Republic of; [2]Biomed. Engin., Sungkyunkwan Univ., Suwon-si, Korea, Republic of

Abstract: Accuracy of the tool end-effector control is an essential requirement for precision tasks, such as medical surgery and precision sports, while control accuracy is fundamentally limited as the tool end-effector is a certain distance away from the body. Visual assistance has been typically employed to improve the tool end-effector control accuracy, such as the cases of image-guided surgery and online golf instructors. However, visual feedback accompanies with intrinsic limitations for the engagement of the vision on the motor task itself, and further, it heavily occupies neural resources to interpret the vast amount of information.
The objective of this study was to test the hypothesis that location-based electrotactile feedback

enhances the training effectiveness of visual feedback on increasing the hitting accuracy of the golf club head at approach swing (short-distance swing). This is because electrotactile feedback is not intrinsically engaged in the real-time motor task and less cognitive than visual feedback. 15 healthy young human subjects participated in the experiment of virtual-reality (VR) golf game, where each subject went through baseline test session, two consecutive training sessions, 5-min post-training session, and 24-hr post-training session. Each session was composed of 50× approach swings at VR environment, with a target location of the golf club head given by visual feedback at the beginning of each session. 15 subjects were divided into three groups according to the type of feedback about the hitting location, provided during the training sessions: no feedback, visual feedback, and visual + electrotactile feedback. Hitting accuracy was calculated during each session, and proprioceptive drift was measured for three times before baseline session, after 5-min post-training session, and after 24-hr post-training session, respectively. Experimental results showed that "visual + electrotactile" training enhanced hitting accuracy by 53.6±0.1% (AVR±STE), which is 32% higher than the enhancement by "visual" training. This result suggests that location-based electrotactile feedback is effective on increasing the training effectiveness of the golf club head hitting accuracy. We also observed -8.2±3.9% proprioceptive drift after training sessions for "visual + electrotactile" training group, while there was no proprioceptive drift for control group and "visual" training group (2.5±6.7% and -1.9±5.3% respectively). Here, negative proprioceptive drift implies the extension of body schema toward the golf club head. This result suggests that body schema extension is advantageous to the hitting accuracy improvement.

Disclosures: K. Youngdeok: None. **C. park:** None. **H. Park:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.16/F32

Topic: D.08. Multisensory Integration

Title: Visual Speech Differently Restores Temporal and Spectral Speech Information in the Auditory Cortex

Authors: *C. CAO, K. GANESAN, A. JAHN, D. BRANG;
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Seeing a speaker's face facilitates accurate speech perception. Previously, research has shown that listeners use lipreading to restore degraded auditory speech information, but these influences fail to account for the full benefits from visual speech. Prior work has hypothesized that visual speech can restore information from two additional features of speech: spectral and temporal information. For example, listeners can recover spectral information using speakers' mouth width and the speaker's lip closure helps listeners parse the temporal boundary between words. However, it remains poorly understood whether, and how, spectral and temporal

information is restored in the auditory cortex. In the current study, we asked two questions: first, is visual speech integration regionally specific, where auditory temporal and spectral information is restored in separate areas, or regionally nonspecific, where the same region restores both kinds of information? Second, how does visual speech alter the spatial pattern of auditory system activity to improve audibility of speech? We hypothesized that visual speech restores the spatial pattern of auditory activity evoked by degraded auditory speech, making it more similar to the pattern of activity evoked by clear speech. We collected functional magnetic resonance imaging (fMRI) data from 64 subjects who listened to 200 sentences presented across five conditions--auditory-alone original, auditory-alone temporally smeared, auditory-alone spectrally smeared, audio-visual temporally degraded, and audio-visual spectrally degraded. Univariate contrasts reveal that the same visual signal has different effects on auditory processing depending on the degraded auditory feature. Visual speech increased BOLD in the STG for both types of degraded speech, but differed across conditions in other regions: AV spectral recovery increased BOLD in Heschl's gyrus whereas AV temporal recovery increased BOLD in anterior STG. Second, we used Representational Similarity Analysis to compare fMRI data of audiovisual conditions to the auditory unfiltered condition. Our preliminary results from RSA analysis suggest that visual restoration of speech uses distinctly different mechanisms from auditory speech perception. To verify this finding, we plan to build a single-trial-based GLM regressor to examine if representational distance is closer between audiovisual filtered speech and original auditory speech than that of audio-alone filtered speech. Together, we show that auditory cortex uses visual speech signals to selectively recover features of the auditory signal that have been degraded.

Disclosures: C. Cao: None. **K. Ganesan:** None. **A. Jahn:** None. **D. Brang:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.17/F33

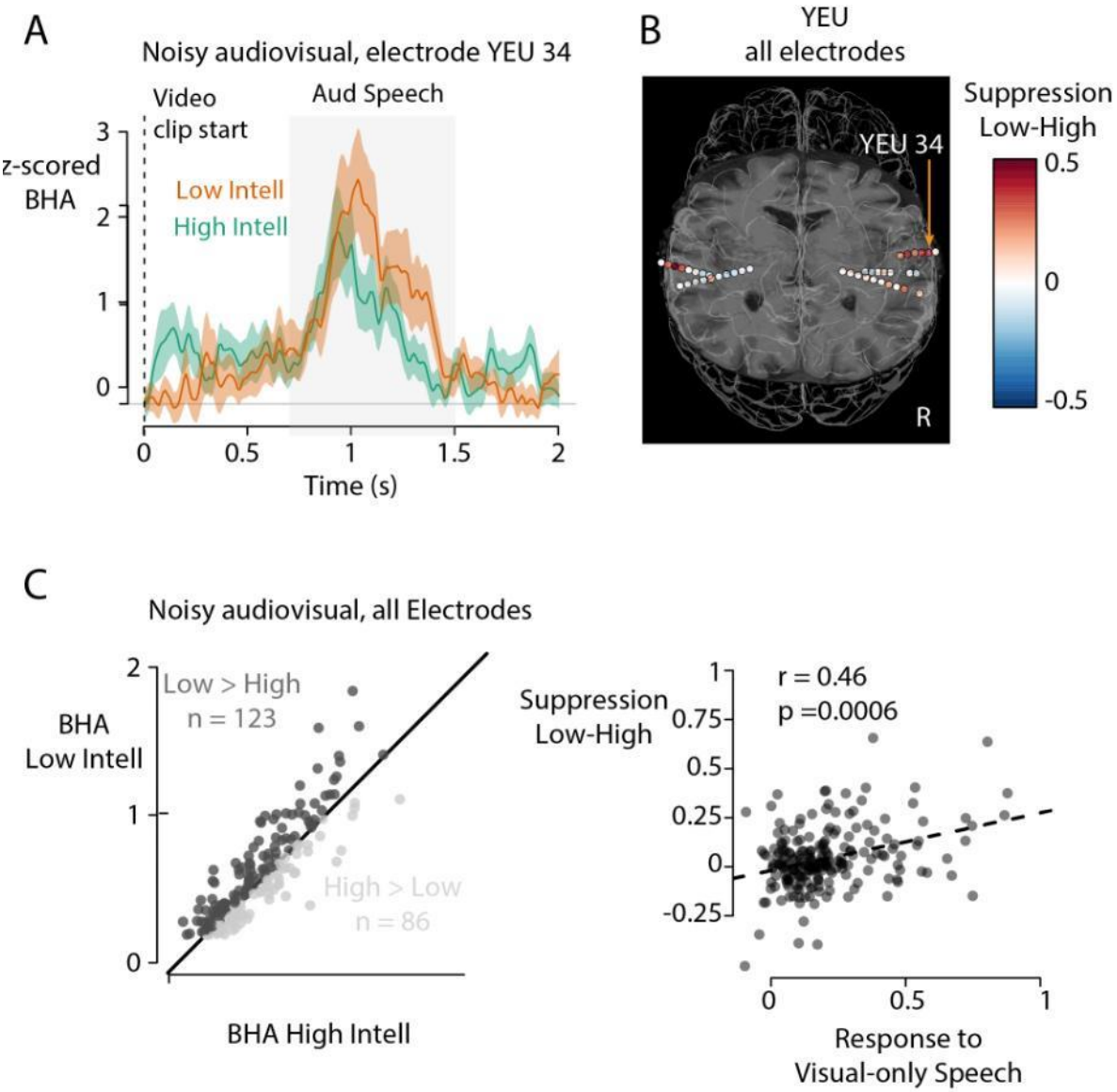
Topic: D.08. Multisensory Integration

Support: U01NS113339
R01NS065395

Title: Ieeg correlates of noisy audiovisual speech perception in superior temporal cortex

Authors: *Y. ZHANG[1], J. F. MAGNOTTI[2], X. ZHANG[3], Y. YU[2], D. YOSHOR[4], S. A. SHETH[5], H. CHEN[3], K. DAVIS[3], Z. WANG[3], M. BEAUCHAMP[3];
[1]Baylor Col. of Med., Houston, TX; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [5]Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: A key function of human superior temporal cortex is to decode the rapid stream of language elements that constitute speech. Speech perception is especially difficult under noisy listening conditions, but seeing the face of the talker improves intelligibility. To study the neural mechanisms of audiovisual speech perception, we measured z-scored broadband high-frequency activity (BHA, 70 - 150 Hz) in epilepsy patients implanted with stereotactic EEG electrodes. Each patient was presented with 50 different words with pink noise added at a signal-to-noise of -4 dB. Words were presented in noisy auditory-only (An), noisy audiovisual (AnV) and visual-only (V) formats. The ***RAVE*** iEEG software toolbox was used to analyze all data (see poster from Wang *et al.*). Participants repeated each word after presentation. An accuracy score was calculated based on the phoneme correspondence between the stimulus and response words. Accuracy was entered into a linear mixed effects model (LME) as the dependent measure; stimulus format (An, AnV) were fixed factors; word and participant were random factors. As expected, accuracy was significantly higher for AnV than An words, 86% *vs.* 71%, $p = 10^{-16}$. Across 17 participants, 209 electrodes were identified in the posterior superior temporal gyrus and sulcus (pSTG/S) that showed a significant response to speech. The BHA response to each word (800 ms before clip onset vs. after auditory onset) was calculated and entered into an LME with fixed effects of accuracy and format and random effect of electrode. There was a significant interaction between accuracy and format ($p = 10^{-4}$) caused by a negative correlation between perceptual accuracy and neural response for AnV words ($r = -0.04$) but a positive correlation for An words ($r = 0.01$). Across electrodes, the BHA response to V-only words moderated the correlation for AnV ($p = 10^{-4}$), but not An words ($p = 0.09$). These results are consistent with a model in which visual speech suppresses responses to auditory phonemes that are incompatible with the viewed speech (e.g. a closed mouth shape is incompatible with /da/, Karas 2019).



Disclosures: Y. Zhang: None. **J.F. Magnotti:** None. **X. Zhang:** None. **Y. Yu:** None. **D. Yoshor:** None. **S.A. Sheth:** None. **H. Chen:** None. **K. Davis:** None. **Z. Wang:** None. **M. Beauchamp:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.18/F34

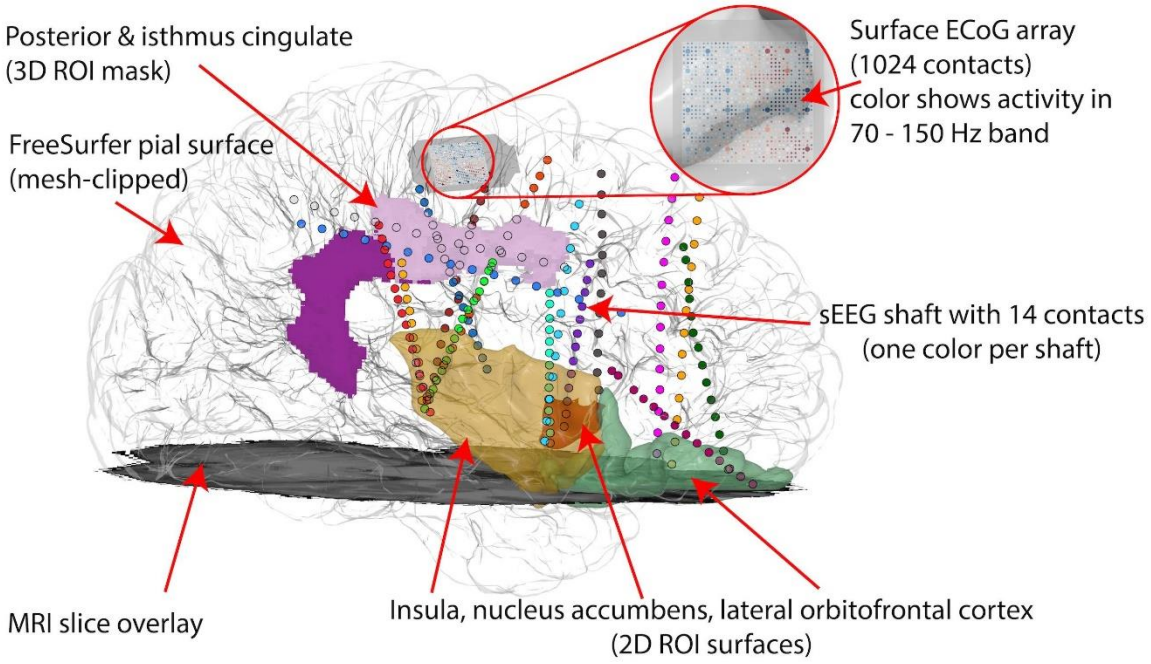
Topic: D.08. Multisensory Integration

Support: NIH Grant 5U01NS113339-05

Title: New tools for iEEG analysis and visualization in RAVE and YAEL

Authors: *Z. WANG, J. F. MAGNOTTI, X. ZHANG, M. S. BEAUCHAMP;
Dept. of Neurosurg., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Intracranial electroencephalography provides a unique opportunity to record from small populations of neurons in the human brain using implanted electrodes. RAVE (Reproducible Analysis and Visualization of iEEG) is a comprehensive toolbox that includes YAEL (Your Advanced Electrode Localizer). YAEL incorporates a powerful viewer for simultaneous viewing of 3D cortical surface models and 2D MRI slices together with all electrodes. RAVE and YAEL are open source and freely available from https://rave.wiki and https://yael.wiki .
Recently added features of RAVE and YAEL include:
1. Standalone viewer for easy sharing: Patient imaging data, electrode locations and the results of iEEG analyses can be packaged into an HTML file that can be distributed and opened by anyone with a web browser, without installing RAVE. This greatly simplifies collaboration and data sharing.
2. Automatic localization of sEEG electrodes: Manually localizing sEEG contacts from a CT scan is time-consuming and error prone. YAEL streamlines this process with automated electrode localization. RAVE can create surface models of ROI (such as thalamic or other subcortical nuclei) and display alongside sEEG shafts to allow for precise delineation of the location of each contact.
3. Support for next-generation electrodes: New electrode technologies are rapidly emerging, such as the thin-film micro-array with 4096 contacts. YAEL allows users to define and localize complex electrode arrays and display them on the cortical surface model.
4. Support for user-generated 2D and 3D datasets and ROIs: Any 2D or 3D dataset can be displayed by dragging and dropping the file onto the viewer such as activity or connectivity at individual surface nodes or brain voxels.
5. Electrode line of sight visualization: In addition to the canonical 3-plane MRI view, users can reslice the MRI along the electrode insertion trajectory, providing a new perspective on subcortical targets. The 3D surface model can also be sliced along any plane to combine the benefits of surface model and MRI slice visualizations.



Disclosures: Z. Wang: None. **J.F. Magnotti:** None. **X. Zhang:** None. **M.S. Beauchamp:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.19/F35

Topic: D.08. Multisensory Integration

Support: Nancy Lurie Marks Family Foundation

Title: Clinically Interpretable Biometrics to Assess Internal States of Distress in Autism and the General Population

Authors: *M. ELSAYED[1], E. B. TORRES[2];
[1]Rutgers Univ., Piscataway, NJ; [2]Psychology Dept, Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: Studies of human behavior often do not consider underlying physiological processes which reveal pertinent information about internal cognitive and emotional states. By exploring the biophysiological signatures of the individual through analysis of various layers of the nervous systems and leveraging the digital revolution, we can begin to develop an understanding of how internal states influence affective responses and physical actions. This is especially important for characterizing stress, pain, and anxiety in autism and in nonspeaking individuals who may experience trouble conveying such internal states. Cardiac rhythms alone open a

window into understanding such states, providing meaningful insight regarding an individual's self-control, emotional regulation, and ability to adapt to novel circumstances. Autonomic states can also influence sensory sensitivity levels, daily social interactions, and overall well-being. The balance between sympathetic and parasympathetic/vagal activity within the autonomic system can ultimately guide an individual's decisions and actions. An imbalance between these systems can lead to cascading effects on the mind and body, influencing behavioral adaptation, social engagement, and overall cognition. Current assessments of pain and distress rely on self-reports (which assume a capacity to understand and verbalize mental/emotional states) and behavioral observation which can be subject to limitation and misinterpretation. This in turn influences quality of care and delays appropriate diagnosis and treatment. Thus, there is an urgent need to develop objective methods to characterize and understand pain and heightened distress/arousal in those who have trouble communicating such experiences and internal states to others. We here offer new personalized analyses of biophysical data revealing changes in autonomic reactivities/imbalances and peripheral responses that are clinically relevant to autism and other neurological disorders. In 195 individuals (69 with neurological diagnoses, 131 controls) we uncover a single stochastic parameter amenable to define individualized pain/anxiety thresholds potentially explaining differential pain sensitivities across the population. Our integrative, multi-sensory, multi-layered approach offers new avenues to ultimately aid in the development of personalized assessments and the tailoring of treatments.

Disclosures: M. Elsayed: None. **E.B. Torres:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.20/F36

Topic: D.08. Multisensory Integration

Support: The Nancy Lurie Marks Family Foundation Research Career Award

Title: Towards a Model of Digital Wellbeing

Authors: *E. TORRES[1], T. BERMPERIDIS[2], M. ELSAYED[3];
[1]Psychology, CS, Rutgers Ctr. for Cognitive Sci., Rutgers the State Univ. of New Jersey, New Brunswick, NJ; [2]Psychology, Rutgers Univ., New Brunswick, NJ; [3]Rutgers Univ., Piscataway, NJ

Abstract: The wearable sensors revolution and the current revolution in machine learning and artificial intelligence have enabled scalability of our science while encouraging new participatory, community drive research whereby the participants are not mere subjects but rather integral active contributors and collaborators of our research programs. While data acquisition techniques involving the tracking of nervous systems biorhythms have improved significantly, new briefer, simpler assays are needed along with analytics leading to parameter identification

that enable personalized assessments while providing standardized population scales. Furthermore, such digital data ought to be informed by clinical criteria if adoption of our new digital tools is to be realized at clinical, school and home environments where naturalistic activities of daily life occur. I here introduce a research program from my lab that explores three axes of data acquisition and analyses aimed at the development of a new highly scalable model of digital wellbeing of our community. The first axis is along the neurodevelopmental pathway, spanning from neonatal stages to school age, where we introduce new screening methods with the potential to stratify disorders of the nervous systems on a spectrum. The second axis pertains to the assessment of dysregulation and stress, inclusive of pain, amenable to assess individualized sensitivity thresholds and aid regulation during interventions. The third includes the digitization of common neurological cognitive assessments using the motor code, useful to assess the intersection of cognitive, memory and motor control performance, inclusive of patients with early signs of dementia (e.g., Parkinson's disease, Alzheimer's disease.) I present data from my lab on all three axes and invite feedback on implementation of our program at scale.

Disclosures: E. Torres: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Books, patents, Elsevier, Rutgers Innovation Venture, Neuroinversa. **T. Bermperidis:** None. **M. Elsayed:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.21/F37

Topic: D.08. Multisensory Integration

Support: Nancy Lurie Marks Foundation Research Career Award

Title: Invariant Geodesic Trajectory Properties Derived from Standardized Micro-Movements Spikes of Facial Landmarks Differentiate Autistics from Neurotypicals and Offer a Tool to Assess Treatments' Effectiveness

Authors: *T. BERMPERIDIS[1], E. B. TORRES[2];
[1]Rutgers Univ., New Brunswick, NJ; [2]Psychology Dept, Rutgers Univ. Dept. of Psychology, Piscataway, NJ

Abstract: A myriad of micro-motions produced by motor units' efferent activities across the face and mediated by the afferent inputs from the trigeminal ganglia underlie social-emotional behaviors. In autism spectrum disorders such differences are poorly understood. New non-invasive video-based estimation of facial landmarks, 3D gazes, head orientation and motor units (e.g., Open Face) enable sampling at scale to assess such motor differences in the socio-motor axes of the face. Several challenges however remain, beyond the data acquisition step. Among them are the need for standardized data types enabling comparisons across different age groups, analytics that afford brief assays and offer interpretability about the maturation of the motor

code, to build maps of expected variational ranges as the human nervous systems mature, while also doing so in a personalized manner. Here we offer a versatile data type, the micro-movement spikes (MMS), providing both a real-number version of micro-fluctuations away from the person's mean activity, and a binary spiking version amenable to apply new computational neuroscience techniques, and pair these with new analytics that track the stochastic trajectories, using both the individual's and the population's biorhythmic signals. We test our analytics in different naturalistic contexts (e.g., a school of children with special needs, a conference of non-speaking autistics, therapies at different studios), using a standardized app for brief data acquisition. Three different facial poses (each 5 seconds long) included motions during resting (baseline), instructed smiling and surprise. We transform facial landmark speed profiles to binary MMS and obtain geodesic trajectories in the statistical manifold of positive definite covariance matrices to capture the synchronous dynamics of multiple facial landmarks across neuroanatomical regions corresponding to the trigeminal nerves. Proper representation of these geodesic paths helps us discover self-emerging clusters in the autistic and neurotypical populations. In cases where a therapy was administered, we revealed fundamental pre-post differences that encourages using our new methods as an automated tool to both screen for individual differences at baseline and do frequent tracking of therapy effectiveness.

Disclosures: T. Bermperidis: None. **E.B. Torres:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.22/G1

Topic: D.08. Multisensory Integration

Title: Impact of Variations in Autonomic Activity on the Dorsolateral Prefrontal Cortex - Variations Induced by Microcone Patches and External Environmental Changes-

Authors: *A. KAWASAKI;
Ritsumeikan Univ., Kyoto-Shi, Japan

Abstract: Introduction: Previous studies, including Kawasaki et al. (2024), demonstrated that the microcone patch approach to the auricular branch of the vagus nerve could induce changes in autonomic activity during oral reading and subsequent alterations in blood oxygen saturation in the dorsolateral prefrontal cortex. However, our understanding of how autonomic nervous activity influences the prefrontal cortex during cognitive tasks remains limited. This study aims to further explore this phenomenon through several experiments. **Methods:** In Study 1, twelve typically developing adults with no history of orbital or psychiatric disorders participated. The experiments were conducted under two conditions involving backgrounds of different color tones, controlled for brightness, using a 16-channel NIRS to measure blood oxygen saturation in the bilateral dorsolateral prefrontal cortex during a brief comprehension task. Within-subjects factorial two-factor analysis of variance was performed, analyzing the variation in oxygenated

hemoglobin per channel as the dependent variable. Pupil diameter was monitored as an index of autonomic nervous system activity, alongside the number of correct responses to a reading comprehension task as a behavioral measure. Study 2 included a reanalysis of last year's report and a case study to examine the impact of microcone patches on the results from Study 1. **Results:** The interaction was significant ($p<.05$) with a moderate effect size (partial $\eta^2=.160$) concerning the rate of correct responses under specific experimental conditions (background color and font). Additionally, pupil diameter, a measure of autonomic activity, varied significantly ($p<.05$) with different background colors. Variations in oxygenated hemoglobin in channels reflecting the vicinity of BA10 (ch3: $p=.074$, partial $\eta^2 = .096$) showed a notable trend associated with autonomic activity. **Discussion:** Autonomic activity significantly varied across experimental conditions, correlating with decreases in dorsolateral prefrontal blood oxygen saturation when pupil diameter was enlarged and sympathetic tone was expected to increase. This response might reflect a concentration strategy that allows task completion with less cognitive resource consumption. In Study 2, we hypothesize that the direct effects of microcone patches on autonomic activity may negate these differences, and further case-by-case testing is underway.

Disclosures: A. Kawasaki: None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.23/G2

Topic: D.08. Multisensory Integration

Support: ERC advanced grant: MakingSense 101096659

Title: The role of within and cross sensory binding cues in audiovisual scene analysis

Authors: *Q. WU[1], U. NOPPENEY[2];
[1]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands;
[2]Donders Inst. for Brain, Cognition and Behaviour, Nijmegen, Netherlands

Abstract: To transform the myriad sensory signals into a coherent percept the brain has to integrate signals from common causes and segregate those from separate causes. In complex audiovisual scenes (e.g., a busy restaurant) the brain solves this causal inference problem based on between/within correspondence cues such as temporal coherence or spatial colocation and within-correspondence cues such as similarity in pitch. This study investigated how human observers (N=24) perform causal inference in complex scenes consisting of two visual and two auditory signals by combining within and between-sensory correspondence cues. Observers were exposed to sequences of alternating high- and low-pitched pure tones emanating from the center, either in a purely auditory context or in synchrony with sequences of alternating flashes in the left and right hemifield. Across trials the flashes were presented at three levels of symmetric

eccentricities. The visual eccentricity thus manipulated the visual-visual spatial disparity between two alternating flash streams and the audiovisual (AV) spatial disparity between the central pure tones and the two flash streams. In a subjective explicit causal inference task, participants continuously reported whether they perceived the high- and low-pitched tones as originating from a single or two causes (i.e., auditory stream segregation). In an objective deviant detection task, they detected a loudness-deviant tone inserted randomly within the high-pitched stream. Our results confirm previous findings that larger frequency (pitch) separation enhances auditory stream segregation and thereby facilitates deviant detection. Crucially, auditory stream segregation also depended non-linearly on the visual eccentricity. At minimal eccentricities, left and right flashes were perceived as a single apparent motion visual stimulus and therefore promoted the integration of high- and low-pitched tones into a single stream via AV binding. At moderate eccentricities, flashes were perceived as two distinct visual streams and therefore enhanced auditory stream segregation via AV binding. At high eccentricities, auditory stream segregation was comparable to that observed in a purely auditory context, because the pronounced AV spatial disparities hindered AV binding. Our results show that the brain combines several within- and cross-sensory correspondence cues to attribute sensory signals to their underlying causes. Future research using MEG/EEG will determine the neural mechanisms underpinning audiovisual scene analysis through the complex interplay of multiple binding cues.

Disclosures: Q. Wu: None. **U. Noppeney:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.24/G3

Topic: D.08. Multisensory Integration

Title: Electrical stimulation along the superior temporal gyrus evokes rapid responses in human visual cortex

Authors: *E. CUNNINGHAM, D. BRANG;
Psychology, Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: In natural settings, sounds facilitate visual perception. There is consistent evidence, for example, that concurrent auditory stimuli can reduce visual detection/discrimination thresholds, modulate perceived intensity, and speed responses to visual stimuli. Notably, even in the absence of a concurrent visual target, transient sounds can evoke rapid responses in visual cortex. However, the pathways by which these responses are transmitted remain a matter of debate. In particular, the extent to which human sound-evoked visual responses have subcortical vs. auditory cortical origins is unclear. Although both animal and psychophysical work are consistent with a cortico-cortical route involving projections from early auditory to visual areas, direct causal evidence for/against cortical involvement is limited. One way to obtain such evidence is to take advantage of intracranial EEG recordings from patients receiving direct

electrical stimulation over the superior temporal gyrus (STG). Here we examined existing data from 23 such patients who also had electrode coverage over occipital areas (237 sites across all subjects). Of these patients, 20/23 showed occipital responses to STG stimulation, with responses (significant deviation from pre-stimulation baseline) in ~58% of occipital electrode sites (138/237) after correction for multiple comparisons across time, electrode/subject, and stimulation site. In a subset of individuals, we were able to examine sites located over regions previously shown to exhibit preferential responses to sound (primary visual cortex, N = 3, and probable V5/hMT+, N = 20). Two individuals showed early evoked peri-calcarine responses following STG stimulation, with initial deflections peaking between 20-30ms, and more sustained responses emerging between 100-150ms. As with sound-evoked responses, these responses were more likely to emerge near the anterior portion of the calcarine fissure. STG stimulation also evoked responses near V5/hMT+ in a majority of individuals (with earliest deflections generally emerging between 100-200ms). In both regions, 'crossmodal' stimulation-evoked responses were associated with changes in low- but not high-frequency (gamma-band) activity, consistent with feedback signal propagation. These data provide initial evidence that at least some portion of previously-reported sound-evoked visual responses may originate from auditory cortex.

Disclosures: E. Cunningham: None. **D. Brang:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.25/G4

Topic: D.08. Multisensory Integration

Title: Individual differences in musical experience and autistic traits are associated with multisensory temporal entrainment

Authors: *L. KWAKYE;
Oberlin Col., Oberlin, OH

Abstract: The brain encodes relevant temporal features from rhythmic naturalistic stimuli via neural entrainment in each sensory modality. An individual's ability to precisely code temporal features in each modality and combine this information across modalities to form a coherent neural representation of the outside world is highly variable and influenced by both prior experience and intrinsic differences in neural processing. For example, musical experience has been shown to enhance sensory entrainment and multisensory temporal processing, but autism spectrum disorder is associated with disruptions in these processes. This study seeks to determine whether musical experience and/or autistic traits can explain individual differences in entrainment within modalities (vision and audition) and integration of temporal information across modalities. Participants determined whether ongoing, dynamic audiovisual stimuli were synchronous while electroencephalography (EEG) data was recorded. Audiovisual stimuli were

frequency-tagged to produce steady-state visual and auditory potentials. Participants were classified into high and low musical experience and autistic traits (measured via the autism quotient) groups. The findings of this study suggest no differences in asynchrony detection across musical experience and autistic trait groups. However, EEG findings indicate that experienced musicians demonstrated stronger unisensory encoding than inexperienced musicians. Additionally, low autistic traits were associated with stronger multisensory encoding and increased superadditivity, especially at higher frequencies. Future research in this area will investigate interactions between musical experience and autistic traits to determine whether music training may be used as a therapeutic tool in addressing some of the impairments to temporal encoding in autism.

Disclosures: L. Kwakye: None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.26/G5

Topic: D.08. Multisensory Integration

Support: KU Leuven C14/21/111

Title: Dissociable representations of action category from dynamic shape and motion in the primate action observation network

Authors: *Q. JIN[1], D. CUI[1], K. NELISSEN[2], M. TUNCA[3], A. EROGLU[3];
[1]KU Leuven, Leuven, Belgium; [2]Dept. of Neurosciences, KU Leuven, Leuven, Belgium;
[3]Bilkent Univ., Ankara, Turkey

Abstract: The action observation network (AON), comprising occipito-temporal, parietal, and premotor cortices, is well-documented as critical for processing observed actions in human and non-human primates. However, the specific representational nature of actions within this network and their consistency across human and non-human primate species remain under investigation. Using a comparative fMRI action observation task in three macaque monkeys and 27 human subjects, we addressed whether dynamic shape and motion alone sufficiently define the AON's action representations, or if categorical action information plays a distinct role. Whole-brain responses to 144 action video stimuli were analyzed using searchlight-based representational similarity analysis (RSA) and partial correlation. We incorporated four feature-based models (pixel intensity, dynamic shape, dynamic motion, action type) to quantify and compare brain activation patterns. Our results revealed a cross-species consistent representation of action category within the AON, dissociable from those of dynamic shape and motion. While dynamic shape is represented within the occipito-temporal cortex and dynamic motion representation extends to parietal and premotor cortices, distinct regions across the AON

represent action category. These representations are consistent in macaques and humans, suggesting an evolutionarily shared mechanism for perceiving actions.

Disclosures: Q. Jin: None. **D. Cui:** None. **K. Nelissen:** None. **M. Tunca:** None. **A. Eroglu:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.27/G6

Topic: D.08. Multisensory Integration

Support: FWO Postdoctoral Fellowship 12AHE24N

Title: Brain action representation and the dynamic stimulus features associated with behavior

Authors: *D. CUI[1], Q. JIN[1], K. NELISSEN[2];
[2]Dept. of Neurosciences, [1]KU Leuven, Leuven, Belgium

Abstract: Social creatures like primates rely on the ability to recognize others' actions for survival and potential social interactions. How does the brain represent various types of observed actions? Do these representations reflect behavioral discrimination of the various action types? What stimulus features of the observed actions drive the behavioral discrimination that reflects corresponding representations in the brain? To address these questions, we conducted fMRI recordings initially, while three male macaque monkeys observed videos depicting three types of manual actions (grasping, touching, and reaching). Multivariate pattern analysis (MVPA) revealed complete decoding of the three action types in the early visual areas, whereas the action observation network (AON), including the STS and parietal-frontal regions, predominantly classified grasping actions significantly. This consistent grasp representation in the AON aligned with behavioral discrimination in a subsequent action categorization task. Two monkey subjects successfully generalized grasping actions but failed to generalize most touching and reaching actions. Drawing on insights from action recognition in machine vision, we developed a protocol to assess the dynamic spatial-temporal features of action videos, considering the evolution of each action moment over time. Correlation analysis was applied to probe key features of these action videos from the behavioral tasks explaining performance during categorization. Lower-integrative temporal features of effectors (hand or hand-arm), such as optic flow and histogram of optical flow (HOF), exhibited a significant correlation with monkeys' performance. Both lower- and higher-integrative spatial features (histogram of image gradients [HOG] and Hu moments, respectively) of the effectors and the entire body strongly correlated with the behavioral discrimination of the action types. Our results indicate that dynamic spatial and temporal features contribute distinctly to behavioral action categorization, reflecting a consistent pattern of brain representation in the AON. These findings lay the groundwork for studying brain

representations of highly integrative and dynamic stimuli such as actions, using multi-level dynamic spatial-temporal features associated with behavior.

Disclosures: D. Cui: None. **Q. Jin:** None. **K. Nelissen:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.28/G7

Topic: D.08. Multisensory Integration

Support: ERC advanced grant: MakingSense 101096659

Title: Prediction and prediciton errors within and across sensory modalities

Authors: *D. MENG[1], C. DONG[2], U. NOPPENEY[1];
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Abstract: Statistical regularities enable the brain to interact effectively with its environments. Despite a wealth of research on statistical learning within individual senses, it remains unclear how the brain computes predictions and prediction errors when statistical regularities arise across the senses. Does the brain compute predictions independently within each modality, jointly across the senses or via both mechanisms. Independent computations may arise locally in early sensory cortices, while joint computations are likely to occur in multisensory convergence zones. We created sequences of audiovisual (AV) stimuli that factorially combined pure tones varying in pitch (low, middle, high) and Gabor patches varying in orientation (left tilted, horizontal, right tilted). The sequences followed a 1st-order Markov chain with the joint transition probabilities for successive AV stimuli with identical pitch and/or orientation set to zero and the probabilities for the other four transitions set to 0.85 or 0.05. The marginal probabilities of transitioning between A (or V) features irrespective of the other modality were 0.63 vs. 0.37. This Markov chain allowed us to manipulate the expectedness of the AV stimulus components factorially according to i) the joint probability of AV stimulus (AV transition probability: high and low) x ii) the marginal probability of the A feature (A transition probability: high and low) x iii) the marginal probability of the V feature (V transition probability: high and low). The fMRI study presented 24 observers with such AV sequences after ≈3 days of statistical learning. They were instructed to covertly predict the successor stimulus throughout the entire sequence. Their performance on the 15% overt prediction trials confirmed almost perfect learning of the statistical regularities. At the neural level, AV stimuli that were unexpected (i.e. joint AV transition probability of 0.05) enhanced activations in a widespread neural system encompassing frontal and temporoparietal areas, typically involved in error monitoring and cognitive control. Next, we investigated whether the brain independently encodes the marginal probabilities of the A and/or V stimulus components. We found that AV stimuli with an expected A component

increased activations in auditory areas suggesting that at least the brain also encodes the marginal statistical regularities in a particular sensory modality. Collectively, our results suggest that that brain learns and codes statistical regularities in a complex multisensory world both independently and jointly across sensory modalities.

Disclosures: D. Meng: None. **C. Dong:** None. **U. Noppeney:** None.

Poster

PSTR224: Cross-Modal Processing in Humans

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR224.29/G8

Topic: D.08. Multisensory Integration

Support: ERC advanced grant MakingSense, 101096659

Title: An expected visual location biases observers' perceived sound location

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Abstract: To interact effectively with the multisensory world the brain combines noisy sensory signals with prior knowledge weighted by their precisions into a coherent percept. While integrating information from multiple sources reduces observers' uncertainty about the current state of the world, it can introduce perceptual biases as illustrated by the ventriloquist illusion: When presented with spatially disparate audiovisual signals, observers perceive the sound as shifted towards the visual location and vice versa depending on their relative precisions. This study investigated whether prior expectations about the location of a visual stimulus biases an observer's perceived sound location. Participants (N=10, 8 female) were presented with a visual object (a ball) moving from one of 6 possible locations along the azimuth to the bottom of the screen with a constant direction (45 degree tilted to the left or right from vertical) and velocity. The ball then continued its trajectory behind a wall (250 ms). At the time when the object was expected to hit the ground, a sound was presented alone or in synchrony with the ball's reappearance at the expected location. For a particular trajectory, the sound was played from one of three possible locations along the azimuth: (i) the ball's expected location, (ii) the ball's final location before the occlusion or (iii) an unexpected location, equidistant from the final location before the occlusion, but in the opposite direction. On each trial, observers indicated their perceived sound location via a keypress. On trials when the ball reappeared after the occlusion, we observed a pronounced ventriloquist effect, i.e., a shift of observers' perceived sound locations towards the ball's location. When the ball did not reappear, the perceived sound location was shifted towards the ball's final location before the occlusion ($p<0.001$, $t=7.3268$) and most importantly also to the location ($p<0.05$, $t=2.284$) where it was expected to reappear after the occlusion. Our results demonstrate that not only a visual input but also an expected visual location can influence and bias where observers perceive sounds. From a Bayesian

perspective, they show that the brain forms prior expectations based on inputs in one sensory modality that at least to some extent affect perceptual inference of stimuli in another modality. Future research will assess the extent to which priors are modality-specific or generalize across sensory modalities. Moreover, analysis of concurrently acquired EEG data will delineate the neural mechanisms underlying the formation of spatial priors and their influences on perceptual inference within and across the senses.

Disclosures: C. Pleche: None. **U. Noppeney:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.01/G9

Topic: E.01. Eye Movements

Support: McPherson ERI: Kenzie Valentyn Grant

Title: Gaze allocation strategies of autistic and non-autistic young adults during simple and complex motor dual-tasks

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Abstract: ***Introduction:*** Previous literature has suggested that individuals of all ages and abilities experience significant motor performance decline when simultaneously performing two tasks, such as walking while carrying an object (Abbruzzese et al. 2014; Cherng et al. 2007). While several studies have measured motor declines during dual tasks, the role of visual attention on dual-task motor performance, particularly in autistic individuals, is not well understood. We conducted the current study to measure dual-task effects on gaze allocation while neurotypical and autistic individuals performed four different walking and bimanual carrying tasks, ranging from simple to complex. ***Methods:*** Sixteen non-autistic (9 male, M = 24.55 ± 1.2 years) and twelve autistic young adults (4 male, M = 23.16 ± 0.8 years) participated in this study. Participants walked across an instrumented walkway while performing ten trials of four motor tasks: A) overground walking, B) walking while carrying an empty tray, C) walking while carrying a tray with unstacked wooden blocks, and D) walking while carrying a tray with stacked wooden blocks. A Pupil Core eye-tracking headset was used to record the gaze activity of participants as they performed each task. The mean total gaze time (TGT), average single gaze duration (SGD), and number of gazes per trial at three locations of interest: 1) front wall, 2) floor, and 3) tray/blocks, were calculated for each task. Gaze locations were determined during post hoc video analysis. ***Results:*** For both mean total gaze time and average single gaze duration per trial, significant main effects of task complexity were found in both groups, with decreased gaze time and single gaze duration at the wall (both TGT and SGD: $p < 0.001$) and the floor

(TGT: $\chi^2 = 13.8$, $p < 0.001$; SGD: $p < 0.001$), and increased gaze time and single gaze duration at the tray/blocks (TGT: $p < 0.001$; SGD: $\chi^2 = 43.28$, $p < 0.001$), with increased task complexity. Moreover, significant main effects of complexity were found in both groups for the number of gazes, with fewer gazes at the wall ($p < 0.001$) and the floor ($\chi^2 = 9.515$, $p < 0.05$), and more gazes at the tray/blocks ($p < 0.01$) with increased complexity. However, no main effects of group or group x condition interactions were found. ***Conclusions:*** Increasing the complexity of a secondary task performed while walking results in fewer gazes and greater gaze time directed to the secondary task in young adults, with similar effects on both autistic and non-autistic groups. These findings provide a better understanding of how visual attention is allocated by both neurotypical and autistic young adults to successfully perform different dual motor tasks.

Disclosures: R.W. Nelson: None. **K.A. Pickett:** None. **B.G. Travers:** None. **A.H. Mason:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.02/G10

Topic: E.01. Eye Movements

Support: ZIAMH002909

Title: Pre-saccadic information interacts with post saccadic processing in V1

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Abstract: Saccadic eye-movements shift visual input in the primary visual cortex (V1). Using fMRI, we demonstrate pre-saccadic information is transferred to new positions in V1 to support post-saccadic processing. Participants (collected=18; analyzed=12, two to three sessions each) viewed a face or house stimulus 3° in the periphery of their left visual field, which is processed by right hemisphere V1. Participants were then cued to make a horizontal saccade across the stimulus, bringing the post-saccadic image into the right visual field, which is processed in left hemisphere V1. During the saccade, contingent on saccade onset, the stimulus either 1) remained the same, 2) changed, or 3) disappeared. Using a pattern classifier on voxels in the retinotopic position corresponding to the post-saccadic stimulus, we could decode images that remained the same or disappeared. However, when the stimulus changed during the saccade, decoding was reduced, yet still above chance. Successful decoding when the stimulus disappears during the saccade suggests stimulus information encoded prior to the saccade is transferred across hemispheres. This information transference led to facilitation of post-saccadic decoding when the

stimulus remained the same. Comparably, when the stimulus changed, decoding accuracy decreased, suggesting interference between transferred pre-saccadic information and the processing of post-saccadic input. Our results suggest that stimulus feature information is transferred to new retinotopic positions in V1 with each saccade. Importantly, this information is likely projected to V1 from extrastriate cortex since there are no direct anatomical connections between homotopically-aligned portions of right and left hemisphere V1.

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Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.03/G11

Topic: E.01. Eye Movements

Support: NIH Grant EY025172

Title: Urgency reveals dynamics of attentional deployment and evolving perceptual judgments in the frontal eye field

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Abstract: Primates rely on saccadic eye movements to gain information about the visual environment, with each saccade being an overt expression of the covert interplay between sensory, cognitive, and motor processes. The lateral intraparietal area (LIP) and frontal eye field (FEF) are well-known as key hubs within the network responsible for implementing the necessary visuomotor transformations, yet some aspects of their putative roles remain open to debate. In particular, the spatially selective presaccadic activity of neurons in these regions has been interpreted in different studies to represent the deployment of spatial or feature-based attention, saccadic motor planning, and/or the accumulation of perceptual evidence that guides the eventual saccade. Distinguishing among these alternatives is difficult when using serialized laboratory tasks for which we can expect all of these processes to point toward the same spatial goal. In a recent study, our lab addressed this issue by recording from LIP neurons while monkeys performed an urgent version of the random dot motion (RDM) perceptual decision task. Unlike the standard task, the “compelled” RDM (CRDM) task places the dual imperatives of allocating spatial attention parafoveally (to the dots) and eccentrically (to the saccade goal) in direct conflict. The urgent RDM task yields a psychometric function (tachometric curve) that varies from chance to a performance asymptote as a function of raw processing time (rPT), which is the time between dot motion onset and the saccade choice. As such, the tachometric

curve tracks the rPT-dependent transition in accuracy for saccade choices made on the basis of less (short rPTs) versus more (long rPTs) perceptual evidence. For LIP neurons, spatial selection of the goal was found to be inversely related to performance accuracy, a finding at odds with a signal of perceptual evidence but consistent with one of spatial attention. Here, we focus on the role of FEF, and report preliminary data based on single neurons (n=57) recorded from two monkeys performing the CRDM task. Initial findings indicate that spatial differentiation of the goal remains stable, or diminishes slightly, as rPT, evidence, and behavioral performance all increase. This suggests that, similarly to LIP, spatially selective activity of FEF better reflects the allocation of spatial attention than the accumulation of perceptual evidence.

Disclosures: E.A. Kattner: None. **E. Salinas:** None. **T.R. Stanford:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

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Program #/Poster #: PSTR225.04/G12

Topic: E.01. Eye Movements

Support: HAI/Wu Tsai Neurosciences Institute Grant

Title: A Bayesian framework for disentangling eye movement strategies during naturalistic search

Authors: *H. GU, J. L. GARDNER;
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Abstract: Complex visual tasks in natural contexts often necessitate visual search. A Bayesian ideal observer model of eye movements (Najemnik & Geisler, 2005) offers an optimal search policy under the constraint of peripheral visibility, serving as a framework useful for comparing different fixation selection strategies. By design, the model uses a target-identical matching scheme with a visibility function psychophysically measured using simple stimuli. However, generalizing this model to naturalistic stimuli poses challenges. In natural vision, (i) targets often vary in appearance, prohibiting identical target matching, and (ii) peripheral objects are often hard to identify when cluttered (crowding effect), which is not explicitly captured in the simple visibility function. We present a generalized model that leverages features of deep neural networks, approaching these challenges with (i) template matching in the space of latent features and (ii) eccentricity-dependent probabilistic pooling. This framework enables us to test different hypotheses of fixation selection with human eye movement data during a naturalistic visual search.

Disclosures: H. Gu: None. **J.L. Gardner:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.05/G13

Topic: E.01. Eye Movements

Support: NIH Grant EY030669

Title: Saccadic decision making based on auditory spatial cues in the monkey superior colliculus

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Abstract: When perceptual decisions about visual stimuli are reported with eye movements, signals related to decision formation are evident in the superior colliculus (SC). Whether such signals are evident in the SC when saccadic decisions are based on nonvisual sensory inputs, however, is unknown. The SC receives sensory inputs from multiple auditory areas, specifically, auditory spatial inputs thought to mediate reflexive orienting behaviors. It is possible, therefore, that the SC plays a role in the evaluation of auditory inputs during the formation of saccadic decisions based on auditory spatial cues. To investigate this possibility, we recorded individual SC neurons in a rhesus monkey that was making decisions about where to look based on auditory spatial cues. On each trial, the monkey fixated a visual stimulus located at the center of a visual display, and a sequence of brief, auditory-white-noise bursts (with noisy spatial locations) was presented along the frontal-horizontal plane. The monkey's task was to decide if the acoustic source that generated the sequence was located to the left or right of the frontal midline and to report that decision by making a saccade to one of two visual targets. The location of the correct target was spatially dissociated from the acoustic source (within a hemifield), thus requiring a flexible transformation of the auditory cues into an overt action plan (i.e., the monkey did not make a saccade directly to the perceived location of the acoustic source but rather interpreted that location to select the appropriate saccade plan). The monkey's performance on this task varied systematically with evidence strength: sensitivity (*d'*) increased as the location of the acoustic source moved away from the frontal midline, providing more robust auditory spatial cues for the selection of a saccade plan. A subset of SC neurons exhibited activity during task performance that suggested a potential involvement of those neurons in saccadic-decision formation, meaning modulations of their activity patterns by auditory-evidence strength and/or nonsensory factors related to task performance. This suggests that the SC may play a role in the evaluation of auditory cues when such cues are required for saccade planning. It also suggests a more general role for the SC, and perhaps other oculomotor structures, in the formation of saccadic decisions based on nonvisual sensory inputs.

Disclosures: C. Conroy: None. **Y.E. Cohen:** None. **R.M. McPeek:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

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Program #/Poster #: PSTR225.06/G14

Topic: E.01. Eye Movements

Support: NIH Grant EY027373

Title: Structured sensory inputs drive frontal cortical computation of flexible motor policies

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Abstract: Frontal cortical neurons enact sensory-motor policies that are matched to behavioral context based on inference from sensory inputs. How sensory inputs to the frontal cortex are weighted to support this flexibility, however, remains unclear. Under one extreme hypothesis, the weighting of the connections of a given sensory neuron to different target neurons are broadly distributed, resulting in multiple input streams to the downstream population. At the other extreme, the weights are identical, resulting in a single input stream. To determine the degree to which frontal cortical computations rely on (un)structured inputs, we leveraged our understanding of the neural circuit for smooth pursuit eye movements. In this circuit, neurons in the smooth pursuit region of the frontal eye fields (FEFsem) adjust the strength, or gain, of visual-motor transmission for pursuit. They achieve this, in part, by integrating information about target speed and motion reliability from neurons in area MT. Previous experiments controlled motion reliability by manipulating the coherence of motion within dot patches used for pursuit and established that realistic pursuit behavior could be computed with a gain system that weights MT inputs according to the logarithm of each neuron's preferred speed. This predicts that the input to FEFsem neurons from MT will be highly structured, resulting in a uniform population response in FEFsem. To test these predictions, we had monkeys pursue patches of dots that moved at different speeds and different levels of coherence while recording from FEFsem neurons. The gain measured from behavior strongly correlated with a model that assumes structured inputs from MT. However, recordings from FEFsem neurons revealed a diversity of gain-related responses not predicted by our model of MT inputs. We therefore relaxed our assumptions about the structure of MT inputs to FEFsem and allowed for a diversity of input weights. By systematically varying the degree of structure imposed on the connectivity, we found that two MT input streams were sufficient to predict FEFsem responses - one stream with weights set according to each MT unit's preferred speed and a second stream that weighted each MT unit equally. These results suggest that the computations in the frontal cortex that enable flexible sensory-motor policies rely on structured inputs that can be derived from the response properties of neurons in the sensory population.

Disclosures: S.W. Egger: None. **S.G. Lisberger:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

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Topic: E.01. Eye Movements

Support: NIH Grant 1R01MH113701-05

Title: Neural correlates underlying erroneous perceptual decisions in individuals with psychosis

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Abstract: Moving the eyes to sample the environment disrupts the flow of incoming visual information and subsequently the perception of a continuous scene. Compensating for these disruptions across eye movements is hypothesized to be aided by the integration of external sensory and internally-generated information. In patients experiencing psychotic symptoms (e.g., Schizophrenia and Bipolar Disorder), current evidence suggests that this integration may likely be defective thus resulting in improper perceptual judgments after visual disruptions. We investigated the neural mechanisms underlying erroneous perceptual decision making in a visual perception task. We recruited two participant samples: the first consisting of patients experiencing psychotic symptoms (N = 75); the second consisting of healthy control participants (N = 40). The goal of the experiment was to track a screen target that changed position along the horizontal plane during eye movements. This task was conducted concurrently with functional magnetic resonance imaging (fMRI) to capture the neural correlates of perceptual decision making. We monitored eye movements and the direction subjects reported for the target change ("left" vs. "right"). Behavioral responses were made by pressing one of two buttons indicating the target shift direction, and were used to quantify perceptual bias and threshold. We found that patients were characterized by a more negative bias and greater perceptual threshold indicating that they were less sensitive to target shifts, and that the visual error was likely overemphasized in forming their perceptual decisions. Reaction time, saccade precision, and manual response directions were used as model inputs to predict the perceptual accuracy with a drift diffusion model (DDM). We found that patients' visual error tended to bias DDM parameters to a greater degree relative to healthy controls. We present task-based fMRI univariate contrasts that highlight group differences correlating with such biasing effects. We expect such contrasts to help uncover the neuronal mechanisms that contribute to impaired saccadic corollary discharge and overreliance on visual feedback in psychotic patients. Such understanding will be critical for future clinical care and translational applications.

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Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

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Topic: E.01. Eye Movements

Support: SickKids POS Summer Studentship
SickKids Lunenfeld Summer Studentship
Internal funds from Dr. George Ibrahim

Title: A hands-free visual search task to assess selective attention and working memory in children with disabilities

Authors: *W. B. MAZIN[1], S. M. WONG[1,3], K. MITHANI[2,3], M. EBDEN[1], V. LI[1], N. M. WARSI[2,3], G. M. IBRAHIM[2,3,1,4];
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Abstract: The last decade has seen a rapid emergence of targeted neurotherapeutics for the treatment of neurological disorders in children, including for example deep brain stimulation. Evaluating the cognitive impacts of these interventions, while imperative, is often not feasible with traditional neuropsychological testing due to physical or intellectual disability. There is an unmet need for inclusive and accessible approaches to evaluating cognitive function in these children to guide evaluations of intervention safety and efficacy, as well as inform patient selection and counselling. To this end, we developed a hands-free visual search task to assess a range of cognitive functions including working memory, and selective attention in children with the exclusive use of eye tracking. A series of illustrated cluttered scenes are presented, with instructions to locate a familiar cartoon character as quickly as possible. Throughout the task, gaze and pupil size are recorded at 60 Hz using an eye tracker (Tobii Pro Nano, Tobii Technology Inc., Stockholm, Sweden). The task consists of 30 scenes, each containing two target stimuli with participants completing all scenes twice with prompts indicating the target character for each scene, leading to a total of 60 trials, with a randomized order of target stimuli between participants. To validate this task, we recruited healthy young adults (n = 13, average age 25.9 ± 3.5 years) to complete the task while undergoing simultaneous scalp electroencephalography. To evaluate task performance, search time, pupil size, and productivity metrics were studied. Productive time is a novel metric used to index cognitive function, specifically working memory, based on the proportion of time participants spend searching for the target in unseen locations, as opposed to redundantly directing their gaze to previously searched areas. Across all trials and participants (n = 780), the average time spent on each trial was 3.12 ± 1.14 seconds. Mean pupil variance over the course of each trial was 0.048 ± 0.030 mm with a mean pupil diameter of 3.65 ± 0.69 mm across participants. Finally, participants spent an average of 89.1 ± 4.1% of their time productively. These results demonstrate the feasibility of using this task to study a range of cognitive functions indexed by eye-tracking metrics. Ongoing efforts are aimed at applying this

task in pediatric patients with physical disabilities or developmental delay, particularly in evaluating the effect of neurotherapeutic interventions on cognitive function.

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Poster

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Title: Modulation of post-saccadic traveling brain wave patterns in the marmosets by visual stimuli

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Abstract: Traveling waves (TW) are identified as local field potential that spreads across the brain tissue in a topological order. In the past decade, different patterns of TWs have been widely studied and related to motor, sensory, and cognitive brain functions. Recent studies showed that after saccadic eye movements (saccades), large TWs originate from the primary visual area (V1) and propagate rostrally in an order consistent with the visual hierarchy. However, it remains elusive whether these TWs result directly from the motor command of saccades, the accompanying retinal shift of visual stimuli, or both. To answer this question, we implanted hemispheric electrocorticography arrays on two common marmosets, and trained them to perform a visually-guided saccade task (VGS) as well as a free viewing task of pictures (FV_p) or uniform gray background (FV_g). The event-related potential aligned on saccade offset shows a triphasic response during 0~100 ms, whose amplitude is the strongest in FV_p and weakest in FV_g. In the time-frequency analysis, we confirmed that this response corresponds to a peak of phase-locked power at 20~50 Hz. To analyze the spatiotemporal dynamics of this activity, we applied our previously developed graph-based algorithm for trial-by-trial detection and

quantification of the TWs. We further calculated the probability for a wave to asymmetrically flow between each neighboring electrode pair at each moment around saccade offset. In VGS and FV_p, the post-saccadic activity is mainly a TW going from V1 to temporal areas. In VGS, the latency, origin, and direction of TWs are significantly modulated by saccade directions, whereas in FV_p such modulation is absent. In FV_g, the post-saccadic TWs tend to originate from parietal areas, propagate caudally to dorsal occipital areas, and then ventrally to ventral occipital and temporal areas. Our results show that the amplitude and spatiotemporal pattern of the post-saccadic TWs are strongly influenced by visual stimuli either at the saccade target or on the background, which supports the hypothesis that retinal shift of visual stimuli dominates the generation of the post-saccadic TWs. Nevertheless, the weak but significant post-saccadic TWs in FV_g share a common later spatiotemporal dynamics with VGS and FV_p, which indicates that the motor component of saccades may interact with the sensory component in a cooperative manner.

Disclosures: K. Ho: None. **C. Chen:** None. **H. Onoe:** None. **T. Isa:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.10/G18

Topic: E.01. Eye Movements

Support: Department of biotechnology No. BT/PR17550/MED/30/1755/2016

Title: Comprehensive analysis and statistical modelling of candidate genes and biomarker in Age-related Macular Degeneration

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Abstract: ABSTRACTComprehensive analysis and statistical modelling of candidate genes and biomarkers in Age-related Macular Degeneration Priya Battu[1], Ramandeep Singh[2], Suresh Kumar Sharma[3], Akshay Anand[1*]
[1]Neuroscience Research Lab, Department of Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.[2]Advanced Eye Centre, Department of Ophthalmology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.[3]Department of Statistics, Panjab University, Chandigarh, India.
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PURPOSE: Age-related macular degeneration (AMD) is a sight-threatening ocular disorder. In

addition to genetic risk, environmental factors are also implicated in altering the susceptibility to AMD. In this study, we have comprehensively investigated the SNP association and serum expression of AMD associating genes and further evaluated the risk and predictive potential by statistical modelling.**METHODS:** A total of 262 participants were recruited for the study. We employed an Enzyme-linked immune sorbent assay to analyse the expression of the proteins in serum. SNP genotyping was done by using TaqMan assays. Bivariate logistic regression was used to analyse the association of the genetic variants with AMD. We further analysed the predictive ability of our genetic and serum expression data in predicting AMD cases through statistical modelling.
RESULTS: The serum levels of COL10A1 were upregulated, and RAD51B, TGFBR1, TNFRSF10A1 and VEGFA were downregulated in AMD patients. The ARMS2 and VEGF variants, T and C, respectively, conferred risk, whereas variant G in COL10A1 conferred protection for AMD in the studied population. Statistical modelling revealed that serum levels of TNFRSF10A and COL10A1 are good predictors, with 93.8% accuracy and VEGF and COL10A1 genotypes are good predictors of AMD cases, with 69.2% accuracy.**CONCLUSION:** The genes studied play a vital role in AMD pathogenesis and should be further explored to decipher its pathophysiology.

Disclosures: P. Battu: None. **R. Singh:** None. **S. Sharma:** None. **A. Anand:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

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Program #/Poster #: PSTR225.11/G19

Topic: E.01. Eye Movements

Support: NIH RO1-EY08890
P30-EY008126

Title: Mapping visual search errors to covert operations with frontal eye field neurophysiology and double factorial design

Authors: *W. LYU, J. D. SCHALL;
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Abstract: Behavior is the outcome of covert perceptual, cognitive, and motor operations that can be described by mathematical models and are produced by brain systems comprised of diverse neurons. Using the logic of selective influence, we previously distinguished the stages of processing supporting visual search (Lyu, Reppert, Schall, 2023). In that study, macaque monkeys searched for a color singleton among distractors. Two operations necessary for the task were independently manipulated. Singleton localizability was manipulated by varying the similarity between singleton and distractor colors. Stimulus-response mapping was manipulated by varying the discriminability of search array shape, signaling GO/NOGO response. The

organization and termination rule of the two operations were determined using System Factorial Technology (SFT; Lowe et al, 2019). The necessary next step in this research is to account for performance errors in this difficult task, which influence the logic of the SFT diagnosis. Monkeys made two key errors: on GO trials, monkeys occasionally shifted gaze to a distractor due to unsuccessful localization (GO error). On NOGO trials, they failed to inhibit their saccade towards either the singleton or the distractors (NOGO errors). NOGO errors reflect failure in discrimination alone or both operations, respectively. We probed the neural sources of these error saccades using single-unit spiking in frontal eye field. Neurons representing stimulus salience were distinguished from neurons mediating saccade preparation. Our data suggest that GO errors occur when visual salience neurons misrepresenting the distractor as the singleton. NOGO errors to singleton arise from incorrect discrimination by saccade preparation neurons whereas NOGO errors to distractor arise from inaccurate response from both neuron types. The convergence of performance and neural results on error trials offer constraints to mathematical models and provide evidence so that distinct operations and their organization during visual search can be resolved.

Disclosures: W. Lyu: None. **J.D. Schall:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.12/G20

Topic: E.01. Eye Movements

Support: R01 NS125843-01

Title: Projection patterns to superior colliculus of the non-human primate: comparison of rAAV2-retro with a CAG and CBA promotor

Authors: *M. BOHLEN[1], J. M. HASSE[2], A. RUDZITE[3], M. A. BASSO[4], L. N. KATZ[5], R. J. KRAUZLIS[6], M. A. SOMMER[7], K. RITOLA[8];
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Abstract: Background: Optogenetic technologies offer unprecedented insights into neuronal circuit function. However, their application in macaque monkeys has been limited. While achieving behavioral perturbation through actuators remains elusive, it is possible with the help of opsins to photosensitize neurons. A promising method involves using retrograde viruses to photosensitize neurons in areas distant from but projecting to the injection site, a technique known as “photo- or opto-tagging with projection targeting.” A significant challenge in this approach is characterizing transduction profiles. To address this issue, we provide a

comprehensive anatomical characterization of rAAV2-retro with different promoters in the macaque model. We present the results of mapping the biodistribution and transduction profiles of rAAV2-retro-CAG and rAAV2-retro-CBA following injections into the superior colliculus.
Results: Our preliminary data reveal an intricate neuroanatomical expression atlas for rAAV2-retro, which includes several structures known to project to the superior colliculus, for example, bilateral projections from the frontal eye fields, confirming successful vector transduction. Additionally, we weak to no observable labeling in some structures previously identified as having strong projections to the superior colliculus using conventional neuroanatomical tracers, for example, visual cortical area V1. Finally, there were a few circuit differences that were observed between CAG and CBA promoters suggesting new, additional promoter capsid interactions that add to those we have previously described when comparing transduction profile differences between CAG and hSyn, for example, cerebellotectal and the peripeduncular nucleus (not observed with CAG or hSyn).
Conclusion: This research establishes the necessary anatomical foundations for physiological experiments targeting SC-projecting neuronal populations. The promoter capsid interaction underscores the importance of neuroanatomically assessing viral transduction patterns before attempting any circuit-specific manipulations, in the macaque brain. Furthermore, our observations indicate that promoter-capsid interactions play a crucial role in determining transduction profiles, since only the promotor differed between constructs, while the capsid was constant. By employing projection targeting, we can now physiologically discern the information transmitted through individual circuits to the superior colliculus, thereby gaining new insights into its role in visual and visuomotor behaviors.

Disclosures: M. Bohlen: None. **J.M. Hasse:** None. **A. Rudzite:** None. **M.A. Basso:** None. **L.N. Katz:** None. **R.J. Krauzlis:** None. **M.A. Sommer:** None. **K. Ritola:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

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Topic: E.01. Eye Movements

Support: P30EY08098
F32EY035134
Whitehall Foundation

Title: Neuronal correlates of spatial attention are robust to the presence and direction of microsaccades

Authors: *S. WILLETT[1], J. MAYO[2];
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Abstract: Visual spatial attention and microsaccades are thought to interact. Attending to a location can bias microsaccades toward that location (Hafed & Clark, 2002), and microsaccades towards a location may gate attentional modulation of cortical neurons that represent that location (Lowet et al., 2018). However, we recently observed that there was little to no interaction between the location of spatial attention and the direction of microsaccades in a variably cued attention task (Willett & Mayo, 2023). Here, we extend our behavioral work by investigating whether the presence and direction of a microsaccade alters the attentional modulation of ongoing population activity in visual area V4.
We measured microsaccades using scleral coils and attentional modulation of V4 activity using two 48-channel Utah microelectrode arrays (one in each hemisphere) while monkeys performed a visual-spatial attention task. Monkeys were trained to report, via saccade, an orientation change in one of two simultaneously presented sinusoidally counterphased Gabor stimuli. Attention was cued using an 80% valid cue presented during a brief set of instruction trials prior to a block of no-cue test trials. We time-locked neuronal activity to microsaccades measured during fixation, 500 milliseconds after stimulus onset and 200 milliseconds prior to the orientation change.
The average microsaccade-aligned population response was larger when monkeys attended to a target in or near the recorded receptive fields versus when they attended to a target in the opposite visual hemifield. This finding was largely unaffected by the presence or direction of a microsaccade. However, we were concerned that averaging over the population could occlude effects present in the minority of the population, which would mask potential microsaccade-related gating of attentional modulation. To mitigate this concern and to allow each neuron to meaningfully contribute to the population response, we preformed de-mixed principal component analysis (dPCA). dPCA was performed on each recording session to extract the neural componentdimension that explained the most attention-related variance related to attention. We projected trial-averaged and single-trial neuronal responses onto this attention component and found no difference between trials with or without microsaccades. Additionally, we found that the projection onto the attention component was flat before, during and after the microsaccade. These results suggest that the modulation of V4 firing rates related to attention is consistent regardless of the presence or absence of microsaccades.

Disclosures: S. Willett: None. **J. Mayo:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.14/G22

Topic: E.01. Eye Movements

Support: NIH Grant R01EY034626
Smith-Kettlewell Eye Research Institute
Wright State University

Title: Phoria in neurotypicals is dynamic during monocular smooth pursuit

Authors: *S. HEINEN[1], A. CHANDNA[1], D. SINGH[1], S. N. WATAMANIUK[2];
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Abstract: Phoria is an eye misalignment revealed by covering that eye during “static” fixation. Phoria of one or both eyes occurs in most neurotypicals, yet it may progressively increase, potentially leading to strabismus. While sometimes assumed to be caused by in imbalance in muscle tension, phoria’s origin is unknown. Here we test this muscle theory by measuring phoria during voluntary eye movements. Observers pursued a small (.2 deg) white target moving horizontally across a dark screen with either a pure sinusoidal velocity profile, or a more difficult “noisy” sinusoidal profile in which it reversed direction at random times. Peak velocity was either 5 or 25 deg/sec. Stimulus type and speeds were blocked. Each trial began with a randomized (1-1.5 sec) fixation period during which phoria was measured. Viewing was binocular, or monocular with an eye occluded by an infrared pass filter. Binocular eye movements were recorded with an Eyelink 1000 plus. Muscle imbalance should cause constant phoria in the covered eye during fixation and pursuit. Instead, there were a wide range of behaviors. Some observers maintained a constant phoria during pursuit. In others, the phoria fluctuated. Surprisingly, phoria magnitude during fixation and pursuit were correlated. The results suggest that neural factors related to task difficulty and expectation contribute to phoria, which forms the foundation of general oculomotor control and developed strabismus.

Disclosures: S. Heinen: None. **A. Chandna:** None. **D. Singh:** None. **S.N. Watamaniuk:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

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Program #/Poster #: PSTR225.15/G23

Topic: E.01. Eye Movements

Support: CIHR Grant MOP-FDN-148418

Title: Blinks as a critical oculomotor effector: comparison of eye blink parameters measured by video-based eye-tracking and electromyography

Authors: *I. C. PITIGOI, D. C. BRIEN, B. C. COE, D. P. MUNOZ;
Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada

Abstract: Blinks are strategically timed to maintain visual efficiency by limiting co-occurrence with important visual or auditory events. This implies integration of both bottom-up sensory signals and top-down conscious goals to prioritize efficient information gathering. We have previously shown that subjects blink at implicit breakpoints in visual attention during both a structured pro/anti-saccade and unstructured video-viewing task (Pitigoi et al., 2024, doi:10.1523/ENEURO.0296-23.2024) and that this behaviour is quickly learned. Here we will present new electromyographic (EMG) data from orbicularis oculi (OO) muscles to demonstrate

the accuracy of blink latency and duration measures obtained from Eyelink-1000 video-based eye-trackers. OO-EMG data was recorded from surface electrodes directly beneath the eye in 22 healthy participants (11 females, aged 18-24 years) performing both a structured voluntary blink task and an unstructured video-viewing paradigm eliciting spontaneous blinks. Across subjects and tasks, there was remarkable consistency in the timing of blink onset: the OO-EMG burst started ~50ms before Eyelink pupil data loss. The durations of the OO-EMG burst were highly variable between subjects yet, correlated strongly to the overall duration of Eyelink pupil data loss, although they were substantially shorter. These results show the periods of data loss obtained through the Eyelink eye-tracker are a reliable measure of blinks. A clearer understanding of these timing considerations will allow for more precise quantification of blink behaviour, which is becoming increasingly important as a supplement to other oculomotor effectors (saccade, pupil) obtained from eye-tracking. These findings are therefore crucial to establish the validity of blink measures, particularly onset and duration, as the literature expands in this exciting new direction.

Disclosures: I.C. Pitigoi: None. **D.C. Brien:** None. **B.C. Coe:** None. **D.P. Munoz:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.16/G24

Topic: E.01. Eye Movements

Support: NIH Grant 1R01EY034626

Title: Microsaccades are conjugate in time but not space during fixation in the dark (and the light!)

Authors: *S. N. J. WATAMANIUK[1,2], D. SINGH[3], A. CHANDNA[3], S. J. HEINEN[3]; [1]Wright State Univ., Dayton, OH; [2]Smith-Kettlewell Eye Research Institute, San Francisco, CA; [3]Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Previously we demonstrated that during fixation, the eyes drift independently while microsaccades were always triggered simultaneously in both. When we covered one eye during fixation, *both* eyes became less well-controlled increasing their bivariate contour ellipse area (BCEA). These data challenge Hering's Law of unitary neural eye movement commands. Here, to test intrinsic eye movement control without the asymmetric input of monocular viewing, we characterized the stability of fixational eye movements in the dark. While seated in a dark room, observers were shown a small grey fixation spot (black background) on a computer monitor for 500 ms after which the spot was extinguished, and observers held fixation at the remembered location for 19.5 sec. In a control experiment, observers fixated the same visible spot target for the entire test period. Binocular eye movements were recorded with an Eyelink 1000+ eye tracker at 1000 Hz. Perhaps unsurprisingly, in the dark the eyes were less well controlled than

during binocular viewing, showing significantly larger BCEAs. These BCEAs were even larger than our previously reported covered eyes' during monocular viewing. A closer examination of microsaccades showed that in the dark, though they were initiated simultaneously in both eyes their direction and magnitude were surprisingly different (up to X deg in direction and x deg in magnitude). This effect was also evident during binocular viewing. Consistent with independent control of slow drifts, microsaccade spatial dynamics are influenced by independent control, however their timing is conjugate.

Disclosures: S.N.J. Watamaniuk: None. **D. Singh:** None. **A. Chandna:** None. **S.J. Heinen:** None.

Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

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Title: Brain Structural Correlates of Ocular Tracking in Preadolescent Children and Young Adults

Authors: *W. HUANG[1,2], B. HONG[1,2], J. CHEN[3,2], L. LI[3,2];
[1]Sch. of Psychology and Cognitive Sci., East China Normal Univ., Shanghai, China; [2]NYU-ECNU Institute of Brain and Cognitive Science at New York University Shanghai, Shanghai, China; [3]Fac. of Arts and Sci., New York Univ. Shanghai, Shanghai, China

Abstract: Although ocular tracking involves widespread brain regions, the neural basis for the development of ocular tracking abilities remains unknown. We addressed this issue by investigating the relationship between ocular tracking abilities and gray matter volume in preadolescent children and adults. Participants performed an 8-min ocular tracking task in which they used their eyes to track the step-ramp motion of a target (0.64°H × 0.64°V) with its speed (16°/s-24°/s) and direction (2°-358° in 4° steps, no repeats) varying randomly across trials. Eighty-one children aged 8-9 years (47F/34M) and 77 adults aged 18-30 years (43F/34M) completed the task, with 52 children (34F/18M) and 72 adults (42F/30M) providing valid

structural magnetic resonance imaging (MRI) data. Our task provided a comprehensive assessment of ocular tracking abilities including smooth pursuit, the coordination of pursuit with saccades, and dynamic visual motion processing with 12 oculometric measures that could be combined into one overall performance index. Regarding MRI data, we transformed the cortical grey matter volumes of 34 brain regions (defined by the Desikan Killiany atlas) into centile scores using the lifespan chart of the human brain derived from the current largest MRI samples. We then correlated ocular tracking performance metrics (the overall performance index and 12 oculometric measures) with the centile scores of the 34 brain regions. We found that children's ocular tracking abilities are inferior to adults. Specifically, the overall performance index and 8 out of the 12 oculometric measures in children related to smooth pursuit initiation, steady-state tracking, pursuit direction-tuning and speed-tuning have not yet reached adult levels. Correlation analysis revealed that the ocular-tracking brain network in adults includes unique regions (e.g., caudal middle frontal, pars opercularis, medial orbitofrontal, entorhinal) not found in children which enter volume reduction stages at older ages. Conversely, the ocular-tracking brain network in children contains unique regions (e.g., superior parietal, pericalcarine, isthmus cingulate) not found in adults which enter volume reduction stages at younger ages. The unique brain regions in adults are correlated with more ocular tracking performance metrics (such as pursuit acceleration and saccade rate) than those in children. These findings suggest that compared to adults, children’s inferior ocular tracking abilities are due to delayed brain maturation. Specifically, children rely on brain regions that developed earlier to substitute for the functions of the later-developing regions in the adult network.

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Poster

PSTR225: Eye Movements: Central Mechanisms, Perception, and Cognition

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR225.18/G26

Topic: E.01. Eye Movements

Support: R01EY024831

Title: Spectral analysis of local field potentials in monkey superior colliculus

Authors: *N. GANDHI[1], B. MOHAN[2,1], S. MAHMOUD[3], N. MCDONALD[1], C. BOURRELLY[1], A. DALLAL[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Mayo Clin., Phoenix, AZ; [3]Cairo Univ., Cairo, Egypt

Abstract: The local field potential (LFP) is a time-varying waveform of voltage aggregated from a volume of tissue surrounding an electrode contact. Often treated as a signature of the presynaptic currents, it presumably reflects the input signal to the recorded site. The spectral representation of LFP identifies its carrier frequencies, power, and phase. This framework has been successfully applied in many cortical regions, where modulations of power in specific

frequency bands have been linked with motor preparation, cognition, as well as feedforward and feedback processes. To our knowledge, however, a similar analysis has not been previously performed for the subcortical superior colliculus (SC) that is crucial for generating visually guided saccades. We seek to fill this gap in knowledge. Spikes and LFPs were recorded using a multicontact laminar probe inserted orthogonally to the SC surface in monkeys while they performed (delayed) saccades. The visual target was positioned either near the neurons' optimal receptive field (RFin) or at the diametrically opposite location (RFout). Spectrograms of trial-averaged LFPs, aligned on target and saccade onsets, were generated for each channel. Normalization by subtracting the log of baseline power revealed broadband power during the initial visual response (RFin), but with a notable reduction in power around 30-40 Hz. Spectral power during the delay period persisted above 40 Hz but was attenuated until movement onset for lower frequencies, including the beta band. The saccade related response (RFin), which extended into the post motor period, was also broadband with the highest power occurring around 50-60 Hz and in channels with the strongest delay period spectrum. For RFout, the activity in this period was weaker and extended until 30 Hz only; there was no obvious structure during the visual and delay periods for RFout data. We also examined separately the effects of removing the aperiodic ($1/f^x$) component while preserving the periodic spectrogram; no baseline subtraction was applied. We observed a robust oscillation around 20 Hz during the baseline and delay epochs, but it was conspicuously absent during the visual period, during which there was a salient attenuation in power for 15-50 Hz. Enhancement, however, was observed in theta (<10 Hz) and high gamma (>100 Hz) bands. Spectral power was highest in the superficial and intermediate layers and decreased gradually for deeper layers. Overall, we found trends that beta band power is attenuated during motor preparation and elevated in gamma band during the delay period, much like observations in the cortex.

Disclosures: N. Gandhi: None. **B. Mohan:** None. **S. Mahmoud:** None. **N. McDonald:** None. **C. Bourrelly:** None. **A. Dallal:** None.

Poster

PSTR226: Cerebellum: Human Studies

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Program #/Poster #: PSTR226.01/G27

Topic: E.02. Cerebellum

Support: DFG FOR MeMoSLAP / FOR 5429 (P06; project number 467143400)
ELAN scholarship for Medical Graduates

Title: Enhancing eyeblink conditioning by focalized cerebellar tDCS: Establishing the eyeblink conditioning paradigm to allow for multiple testing in the MRI scanner

Authors: *M. RIEGER[1], F. SCHLITT[2], G. BATSIKADZE[3], D. TIMMANN[3];
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of Duisburg-Essen, Essen, Germany; [2]Univ. of Duisburg-Essen, Essen, Germany; [3]Dept. of Neurol., Univ. of Duisburg-Essen, Essen, Germany

Abstract: There is significant interest in using cerebellar transcranial direct current stimulation (tDCS) as a tool to understand cerebellar function and treating related disorders. Our project focuses specifically on investigating the impact of cerebellar tDCS on motor learning processes, using an eyeblink conditioning paradigm as a task reliant on intact cerebellar function. Despite documented effects, variability in the outcomes of cerebellar tDCS on eyeblink conditioning prompts further investigation. Our primary study, conducted using a 3 Tesla MRI scanner, will involve participants undergoing both active and sham cerebellar tDCS while performing eyeblink conditioning tasks. This pilot study investigated the feasibility of conducting eyeblink conditioning paradigms in a repeated measures design within the same participant, aiming to maintain consistent learning effects. Additionally, we sought to establish a pupillometry analysis framework as an alternative to the conventional electromyography (EMG) for eyeblink recording in the MRI setting. During the experiment participants were presented with an unconditioned stimulus (US) as air puffs directed towards the eye, paired with conditioned stimuli (CSs) of visual or auditory modality. To further minimize learning transfer between sessions, we kept learning phases brief, utilized partial reinforcement, and included a prolonged extinction phase. Three sessions were spaced one week apart from each other. A baseline session, a shorter task version performed on the first day with the aim to stratify individual learning capabilities, revealed low conditioning rates, potentially attributed primarily to spontaneous blinking. Analysis of combined data from the two following task sessions indicated significant learning effects on both days, with auditory CSs yielding a higher incidence of conditioned responses, especially early in acquisition. Learning transfer was observed between baseline and task sessions, particularly with baselines and task sessions with auditory CS, suggesting a potential carryover effect. Comparison of data obtained by EMG and pupillometry revealed similar outcomes, affirming pupillometry's suitability for eyeblink recognition. In conclusion, multiple testing of eyeblink conditioning is feasible, as both task sessions showed significant learning rates. Next step is to test the paradigm in the MRI scanner without the baseline session to further reduce carryover effects.

Disclosures: M. Rieger: None. **F. Schlitt:** None. **G. Batsikadze:** None. **D. Timmann:** None.

Poster

PSTR226: Cerebellum: Human Studies

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Program #/Poster #: PSTR226.02/G28

Topic: E.02. Cerebellum

Support: NS116883
NS105839

Title: Cerebellar Ramping Activity as a Scaffold for Temporal Processing

Authors: *T. WANG[1], R. IVRY[2];
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Abstract: The cerebellum is essential for temporal processing. Recent theories have hypothesized that the cerebellum may also play a key role in learning the statistical properties of temporal contexts (such as the temporal variability of an event). We explored this issue by studying cerebellar degeneration patients.
Patients and age-matched controls performed a ready-set-go temporal reproduction task with durations derived from various prior distributions. Patients displayed a stronger central tendency, where they were more likely to underestimate longer durations and overestimate shorter ones. A Bayesian model suggests that the stronger central tendency might be a consequence of a larger perceptual noise. Specifically, patients showed a less accurate perception of the current duration, so they have likely skewed their estimations towards the prior, intensifying the central tendency. Following this idea, the cerebellum may only process the current duration, with the cortex representing the "prior". An alternative hypothesis posits that the stronger central tendency could stem directly from cerebellar deficits, irrespective of perceptual noise.
To differentiate these hypotheses, we conducted a second experiment on healthy participants, introducing a secondary task in 25% of trials to increase perceptual noise. Despite increased variance in trials with a secondary task, the central tendency was consistent across trials. The decorrelation between the perceptual noise and central tendency challenges the Bayesian model and indicates that the pronounced central tendency in patients may be a specific cerebellar anomaly.
To account for those results, we proposed a cerebellar ramping model, positing that cerebellar ramping reduces perceptual noise and decreases central tendency. This model can explain how the central tendency is modulated by prior distributions as well as several contextual effects that contradict the Bayesian perspective. According to this model, reduced ramping activation in CD patients results in higher variance and stronger central tendency. For healthy participants, although attentional manipulation increased variance, unchanged cerebellar ramping meant the central tendency remained stable. Our findings together provide a new perspective on how the cerebellum supports temporal perception.

Disclosures: T. Wang: None. **R. Ivry:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RI is a co-founder with equity in Magnetic Tides, Inc..

Poster

PSTR226: Cerebellum: Human Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR226.03/G29

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Title: Brainstem and Cerebellar Contributions to Facial Expressivity in Autism

Authors: *B. TRAVERS[1], C. J. CASCIO[2], J. GUERRERO-GONZALEZ[3], S. KECSKEMETI[1], A. ALEXANDER[1], D. DEAN[1], N. ADLURU[4], K. S. BRESS[5], J. QUINDE ZLIBUT[5], G. KIRK[1];
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Abstract: Facial expressivity is widely acknowledged to differ in autistic individuals (Trevisan et al., 2018), but the underlying neural circuitry of these differences is unclear. In animal models, the brainstem's pontine and medullary reticular formation has been associated with facial movement (Schulz et al., 1979; Siegel et al., 1983). In humans, electrical stimulation of cerebellar lobule VI elicited face/mouth movements that were brainstem mediated (Mottolese et al., 2013). These data underscore the need to understand cerebellar and brainstem contributions to facial movements in autistic individuals. While the brainstem has been difficult to image in autistic individuals, our group recently has implemented specialized image acquisition and post-processing techniques that enhance images of the brainstem. In a sample of 74 autistic children (6.0-10.9 years, 80% male), participants completed T1- and diffusion-weighted imaging with acquisition and post-processing techniques aimed to improve brainstem images (Guerrero-Gonzalez et al., 2022). Data reduction of autism features and microstructural measures of 22 brainstem nuclei (Singh et al., 2022) was performed via principal component analyses. As there was no direct measure of facial expressivity, we z-scored and combined 5 items that addressed facial expressivity across two standardized measures of autistic behaviors (SRS-2 and SCQ). Partial correlations examined associations among facial expressivity and brainstem cluster values, while controlling for age, sex, and head motion. The results found that facial expressivity was related to a cluster of pontine nuclei (parvicellular reticular formation, subcoeruleus, and pontine reticular nucleus), r=-.30, p=.01. These results mirror animal studies suggesting brainstem involvement in facial expression and suggest that microstructural differences in these brainstem regions may help account for individual differences in facial expressivity in autistic individuals. Future research using more fine-tuned measures of facial expressivity will be needed to further examine this effect.

Disclosures: B. Travers: None. **C.J. Cascio:** None. **J. Guerrero-Gonzalez:** None. **S. Kecskemeti:** None. **A. Alexander:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ImgGyd, LLC. F. Consulting Fees (e.g., advisory boards); Independent consulting business. **D. Dean:** None. **N. Adluru:** None. **K.S. Bress:** None. **J. Quinde Zlibut:** None. **G. Kirk:** None.

Poster

PSTR226: Cerebellum: Human Studies

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR226.04/G30

Topic: E.02. Cerebellum

Support: NIH K12HD001399

Title: Perturbations of steroid metabolism are linked to cerebellar injury in preterm neonates with necrotizing enterocolitis

Authors: *C. BYRD[1], G. SANIDAS[1], N. WOLFF[1], J. GHAEMMAGHAMI[1], G. SIMONTI[1], V. GALLO[2], P. KRATIMENOS[3];
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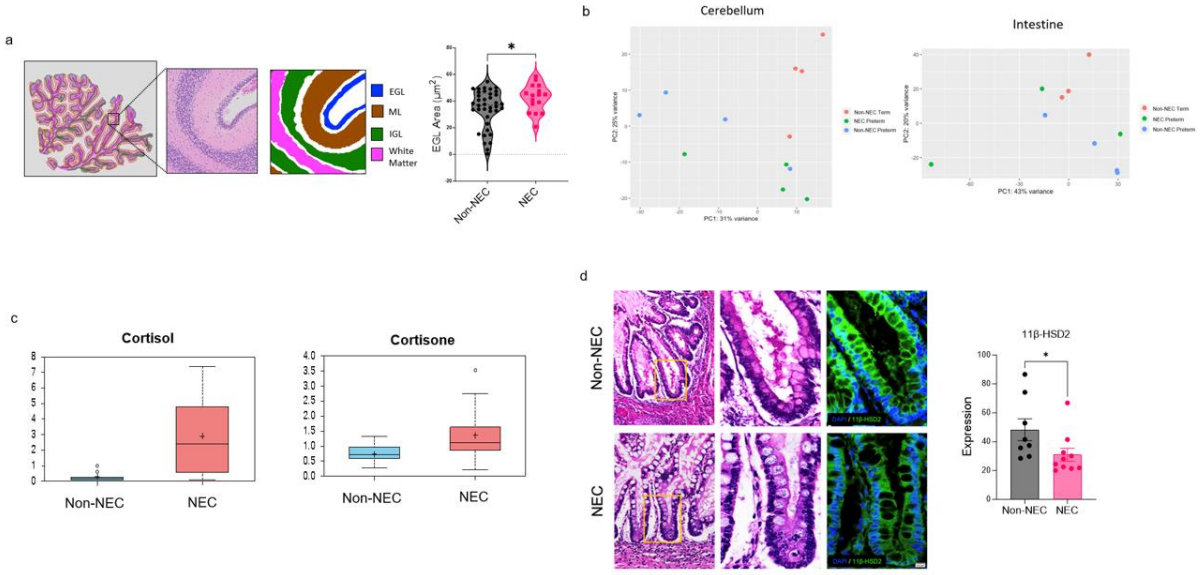
Abstract: Necrotizing enterocolitis (NEC) is a complication of prematurity and is independently linked to severe neurodevelopmental deficits. Perinatal steroid administration aims to accelerate fetal lung maturation before preterm deliveries but comes at the cost of disrupting immune function and development of major organs. The timing of the perinatal steroid use often coincides with the enteric mucosal immune system maturation and the rapid in-utero cerebellar development, a period of which preterm neonates are partially deprived. Moreover, cerebellar development is heavily dependent on Sonic hedgehog (Shh) signaling, which is in-turn regulated by steroid metabolism. We performed rigorous histopathological analysis on postmortem intestine and cerebellum using whole-slide imaging and semi-supervised machine learning quantification (n=16 NEC, 16 control), serum metabolomics (n=20 NEC, 10 control), and cerebellar and intestinal transcriptomics (n=4 NEC, 4 control). Age and gender-matched preterm neonates without NEC served as controls. We demonstrated that NEC delays the differentiation and migration of cerebellar granule cells (GCs), independent of prematurity [Fig1a]. Transcriptomic analysis of the cerebellum and intestine in preterm neonates with NEC revealed increased differential expression of canonical pathways associated with immune regulation, steroid metabolism and Shh [Fig1b]. Serum metabolomic analysis, in line with the transcriptomic data, demonstrated a hypercortisolemic state in the NEC group [Fig1c]. Importantly, NEC intestine, compared to non-NEC, revealed decreased expression of 11βHSD2, an effector molecule of Shh signaling pathway protection against steroid-induced cerebellar injury [Fig1d]. In conclusion, NEC is linked to cerebellar injury independently of prematurity. Reduction in intestinal 11βHSD2 expression in neonates with NEC may further contribute to stress-induced systemic hypercortisolemia, deactivation of Shh, prevention of GC maturation and ultimately disrupted circuitry and neurodevelopmental impairment.

Figure 1



Disclosures: C. Byrd: None. **G. Sanidas:** None. **N. Wolff:** None. **J. Ghaemmaghami:** None. **G. Simonti:** None. **V. Gallo:** None. **P. Kratimenos:** None.

Poster

PSTR226: Cerebellum: Human Studies

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Program #/Poster #: PSTR226.05/G31

Topic: E.02. Cerebellum

Support: Raynor Cerebellum Project
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Title: Preterm birth prevents cerebellar growth and disrupts cell cycle regulation of the neuronal precursors

Authors: *G. SANIDAS[1], C. BYRD[1], G. SIMONTI[1], M. TRIANTAFYLLOU[1], N. WOLFF[1], *J. GHAEMMAGHAMI[1], I. KOUTROULIS[1], V. GALLO[2], P. KRATIMENOS[1];
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Abstract: Title: **Preterm birth prevents cerebellar growth and disrupts cell cycle regulation of the neuronal precursors** Background: The boundaries of viability for preterm infants are constantly extended, raising concerns about their neurodevelopmental outcomes. The cerebellum undergoes significant development in the third trimester of gestation, a critical phase missed by preterm newborns. In addition, preterm survivors exhibit cerebellar impairment which persists until adulthood. However, the cellular and molecular landscape of human preterm cerebellar

development remains unexplored. Methodology: Here, using sonographic imaging we measured the cerebellar growth at term equivalent age(TEA) in preterm(n=301) and age- and gender-matched term(n=392) infants. We utilized whole slide imaging combined with machine learning, to perform rigorous neuropathological postmortem analysis of preterm(n=51) and term(n=35) human neonates. We determined the molecular profile of the morphological alterations (spatial-RNAseq) related to preterm birth and defined the developmental maturation of the cerebellar cortex by projecting established developmental single-cell transcripts (scRNASeq) on the enrolled subjects.Results: The *ex-utero* growth resulted in decreased cerebellar dimensions at TAE, correlating with gestational age**.** This reduced growth was associated with decreased overall cortical foliation, decreased internal granular layer(IGL) and molecular layer (ML) surface areas and granular cell(GC) depletion. In contrast, external granular layer(EGL) was retained for a longer period in preterm infants compared to term**.** Preterm neonates exhibited decreased complexity and branching of the Purkinje cell(PC) arbors**.** Preterm granular cell precursors(GCPs) and PCs (vsTerm) exhibited differential expression of pathways related to metabolism and cell cycle signaling. Cell cycle assays confirmed dysregulation at G2M checkpoints, with increased levels of cell cycle inhibitory proteins (p21 and p27) in preterm vs. term**.** Developmental analysis using scRNASeq identified the EGL-GCPs and PCs as main areas of the arrested development**.** Conclusion: We demonstrated that gestational age correlates with cerebellar growth in infancy. Preterm birth impacts the PCs and EGL-GCs development, with cell-cycle dysregulation playing a critical role in this process.

Disclosures: G. Sanidas: None. **C. Byrd:** None. **G. Simonti:** None. **M. Triantafyllou:** None. **N. Wolff:** None. **J. Ghaemmaghami:** None. **I. Koutroulis:** None. **V. Gallo:** None. **P. Kratimenos:** None.

Poster

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Topic: E.02. Cerebellum

Support: NIH Grant MH124004
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Title: Evidence for Segregation of Distinct Cerebellar Association Networks in the Pons and Dentate

Authors: *V. TRIPATHI[1], H. L. KOSAKOWSKI[1], N. SAADON GROSMAN[1], R. L. BUCKNER[1,2,3];
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Abstract: The cerebellar cortex receives input from the cerebral cortex via the pons and projects its output to the deep cerebellar nuclei then back to the cortex via the thalamus (Schmahmann et al., 2019; Strick et al., 2009). Relative enlargement of the dentate nucleus, the deep cerebellar nucleus linked with cerebral association cortex, was offered by Leiner, Leiner, and Dow (1986) as initial evidence that the human cerebellum might support cognition. Using precision neuroimaging, we have recently discovered multiple juxtaposed higher-order association networks in the cerebellum that echo cerebral organization (Du et al., 2024; Saadon-Grosman et al., 2023; Xue et al., 2021). Guided by anatomy and these recent discoveries, we explored in intensively sampled individuals whether distinct association networks are also segregated within the pons and dentate. Using functional connectivity MRI, data from Xue et al. (2021) were reanalyzed (each individual was scanned across 31 sessions yielding ~60 functional runs). In each individual, seed regions placed within subregions of the pons and dentate were able to selectively reproduce five distinct cerebral association networks including those linked to cognitive control, language, social inference, and episodic/spatial functions. The results were replicated in multiple individuals from an independent sample of 15 participants (Du et al., 2024; each individual scanned across 8+ sessions). The finding that higher-order association networks remain distinct within the pons and dentate is consistent with the hypothesis that parallel higher-order cerebral-cerebellar networks remain anatomically segregated (or partially segregated) throughout their entire distributed extents.

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Poster

PSTR226: Cerebellum: Human Studies

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Program #/Poster #: PSTR226.07/G33

Topic: E.02. Cerebellum

Support: FWO-funded project G095121N

Title: The cerebellum as a potential key region for motor reserve in healthy aging

Authors: *A. MATTHIJS[1,2], A. DE WITTE[1,2], D. MANTINI[1,2], J. GOOIJERS[1,2], J.-J. ORBAN DE XIVRY[1,2];
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Abstract: Healthy aging can impact brain structure. However, neuronal loss in motor areas is not always associated with a decline in motor function. This is because, some older adults' motor function is more resilient to aging. More resilient adults are thought to have a higher level of motor reserve, which will help maintaining motor function against several stressors. A stressor can be age-related brain degeneration, but also a task-related physical stressor. We focus on

resilience of cerebellar function to aging. While this structure is heavily affected by aging, motor performance on tasks relying on cerebellar internal model function is found to be preserved. Therefore the cerebellum might be a potential key region for motor reserve, supporting physical resilience in older adults.
We investigated 50 young (20-35 y/o), 80 old (55-70 y/o) and 27 older old (>80 y/o) adults. Participants performed 2 tasks relying on cerebellar internal model function (inter-joint coordination & motor timing task), and 1 task relying on accurate signals from multiple systems (postural control). To test resilience of the (cerebellar) motor system, in each task a standardized physical stressor was added such that every task was performed at 2 levels of difficulty. Participants with small differences in motor performance between both difficulty levels of the same task were associated with higher levels of physical resilience, since they can resist the physical stressor better, and vice versa. To evaluate participants' cerebellar functional resistance against cerebellar structural degeneration, we compared motor function outcomes with the cerebellar grey matter volume, obtained via structural T1 images.
Our results showed no significant differences between all age groups for functional outcomes on the cerebellar-dependent tasks. However, for the postural control task we did find that older adults performed worse compared to younger adults. Furthermore, for the cerebellar-dependent tasks the effect of the stressor was the same for young and (older) old adults, but for the postural control task older adults performed significantly worse if the physical stressor was added. The grey matter analysis of the cerebellum confirms that, in our sample, there was a significant decline in cerebellar volume with older age.
These results suggest that cerebellar motor function is more resistant to aging and to physical stressors than motor function in general despite the pronounced age-related decline in cerebellar structure. This supports the hypothesis that the cerebellum can act as a motor reserve, such that at older age cerebellar motor function is resistant to age-related structural degeneration.

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Poster

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Program #/Poster #: PSTR226.08/G34

Topic: E.02. Cerebellum

Title: Age-related decline in cerebellar structure does not lead to a decline in cerebellar motor function

Authors: *A. DE WITTE[1,2], A. MATTHIJS[1,2], J. GOOIJERS[1], D. MANTINI[1], J.-J. ORBAN DE XIVRY[1,2];
[1]KU Leuven, Leuven, Belgium; [2]Leuven Brain Institute, Leuven, Belgium

Abstract: The cerebellum plays an important role in many motor and non-motor functions. Patients diagnosed with cerebellar degenerative disease, suffer from severe problems with movement control in several motor domains, such as gait, balance, speech and reaching. The cerebellum is also one of the brain areas that is most heavily affected by age-related degeneration. This raises the question to what extent age-related cerebellar degeneration is responsible for the motor deficits that we see in older people. At the behavioral level, many tasks involves the cerebellar function but none of them relies only on cerebellar function. Furthermore, the cerebellar motor function is quite diverse (timing, coordination adaptation, etc.). Therefore, we wanted to investigate the cerebellar function through a wide variety of tasks to avoid task and domain specificity, and in a diverse sample of older adults, including those 80 years old and above, to go beyond the age span that is often investigated in aging researchWe assessed cerebellar structure and motor and non-motor cerebellar function in healthy adults divided from three age groups: young adults (20-35y/o, N=50), older adults (55-70y/o, N=80), and older old adults (>80y/o, N=26). Structural T1 MR images were acquired and cerebellar gray and white matter volumes were calculated. For cerebellar function assessment we developed a test-battery consisting of six motor and two cognitive tasks, covering the following topics: grip-force load-force coupling, inter-segment coordination, implicit adaptation, sensory attenuation, timing, mental rotation and spatial working memory. All tasks were chosen for their cerebellar dependence, based on literature about deficits in cerebellar patients or correlations with cerebellar activity.We found that both cerebellar gray and white matter volumes were significantly lower in older adults compared to young adults, and that this was even more severe for the older old adults. For the motor tasks from the test battery we did not find any evidence for age-related differences in performance that indicated to cerebellar deficits. In contrast, older and older old adults performed worse than younger adults at the cognitive tasks. Altogether, our results suggest that the age-related decline of cerebellar structure does not necessarily lead to a decline in cerebellar function.Altogether, our results show that age-related decline of the cerebellar structure does not directly lead to a decline of the cerebellar motor function. Our results warn against an oversimplified interpretation of the link between the effect of aging on cerebellar structure and function.

Disclosures: A. de Witte: A. Employment/Salary (full or part-time):; KU Leuven. **A. Matthijs:** None. **J. Gooijers:** None. **D. Mantini:** None. **J. Orban de Xivry:** None.

Poster

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Hessian Ministry of Higher Education, Science, Research and Art

Title: How do we adapt to sensorimotor and inter-sensory delays? Findings from fMRI and tDCS suggest critical roles of the hippocampus and the cerebellum

Authors: *C. V. SCHMITTER, J. SOMMER, B. STRAUBE;
Philipps-Universitat Marburg Fachbereich Medizin, Marburg, Germany

Abstract: Predicting the time at which the sensory outcomes of our actions will occur is a crucial mechanism that helps us to distinguish self- from externally generated sensory inputs. Similarly, the predicted timing between signals from different sensory modalities helps us decide whether they originated from a common source. Importantly, these temporal predictions need to be flexibly adaptable to environmental changes, such as varying sensorimotor or inter-sensory delays. However, the neural correlates underlying these adaptive mechanisms remain poorly understood. Furthermore, ego-disturbances in patients with schizophrenia spectrum disorders (SSD) have been associated with deficits in action outcome prediction. But whether this deficit can be partly explained by the dysfunctional adaptability of this predictive mechanism is unclear. In two studies with healthy subjects, we used fMRI to investigate the neural correlates of the adaptation to sensorimotor and inter-sensory delays. In a third study, we assessed whether delay adaptation is reduced in SSD, and whether transcranial direct current stimulation (tDCS) of relevant brain regions can facilitate adaptation in patients. In all studies, subjects were repeatedly exposed to delays between actively (sensorimotor) or passively (inter-sensory) elicited button presses and auditory or visual stimuli. Effects of this procedure on temporal perception were assessed with a delay detection task. In healthy subjects, adapting to delayed auditory and visual outcomes was associated with activations in bilateral hippocampus and cerebellum (lobules IV and V) in both active and passive movement conditions. Differences in adaptation-dependent activations between movement types occurred, e.g., in frontal and temporal areas. Patients with SSD exhibited no difference in behavioral delay adaptation effects compared to healthy subjects, and, compared to sham stimulation, anodal tDCS of the cerebellum facilitated the adaptation to sensorimotor delays. These findings show for the first time that the hippocampus plays an important role in delay adaptation, presumably related to the updating and encoding of the novel temporal relationship between signals of different sensory modalities. Furthermore, our findings confirm that the cerebellum plays a key role not only in generating but also in adapting predictions about the sensory stimulus timing. Our results further show for the first time that the adaptability of action outcome predictions may be preserved in SSD and that cerebellar tDCS could be a promising tool for addressing deficits in related sensorimotor functions in patients that rely on the cerebellum.

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Poster

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Hans-Böckler-Stiftung

Title: Human cerebellum and ventral tegmental area interact during extinction of learned fear

Authors: *P. PAIS PEREIRA[1,2], E. NIO[1,2], M. PETRENKO[1,2], A. DOUBLIEZ[1,2], T. M. ERNST[1,2], N. DIEKMANN[3], G. BATSIKADZE[1,2], S. MADERWALD[2], C. DEUSCHL[5], S. CHENG[3], C. J. MERZ[4], H. H. QUICK[6,2], D. TIMMANN[1,2];
[1]Dept. of Neurol. and C-TNBS, Univ. Hosp. Essen, Univ. of Duisburg-Essen, Essen, Germany; [2]Erwin L. Hahn Inst. For Magnetic Resonance Imaging, Univ. of Duisburg-Essen, Essen, Germany; [3]Inst. for Neural Computation, [4]Dept. of Cognitive Psychology, Inst. of Cognitive Neurosci., Ruhr Univ. Bochum, Bochum, Germany; [5]Inst. for Diagnos. and Interventional Radiology and Neuroradiology, [6]High-Field and Hybrid MR Imaging, Univ. Hosp. Essen, Essen, Germany

Abstract: Fear extinction involves learning of a new memory trace that inhibits the previously learned fear association and constitutes the fundamental mechanism underlying exposure therapy. The key element for fear extinction learning are unexpected omissions of expected aversive events, which are considered to be rewarding. Given the cerebellum's established role in processing prediction errors and its reception of reward information, we tested the hypothesis that the cerebellum contributes to reward prediction error processing driving extinction learning via its known direct connections with the ventral tegmental area (VTA). To study the proposed interaction between the cerebellum and VTA, 43 young and healthy participants performed a three-day differential fear conditioning paradigm in a 7T MR scanner. Fear acquisition training was performed on day 1, extinction training on day 2, and recall was tested on day 3. Event-related fMRI analyses with model-derived prediction error values as parametric modulations were performed at the time the aversive unexpected stimulus was expected but did not occur. Both the cerebellum and the VTA showed significant activations. Furthermore, increased functional connectivity was observed between the cerebellum and VTA, indicating that during unexpected omissions in extinction, the cerebellum may positively modulate VTA activity, possibly facilitating dopaminergic signaling during fear extinction learning. These results suggest that an interaction between the cerebellum and VTA should be incorporated into the existing model of the fear extinction network.

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Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

Support: JSPS 24H00064
JST-CREST JPMJCR23P3

Title: Dynamic changes in cortico-striatal transmission associated with striatal beta oscillations in monkeys

Authors: *K.-I. OKADA, M. TANAKA;
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Abstract: The basal ganglia play a pivotal role in adaptive behavioral control. Neuronal activity in the striatum flexibly changes both during long-term learning and during rapid changes in task requirements. The long-term changes are known to be associated with the changes in cortico-striatal transmission and synchrony of low frequency oscillations of local field potentials (LFPs). However, it remains unknown whether similar changes can occur rapidly within each trial. To address this, in two monkeys performing oculomotor tasks, we delivered single-pulse electrical stimulation to the supplementary eye field (SEF) and assessed the strength of cortico-striatal transmission by measuring LFPs and single neuron activities in the caudate nucleus. We found that SEF stimulation elicited a triphasic (negative-positive-negative) LFP response in the caudate nucleus. We also examined spiking activity of 137 caudate neurons that responded to SEF stimulation. The majority (72%, 99/137) responded with a short latency (23.8 ± 4.3 ms, mean ± s.d.) around the time of the first negative LFP deflection, and approximately half (53%, 72/137) responded with a longer latency around the time of the second negative LFP component (212.3 ± 97.6 ms). A quarter of neurons (25%, 34/137) showed both the early and late spikes. The very early LFP response preceding the early spikes (5-10 ms after SEF stimulation) was more negative when spikes were elicited, likely reflecting EPSPs. Therefore, we assessed the strength of cortico-striatal transmission by monitoring the occurrence of early spikes and the magnitude of early LFP response. We found that negative modulation of striatal potentials prior to SEF stimulation was more likely to elicit spikes and lead to greater LFP response, suggesting that ongoing oscillatory activity may regulate the efficacy of cortico-striatal transmission. Subsequent analysis revealed that the stimulation effect was highly dependent on the phase and amplitude of striatal beta oscillations (20-40 Hz) just before electrical stimulation, but not on simultaneously recorded beta oscillations in the SEF. Comparison with the shuffled data indicated that the LFP was negatively oriented during this particular phase, but that the SEF stimulation added further negative potentials in the caudate nucleus. On the other hand, spontaneous spikes of these neurons tended to occur at specific phases of striatal theta oscillation (3-8 Hz). These results suggest that cortico-striatal transmission is dynamically regulated by local oscillatory activity, which may underlie the rapid changes in striatal neuronal activity for different task demands.

Disclosures: K. Okada: None. **M. Tanaka:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

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Title: Encoding visual stimuli by striatal neurons

Authors: *J. PÉREZ-BECERRA, L. CARRILLO-REID;
Inst. of Neurobiology, UNAM, Queretaro, Mexico

Abstract: For years visual perception has been mainly understood as a cortico-cortical hierarchical process in which characteristics of visual stimuli are integrated from low-level areas to high-level areas. This oversimplified idea does not explain how specific features of visual stimuli can be preserved during motion, underestimating the potential role of subcortical areas in the representation of visual percepts. In mice, anatomical evidence shows that neurons from primary visual cortex (V1) send functional connections to the dorsomedial portion of the striatum (DMS), a basal ganglia nucleus mainly involved in motor functions. However, how such low-level cortico-striatal interaction modulates the representation of visual information remains unknown. It has been described that populations of neurons in V1 encode specific features of visual stimuli such as the orientation of drifting-gratings. Nevertheless, it has not been reported if the DMS circuit preserves such code. To investigate how visual-oriented drifting-gratings are represented in the electrical activity of DMS neurons, we performed single-unit recordings in DMS with tetrode arrays in head-fixed naive mice during stationary and running states. Next, the orientation selectivity of DMS neurons responding to 4 visually oriented-drifting gratings was determined. We observed specific subpopulations of DMS neurons tuned to different orientations. Orientation encoding was preferentially represented in medium spiny neurons (MSNs) against fast-spiking interneurons. During running, visual evoked activity in MSNs increased, as well as the orientation selectivity. Surprisingly, the enhancement in orientation selectivity was mainly mediated by D1-MSNs from the direct pathway. Our experiments demonstrate that the increased activation of the direct pathway during running improves the codification of visual stimuli in DMS, providing a circuit mechanism that allows the maintenance of visual percepts during motion.

Disclosures: J. Pérez-Becerra: None. **L. Carrillo-Reid:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Fonds de recherche du Québec - Santé (FRQS)
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Brain Canada

Title: Dissecting midbrain dopamine neuron subtypes role in locomotion using novel intersectional tools for selective ablation

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Abstract: Midbrain dopamine (DA) neurons play critical roles in locomotion, reward processing, and addiction. Recent studies have highlighted their molecular diversity and distinct projection patterns, suggesting involvement in various neuronal circuits. However, their specific behavioural functions remain poorly understood. Here, employing an intersectional genetic ablation approach, we sought to elucidate the roles of calbindin (Calb1+) expressing and non-expressing (Calb1-) midbrain DA neurons in locomotion. We engineered autocleavable Caspase3 (taCasp3) into $Cre_{OFF}/Flpo_{ON}$ and $Cre_{ON}/Flpo_{ON}$ intersectional viral constructs. Calb1-ires-Cre;Dat-2A-Flpo mice were injected with either $Cre_{OFF}/Flpo_{ON}$ or $Cre_{ON}/Flpo_{ON}$-taCasp3 AAVs to selectively ablate Calb1- or Calb1+ DA neurons in the substantia nigra pars compacta. In order to label these DA neuron populations, mice were also injected with a $Cre_{ON}/Flpo_{ON}$-EYFP or $Cre_{OFF}/Flpo_{ON}$-mCherry. Three weeks post-injection, mice underwent motor assessments using the rotarod, open field, and cylinder tests. Brains were then collected for histologic analyses of Calb1+ and Calb1- DA striatal fibres and midbrain cell bodies. Our results show that, as expected, $Cre_{OFF}/Flpo_{ON}$-taCasp3 injection ablated preferentially Calb1- DA neurons, while the $Cre_{ON}/Flpo_{ON}$-taCasp3 viral construct preferentially ablated the Calb1+ DA neurons. Behavioural assessment showed that ablation of Calb1- DA neurons resulted in a severe impairment of locomotor learning and performance of the mice, which wasn't observed when Calb1+ DA neurons were ablated. In conclusion, we present here an intersectional tool to selectively ablate molecularly-defined neuronal subtypes and we used it to show that Calb1+ and Calb1- midbrain DA neurons encode different roles in locomotion. We believe these viruses are a new addition to the arsenal already available to investigate the functional roles of neuronal subtypes in behaviour and neurological disorders.

Disclosures: C. Bolduc: None. **C. Oram:** None. **G. Talbot:** None. **S. Martin:** None. **J. Poulin:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR227.04/H3

Topic: E.03. Basal Ganglia

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Title: Tic disorder is caused by disrupted neuronal processing in the cortico-basal ganglia-thalamocortical motor and limbic loops

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Abstract: Tourette syndrome (TS) is a neurodevelopmental disorder characterized by motor and vocal tics, which are sudden, rapid, recurrent, non-rhythmic movements. The TS is also accompanied by cognitive and emotional dysfunctions such as obsessive-compulsive disorder (OCD) and attention deficit hyperactivity disorder (ADHD). Dysfunction of the cortico-basal ganglia-thalamocortical circuits has been suggested to induce these symptoms in TS patients, but the details of neuronal mechanisms underlying tic disorders remain unknown. In this study, we generated drug-induced tic model mice by unilateral local injection of $GABA_A$ receptor antagonist, bicuculline, into the striatal motor region. In this tic mouse model, we detected c-Fos labeled cells not only in motor structures (M1, globus pallidus, substantia nigra pars reticulata, and subthalamic nucleus) but also in limbic structures (insular cortex, cingulate cortex, and amygdala). We then investigated the anatomical route from the basal ganglia output structures (globus pallidus and substantia nigra pars reticulata) to the limbic structures (insular and cingulate cortices) using anterograde and retrograde viral tracers. We found that the intralaminar thalamic nuclei are hub structures, which connect the basal ganglia with the limbic cortex. We also revealed that chemogenetic inhibition of the insular cortex or the thalamocortical (insular) pathway ameliorated the drug-induced tic symptoms. These data suggest that abnormal neuronal processing in motor and limbic domains of the cortico-basal ganglia-thalamocortical loops might be responsible for the generation of tic symptoms.

Disclosures: H. Kuno: None. **K. Kobayashi:** None. **T. Takumi:** None. **Y. Tachibana:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: EWU FGRCW 2023-24

Title: Influence of nicotine and tetrahydrocannabinol on dopamine signaling in the rat dorsal striatum

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Abstract: BACKGROUND: The striatum is highly innervated by dopamine (DA) neurons. The dorsal striatum is involved in the control of voluntary movements and is thought to mediate habit formation. The addictive nature of nicotine and tetrahydrocannabinol (THC) emphasize the importance of understanding their impact on dopamine neurotransmission in the dorsal striatum. The effects of nicotine, delta-9 THC, and delta-8 THC on DA signaling in the dorsal striatum have not been thoroughly investigated *in vivo*. METHODS: Male Sprague-Dawley rats (*Rattus norvegicus*) 325-425 grams were anesthetized with urethane 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. Electrodes were incrementally lowered, and optimized, until clear DA signals were evoked. Once clear and stable DA signals were recorded, nicotine (0.8 mg/kg), delta-9 THC (5 mg/kg), delta-8 (5 mg/kg), or an equivalent volume of vehicle (controls) was administered via intraperitoneal (i.p.) injection. DA signals were stimulated and recorded for 1 h after an i.p. injection. RESULTS: Nicotine significantly decreased electrically-evoked DA signals (peak amplitude) 5 to 55 min post nicotine injection, relative to vehicle-treated controls. In addition, kinetic analysis (Demon Voltammetry and Analysis software) revealed a significant decrease in the upward velocity (DA release) and a significant increase in the downward velocity (DA uptake) 5 to 30 min post nicotine injection. CONCLUSIONS: These data suggest that nicotine decreases dopamine signaling in the dorsal striatum by altering DA release and DA uptake. Delta-9 and delta-8 THC do not impact DA neurotransmission in the dorsal striatum.

Disclosures: D.P. Daberkow: None. **G. Rosenbaum:** None. **J. Alvarez:** None. **J.P. Yorgason:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

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Title: Spatial and temporal changes in mouse striatal dopamine signals during visual learning

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Abstract: Striatal dopamine plays a crucial role in the learning and performance of sensorimotor tasks. Previous fiber photometry results have shown that dopamine axons in the dorsomedial striatum encode cue salience, rather than reward outcome. However, it remains unknown whether these dopamine signals are present from the start of training or how they may emerge as a result of formed associations. Here, we trained mice on a unilateral orientation-change detection task while recording fluorescent dopamine signals bilaterally in the dorsomedial or dorsolateral striatum to explore how task-related dopamine signals evolved with experience and increased performance. Naive mice were injected with dLight1.2 bilaterally in the striatum and then implanted bilaterally with fiber optic cannulae in the dorsomedial or dorsolateral striatum before the start of the training. Head-fixed mice were then trained on a visual perceptual task in which they reported a tilt of the visual stimulus by licking a spout to receive a soy milk reward. Importantly, mice were also required to withhold licks before the stimulus tilt. We found both subregion-dependent and performance-dependent differences in dopamine signals across the course of training. In the dorsomedial striatum, all mice had baseline dopamine responses to the stimulus onset prior to training. In line with previous results, these mice lacked a reward response in the dorsomedial striatum. As performance in the task improved, only expertly performing mice developed a unilateral dopamine signal in response to the reward-predicting cue (stimulus tilt); however, they never developed a reward-locked dopamine signal. Meanwhile, mice implanted in the dorsolateral striatum had bilateral reward responses from the onset of training, though they did not show any baseline stimulus-related dopamine signals. With training, only expertly-performing mice develop dopamine responses to the stimulus tilt in dorsolateral striatum. These results provide new insights into how changes in dopamine signals across the striatum are important for sensorimotor learning, with interesting implications for dopamine-related disorders such as ADHD, OCD, and depression.

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Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

Support: NIH R01 MH099114

Title: Synaptic mechanisms modulate the spatiotemporal dynamics of striatal direct pathway neurons and motoroutput

Authors: *J. J. MARSHALL[1], J. XU[2], N.-H. YEH[2], S. YUN[2], T. NOMURA[3], J. N. ARMSTRONG[2], J. G. PARKER[4], A. CONTRACTOR[2];
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Abstract: Striatal spiny-projection neurons (SPNs) integrate glutamatergic inputs from the motor cortex and thalamus with neuromodulatory signals to regulate motor output. *In vivo* Ca^{2+} imaging has demonstrated that ensembles of direct and indirect pathway SPNs (dSPNs and iSPNs) are coactive during spontaneous movement. Co-activity is statistically greater among nearby neurons, correlates with behavioral state, and undergoes plasticity in an SPN-type-specific manner under pathological conditions. This spatially clustered co-activity could reflect shared excitatory inputs. However, whether and how synaptic mechanisms generate this distinctive spatiotemporal activity is unknown. We found that the Group I metabotropic glutamate receptor 5 (mGluR5), which regulates synaptic strength at corticostriatal synapses, is a key mediator of spatially clustered SPN co-activity. Pharmacological modulation of mGluR5 signaling bidirectionally altered spontaneous movement and spatially clustered neural dynamics, but not the absolute level of activity of dSPNs. Targeted deletion of mGluR5 in dSPNs recapitulated the effects on spatiotemporal neural dynamics and movement demonstrating a striatum-specific effect of mGluR5. Targeted deletion of mGluR5 also produced changes in the synaptic properties of dSPNs. These results demonstrate that the properties of excitatory synapses influence motor function by shaping the characteristic spatially clustered patterns of co-activity that typify dSPN activation *in vivo*.

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Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Program #/Poster #: PSTR227.08/H6

Topic: E.03. Basal Ganglia

Title: Capturing Temporal Dynamics in Human Basal Ganglia Using Low-Dimensional Dynamic Computational Networks

Authors: *M. ASADI[1], *M. ASADI[2], S. JAVADZADEH NO[3], A. SEYYED MOUSAVI[4], T. D. SANGER[5];
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Abstract: Despite extensive research into the dynamics of motor areas in the cortex, the dynamics within the basal ganglia have remained comparatively underexplored. This study aims to determine if the complex neural activities of the basal ganglia can be represented in a simplified, low-dimensional form. Utilizing local field potential data collected from a pediatric patient with Tourette syndrome performing a cued go/no-go task, we implemented a novel machine learning framework that integrates nonlinear autoencoders and simple recurrent neural networks.
Our methodology involves compressing 50-dimensional LFP data to 10 dimensions using a nonlinear autoencoder, followed by the exploration of dynamic structures within these reduced dimensions in a linear space. The initial results indicate that our model achieves an error rate of less than 40% in reconstructing neural activities and predicting subsequent time points.
These preliminary findings suggest that deep brain structures may harbor low-dimensional dynamics that can be effectively revealed through a combination of linear and nonlinear encoding and decoding techniques. This insight underscores the potential for developing more effective treatments and deepening our understanding of the basal ganglia. Future work will focus on refining our model to enhance its accuracy and validate these initial results.

Disclosures: M. Asadi: None. **M. Asadi:** None. **S. Javadzadeh No:** None. **A. Seyyed Mousavi:** None. **T.D. Sanger:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Program #/Poster #: PSTR227.09/H7

Topic: E.03. Basal Ganglia

Title: Go/nogo experiment reveals involvement of basal ganglia regions in initiation and canceling hand reach in human subjects

Authors: *S. JAVADZADEH NO[1], A. SEYYED MOUSAVI[2], M. ASADI[2], T. D. SANGER[3,2]; [1]BME, [2]Electrical engineering department, Univ. of California Irvine, Irvine, CA; [3]Res., Children's Hosp. of Orange County, Orange, CA

Abstract: The basal ganglia-thalamo-cortical circuit is known for its role in execution and cancelation of voluntary movements. It operates through three main pathways: the hyperdirect, indirect, and direct pathways, which regulate movement by exciting or inhibiting the cortex via the globus pallidus internus (GPi), the output nuclei of the basal ganglia. Despite the anatomical connections suggesting a causal link between the basal ganglia (BG) and movement initiation, detecting significant neural activity preceding movement in these regions has been challenging in previous studies. However, post-movement activity in the basal ganglia has been consistently observed.The use of Deep Brain Stimulation (DBS) as a treatment for movement disorders like childhood dystonia provides a unique opportunity to study how BG and thalamic neurons are modulated during movement. During the DBS procedure, electrodes are implanted in various BG

and thalamic nuclei, allowing for the recording of electrical activity. Analyzing this activity can offer insights into the dynamics of dystonia and motor control.In our study, five human participants performed a Go/NoGo task by holding a button until they received a visual cue indicating a "Go" or "NoGo" action. For "Go" cues, they reached for a target button, while for "NoGo" cues, they maintained contact with the initial button until the next cue. Neural activity was recorded from the subthalamic nucleus (STN), GPi, and thalamic regions, which encompass the main pathways of the basal ganglia. Analysis of single-unit activity revealed premovement activity in BG regions, though more pronounced changes occurred after movement initiation. Local Field Potentials (LFPs) from both micro and macro electrodes showed consistent pre movement responses as early as 100ms after cue presentation. Importantly, these LFP responses occurred regardless of cue type (Go/NoGo), suggesting that the basal ganglia may rapidly process cues even before a decision to move is made. Neural activity began to differentiate between Go and NoGo attempts around 300ms after cue presentation.Our results indicate that BG represent both sensory and motor responses with sensory in pre-movement period. While not directly inferred from our results, one possible explanation is that the early activation observed may signify the time needed to kickstart a movement. In this scenario, the BG begins this process upon detecting a cue, then assesses whether to proceed with executing the intended movement or to halt it.

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Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

Title: High-throughput spatiotemporal analysis of signaling dynamics in the striatum during instrumental learning

Authors: *J. JACOBS, J. BERTRAN-GONZALEZ, M. MATAMALES;
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Abstract: The striatum, the entry gate to the basal ganglia, plays a central role in integrating the learning necessary for shaping future behaviour. Current understanding suggests that discrete regions of the striatum underlie specific processes in instrumental conditioning. However, little is known about how these learning processes are encoded in, or communicated between, striatal systems over both space and time. To address this, we mapped the progressive flow of plasticity-dependent molecular activity in the striatum accumulated during learning in transgenic mice undergoing instrumental conditioning. We used a genetically encoded fluorescent kinase translocation reporter (KTR) to measure the transient activation of extracellular signal-regulated

kinase (ERK) *in vivo*, a critical component mediating intracellular signal propagation and molecular activation associated with learning. Cell-specific expression enabled spatiotemporal tracking of ERK dynamics in striatal D1-type spiny projection neurons (D1-SPNs) commensurate with the accumulation of learned experience. Following training and serial confocal imaging, microscopy data passed through a machine-learning algorithm that facilitated high-throughput cell detection and classification of more than 900 D1-SPNs per hemi-section (~5,500 cells/animal). The resulting database was used to engineer a pseudotemporal variable by ordering cells according to the level of KTR-ERK activation, allowing the reconstruction of high-resolution learning-dependent striatal activation motifs. Pseudotemporal dynamics aligned with data obtained from incrementally timed perfusions, validating the construct as a temporal measure reflecting the activation state of D1-SPNs. Directed neural networks were then constructed for each animal through clustering cells by spatial and pseudotemporal dimensions and calculating the degree to which KTR-ERK activity in each node predicted future activity in other nodes. Spatial analysis revealed an overall increase in KTR-ERK activity for the instrumental group (INS) compared to control groups, with this increase localised primarily to the posterior dorsolateral striatum. Network analysis suggested that intra-striatal communication was more refined in the INS group compared to control, and that inactivity of KTR-ERK in the central striatum predicted activity in the posterior dorsolateral and dorsomedial striatum. Taken together, these results support the existing account of functionally defined striatal regions, while also providing an entirely novel insight into how plasticity propagates through the striatal network during instrumental learning.

Disclosures: J. Jacobs: None. **J. Bertran-Gonzalez:** None. **M. Matamales:** None.

Poster

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Title: Cortical projections from M1 and M2 directly excite subpopulations of the Substantia Nigra pars Reticulata, the output stage of the basal ganglia

Authors: *W. THOMPSON, S. GRILLNER, G. SILBERBERG;
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Abstract: The substantia nigra pars reticulata (SNr) is a primary output through which the basal ganglia exert control over motor-related targets in the midbrain, brainstem and thalamus. However, projections from outside the basal ganglia have also been shown to innervate the SNr. Using a virally-targeted optogenetic approach, combined with whole cell patch-clamp recordings from SNr neurons in acute brain slices, we show that projection neurons of the primary and secondary motor cortices (M1 and M2) form functional excitatory synapses with subpopulations of inhibitory SNr neurons. We characterised the kinetic properties and short-term plasticity of this monosynaptic pathway and show that it is capable of effectively increasing SNr neuron firing rate. We investigated the anatomical organisation of cortical input to the SNr by transsynaptically labelling the SNr populations receiving monosynaptic input from either M1 or M2. We found a topographical correspondence between the presynaptic motor cortices and their projections in SNr, and identified distinct downstream targets of the two cortical-recipient SNr subpopulations. Using chemogenetic manipulations *in vivo*, we probed the behavioural roles of the cortical-recipient SNr populations in both spontaneous locomotion and in a learned reaching task. We further investigated the specific contribution of cortical input to these populations by optogenetically silencing synaptic release during behaviour. Our results reveal functional pathways by which M1 and M2 can directly modulate basal ganglia output to different downstream targets.

Disclosures: W. Thompson: None. **S. Grillner:** None. **G. Silberberg:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

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Topic: E.03. Basal Ganglia

Support: R01NS109227

Title: Immediate vs plastic effects of phasic striatal dopamine on rat reach-to-grasp kinematics

Authors: K. CODEN[1], K. ULMER[2], R. LASH[2], H. ZHANG[2], C. BURGESS[3], *D. LEVENTHAL[4];
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Abstract: Striatal dopamine plays an important but incompletely understood role in manual dexterity. Persons with Parkinson Disease (PD) have impaired dexterity that only partially responds to levodopa, suggesting that there are aspects of dopamine signaling not restored by levodopa that are important for fine motor control. We measured phasic dopamine signaling throughout striatum as rats performed a skilled reaching task. Consistent with previous

observations in rats and mice, striatal dopamine increased near reach onset, with post-reach dopamine reflecting the outcome of the reach (grasped/consumed vs dropped). We optogenetically stimulated nigrostriatal pathways either up until or beginning when the paw contacted the pellet. Strikingly, optogenetic manipulations prior to pellet contact had little if any effect on reach kinematics. However, stimulation beginning with pellet contact caused progressive changes in forelimb kinematics across many reaches. These results argue that phasic dopamine signaling provides a feedback signal to adjust coordinated movements with practice, but may not be important for "invigorating" the reach.

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Poster

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Title: Dopamine transients in dorsolateral striatum contribute to age related changes in behavior

Authors: *J. B. ANTHIS[1], W. GILLIS[3], D. R. LEVY[2], S. R. DATTA[1];
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Abstract: Spontaneous behavior is comprised of modular movements or "syllables" which are chosen from a repertoire then sequenced together to form more intricate behaviors. As animals age, the way they behave changes. We've observed that the spontaneous behavior of male mice becomes more stereotyped with age, but it is unknown what neural mechanisms underlie these changes. In adult mice, dopamine systemically fluctuates in the dorsolateral striatum (DLS) as they behave in the absence of any reward or obvious objective. These dopamine signals act as a reinforcer of behaviors while also promoting behavioral variability. We hypothesized that in older male mice, dopamine will still reinforce behaviors but will no longer promote variability. This reinforcement without variability could explain the observed stereotyped behavior. Fiber photometry was used to measure bulk dopamine levels in the DLS in freely behaving mice. To quantify the mice's spontaneous behavior, we used an unsupervised machine learning algorithm utilizing depth footage to extract and identify syllable usage. These recordings were done on male mice ranging from 12 to 98 weeks allowing us to observe any changes in the relationship of dopamine and behavior between mice of different ages. In contrast to young mice, dopamine in older mice was no longer correlated with behavioral variability. We describe how aspects of behavior change with age and provide evidence suggesting that changes in the dopaminergic system in the striatum may support these changes. The investigation sheds light on age-related

changes in brain activity and the direct physiological effects of those changes on the way animals behave. These results raise questions about how the model used for optimizing dopamine evolves with age and how we might reverse aging related effects.

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Poster

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Title: Dopamine encodes trajectory errors based on visual flow and locomotion with varying representations and distinct learning time courses across the striatum

Authors: *E. H. BROWN[1], M.-A. T. VU[2], Y. ZI[1], S. BOUABID[2], B. DEPASQUALE[1], M. HOWE[1];
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Abstract: Dopamine release in the striatum is critical for learning associations between cues, actions and outcomes. Dopamine is well known to encode reward prediction errors, which compute changes in the expectation of an upcoming reward based on external stimuli. Recently however, dopamine signaling across the striatum has been found to vary in its encoding of locomotion, rewards, and stimuli. While these signals hint at distinct functional roles of dopamine across striatal subregions, how spatially varying dopamine release across the striatum contributes to learning and executing context-specific instrumental actions is poorly understood. Here, we used a novel multi-optical fiber array to record high-resolution spatiotemporal dopamine dynamics during a behavioral task which requires mice to run in a particular direction in response to arbitrary visual cues to receive a water reward. We found that dopamine release across the striatum encodes a trajectory error, which compares the current trajectory with the goal trajectory, as indicated by the cue. When the cue indicates a turn in the same direction as the animal's current heading, there is an increase in dopamine release, as the mouse continues in its current direction. However, when the cue indicates a turn in the opposite direction, there is a dip in dopamine release as the mouse switches its running direction. Further, this dopamine trajectory error signal scales with the magnitude of the error. We isolated this novel signal from a trajectory error independent cue response, and found that these two components are separated in

both time and space. Further, positive and negative trajectory error encoding evolved independently across learning, and with distinct time courses across the striatum. Together, these results expand upon our current understanding of what dopamine encodes, its potential behavioral functions, and how its 3-dimensional spatiotemporal dynamics are coordinated across the striatum to support learning.

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Poster

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Topic: E.03. Basal Ganglia

Support: RO1DC017718-01A1

Title: Investigating the modulation of beta bursts in speech and finger tapping across subthalamic nuclei in both hemispheres in Parkinson's disease

Authors: *Z. JOURAHMAD[1], A. H. ROHL[1], J. R. WESSEL[1], J. D. GREENLEE[2];
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Abstract: While the role of the subthalamic nucleus (STN) in speech production has received limited attention, it is integral to the cortico-basal ganglia-cortical circuit, suggesting its involvement in controlling speech motor output. There is evidence that supports the involvement of STN beta-band oscillations (~13-30 Hz) in voluntary movements. Recent studies suggest that STN beta burst activity is more informative regarding pathological conditions and motor behavior compared to average beta power. Therefore, we aimed to investigate the trial-by-trial modulation of STN transient beta bursts identified by calculating the local maxima of time frequency data during two speech tasks and a finger tapping task. We recorded local field potentials (LFPs) from the right and left side STN in 30 participants with Parkinson’s disease (PD) during deep brain stimulation placement surgery. Data was collected from baseline and task periods, which included self-paced interleaved sentence production, syllable repetition and repetitive finger tapping. The power of the beta frequency band was lower during the entire task block (involving speech and finger tapping) compared to the baseline in each hemisphere. The peaks of the relative spectral power of beta frequency bands were higher in left STN vs. right STN. Our findings revealed higher intrahemispheric proportion of trials with beta bursts during the finger tapping task compared to speech tasks, mainly observed in hemisphere contralateral to the tapping hand. This observation can imply the involvement of beta bursts in modifying finger tapping motor behavior but not speech motor behavior. Additionally, in the majority of participants, there was a bi-hemispheric co-occurrence of trials’ beta bursts during the finger tapping task. This was not observed during speech tasks. This finding can suggest the bilateral

involvement of the STN in finger tapping task, consistent with prior research supporting bilateral engagement of STN during motor tasks. Our results demonstrated beta burst modulation predominantly in lower half of beta frequency band on the side contralateral to the tapping hand, with frequencies in higher half of beta frequency band observed on the ipsilateral side. These preliminary findings suggest both unilateral and bilateral involvement of STN beta bursts during motor task but not speech.

Disclosures: Z. Jourahmad: None. **A.H. Rohl:** None. **J.R. Wessel:** None. **J.D. Greenlee:** None.

Poster

PSTR227: Basal Ganglia: Systems Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR227.16/H14

Topic: E.03. Basal Ganglia

Title: Motor alterations and oxidative damage in the substantia nigra after glutamatergic injuries in the red nucleus

Authors: M. RAMIREZ DIONISIO[1,2], ***J. MARTINEZ-LAZCANO**[1];
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Abstract: Functional tractography studies propose a cerebellum-rubro-basal transmission, starting in the deep nuclei of the cerebellum (Cb) that send efferences to the red nucleus (NR) and would end up controlling neurotransmission in the basal ganglia, such as the substantia nigra. (SN). To date, no experimental evidence demonstrates or supports this relay system. As a first approach to establish this possible transmission system, we used glutamatergic lesions directed to the RN with specific glutamatergic agonists for NMDA-type (quinolinic acid; QUIN 45 nmol) and Kainate-type (kainic acid; KA 2.5 nmol) receptors and we evaluated the behavioral alteration, and neuronal-damage marker on the SN. Histological results showed that the lesions are limited to the NR in both groups (QUIN and KA); Injured mice show motor alterations that are maintained for five days after injury in both injured groups compared to the control group; the induction of turning behavior is similar to that presented in lesions directed to the SN, in the QA group; The immunohistology results show decrease in the NR2B subunit of the NMDA-type receptor in the SN only in the group injured with QA. Lipid peroxidation and the generation of reactive oxygen species show increased damage induced by KA, but not in the QA group. In conclusion, the decrease in the density of NMDA-type receptors in the SN, after glutamatergic lesion of the NR could be part of the demonstration of a relay transmission between the SN and the NR, proposed in the different functional tractography studies, this having potential in the treatment of diseases with alterations in the SN.

Disclosures: M. Ramirez Dionisio: None. **J. Martinez-Lazcano:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.01/H15

Topic: E.05. Brain-Machine Interface

Support: Pioneer Fellowship, ETH Foundation: PIO-03 22-2
Bridge Proof of Concept, Swiss National Foundation: 40B1-0_214621
Innosuisse: 113.845 IP-LS
InnoBooster, Gebert Rüf Stiftung: GRS-032/23

Title: Neurophysiological dynamics of transcutaneous auricular vagus nerve stimulation and movement

Authors: C. PERRIN, T. WEILENMANN, *P. VISKAITIS, D. DONEGAN, O. LAMBERCY; ETH Zurich, Zurich, Switzerland

Abstract: Transcutaneous electrical stimulation of the auricular vagus nerve (taVNS) is an emerging non-invasive technology with promising therapeutic applications in neurological diseases. Despite growing evidence that taVNS elicits beneficial neurological effects, the underlying mechanisms are poorly understood as the affected neural structures are susceptible to modulation by internal and external factors, including movement. Understanding the relationship between taVNS and movement can inform both basic neuroscience and therapeutic applications, particularly in cases where motor actions and neural modulation are concurrent or not controlled (e.g., during neurorehabilitation or cognitive tasks). Furthermore, understanding the interplay between motor and autonomic systems can help elucidate mechanisms such as neuroplasticity. This study assessed taVNS effects on autonomic and central nervous system measures during different movement states.

Fourteen healthy adults (23.9 ± 1.8 years; 4 of them females) underwent one session of 156 trials during which heart rate (HR), pupil diameter (PD), galvanic skin response (GSR) and electroencephalography (EEG) were recorded. During each trial, participants received 2 seconds control (no stimulation), 25 Hz aVNS or sham stimulation (at the earlobe), at rest or during right index finger tapping. In movement trials, stimulation was administered before, during, or after movement. A total of 26 randomized conditions were tested.

Analysis revealed widespread and rapid excitatory features in cortical oscillations and brainstem mediated autonomic responses, such as altered PD, GSR and HR. A significant interaction between movement and taVNS was observed in the contralateral motor cortex as measured by EEG alpha power (2-way ANOVA, $n = 14$, $F(1.655, 21.52) = 4.734$, $p = 0.025$). We also discovered a discrepancy in autonomic arousal measures, where changes in HR and GSR were significantly modulated by movement but not stimulation, while PD was also notably influenced by stimulation (2-way ANOVA, $n = 14$, $F(1.919, 23.03 = 6.602$, $p = .006$). Together, these findings reveal differences in taVNS influence on arousal-autonomic measures. Additionally, analysis into timing of taVNS with regards to movement state, revealed that premotor

stimulation resulted in more rapid beta band decrease at movement initiation and greater PD increase, suggesting taVNS may mediate premotor excitatory gain control of movements. These insights contribute to a deeper understanding of how taVNS can be optimized for research and therapeutic applications, emphasizing the importance of considering movement dynamics in its implementation.

Disclosures: C. Perrin: None. **T. Weilenmann:** None. **P. Viskaitis:** None. **D. Donegan:** None. **O. Lambercy:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.02/H16

Topic: E.05. Brain-Machine Interface

Title: Common synaptic input to motor units across the forearm to leverage comprehensive neural drive in stroke and spinal cord injury rehabilitation

Authors: *N. TACCA[1], B. SCHLINK[1], P. PUTNAM[1], S. COLACHIS, IV[1], M. HEIMANN[1], C. DUNLAP[1], M. DARROW[1], L. WENGERD[2], D. FRIEDENBERG[1], E. MEYERS[1]; [1]Neurotechnology, Battelle Mem. Inst., Columbus, OH; [2]NeuroTech Inst., Columbus, OH

Abstract: Non-invasive, high-density electromyography (HD-EMG) provides a simple method to measure a range of neurophysiological motor information. Changes in EMG activity following neurological injury have been shown to provide a quantitative measure of motor function, highlighting the potential for EMG-based features to be used as biomarkers. Recent studies have demonstrated that the common synaptic input to motor units decomposed from non-invasive HD-EMG provides an estimate of neural drive to the muscles, which correlates with functional ability. Typically, motor unit decomposition from HD-EMG has been limited to a single muscle over isometric contractions. However, there has been recent evidence to suggest that pools of motor units within and across muscles are selectively activated as a module by the cortex, which may provide a more comprehensive view of motor function. Here, we demonstrate the decomposition of motor units from a 150-electrode non-invasive HD-EMG sleeve that spans the forearm in individuals with stroke (N=7) and spinal cord injury (N=3) as they attempted various hand and wrist movements. We assess the common synaptic input to the motor units across muscles to extract neural drive, which we show can discriminate motor function groups ($F[2,11]=9.42$; $p=0.0041$) based on Upper Extremity Fugl-Meyer hand sub-score (UEFM-HS; able-bodied, mild: UEFM-HS > 3, and severe: UEFM-HS ≤ 3), and correlates with spasticity clinically measured via the Modified Ashworth Scale ($R^2=0.84$; $p=0.0038$) in individuals with chronic stroke. Furthermore, we show the feasibility of using a real-time estimation of neural drive in an individual with spinal cord injury in conjunction with standard EMG decoding to provide a high degree of freedom (DOF) peripheral “brain”-computer interface to control functional electrical stimulation (FES) that is more closely aligned with the user’s volitional

motor intent. This coupling of motor intent with FES is believed to enhance motor recovery through principles of Hebbian neuroplasticity by synchronizing descending motor commands with the corresponding FES-enabled movement and resultant ascending sensory activation. Therefore, by using a combinatorial approach, there is the potential for a hybrid system that precisely times FES activation with the appropriate inferred cortical activity, while simultaneously enabling high DOF decoding performance to help improve motor rehabilitation.

Disclosures: N. Tacca: None. **B. Schlink:** None. **P. Putnam:** None. **S. Colachis:** None. **M. Heimann:** None. **C. Dunlap:** None. **M. Darrow:** None. **L. Wengerd:** None. **D. Friedenberg:** None. **E. Meyers:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.03/H17

Topic: E.05. Brain-Machine Interface

Title: Wearable, closed-loop functional electrical stimulation technology to improve hand function in individuals with stroke and spinal cord injury

Authors: *B. SCHLINK[1], N. TACCA[1], S. COLACHIS, IV[1], P. PUTNAM[1], C. DUNLAP[1], M. DARROW[1], L. WENGERD[2], E. MEYERS[1], D. FRIEDENBERG[1];
[1]Battelle Mem. Inst., Columbus, OH; [2]Ohio State Univ., Columbus, OH

Abstract: Injuries to the central nervous system, such as spinal cord injury (SCI) and stroke, are devastating and deny individuals the ability to perform basic self-care and leisure activities, reducing their independence and leading to poor psychosocial outcomes. Physical rehabilitation is the gold standard to improve upper limb motor function after SCI and stroke, and it often includes some combination of training with functional electrical stimulation (FES) to supplement and retrain the patient's neuromuscular connections. Recent evidence suggests that pairing FES more closely with the user's volitional motor intent can further improve restoration of hand function following neurological injury by engaging neuroplastic mechanisms, but practical device considerations have limited the widespread adoption of "closed loop" FES therapy. To this end, we recently developed a wearable forearm sleeve with a high-density array of electrodes for simultaneous electromyography (EMG) and FES. The sleeve continuously records the user's muscle activity through the high-density EMG array, decodes muscle activity associated with motor intention (via machine learning algorithms), and delivers FES to the appropriate muscles to evoke the desired movement. Here, we demonstrate preliminary evidence that closed-loop delivery of FES using our wearable system can improve hand function in two cohorts with chronic hemiparesis: 1) Participants recovering from a stroke, and 2) Participants recovering from SCI. Each group went through several weeks of repeated functional task practice while wearing the EMG-FES sleeve. All participants were able to successfully perform tasks that were challenging or impossible to complete without the system. In our cohort of stroke

survivors, we saw improvements on the Action Research Arm Test (ARAT) after repeated closed-loop therapy with our wearable sleeve. In the SCI cohort, we observed improved scores on the Grasp and Release Test (GRT) while using the device. These preliminary data highlight the potential of our closed-loop EMG-FES system as both a rehabilitative tool and a functional orthosis.

Disclosures: B. Schlink: None. **N. Tacca:** None. **S. Colachis:** None. **P. Putnam:** None. **C. Dunlap:** None. **M. Darrow:** None. **L. Wengerd:** None. **E. Meyers:** None. **D. Friedenberg:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.04/H18

Topic: E.05. Brain-Machine Interface

Support: Air Force Research Laboratory
Battelle Memorial Institute internal research and development funds

Title: Natural Communication with Robotic Systems through Gesture-Based Electromyography

Authors: *P. T. PUTNAM, N. TACCA, C. DUNLAP, B. SCHLINK, M. DARROW, E. MEYERS, D. FRIEDENBERG;
Battelle Mem. Inst., Columbus, OH

Abstract: Human-Robotic teaming remains an open challenge and is largely limited by robotic understanding of human actions and intentions. Traditional modalities like computer vision (CV) or motion tracking struggle to capture key features of human intent, such as the amount of force being exerted, require a local environment with external cameras mounted, as well as being susceptible to mistakes due to occlusions and/or lighting. Human-robot collaboration through natural interfaces is a critical for expanding the synergistic partnership between humans and robots, and we hypothesize that high-density electromyography (HD-EMG) wearables have the potential to provide much richer data on human actions without the burden of external infrastructure. To test this, we employed a recently developed wearable forearm sleeve with an array of HD-EMG electrodes, the Battelle NeuroLife® Sleeve, which can sense detailed information about muscle activity underlying hand and wrist movements. Ten able-bodied participants performed 37 hand movements and grasps while EMG was recorded using the HD-EMG sleeve. A CyberGlove hand tracking glove was used to provide ground truth measurements of hand and wrist position, which recorded up to 23 joint-angle measurements at up to 90hz and accuracy of <1 degree. We developed an EMG decoding neural network algorithm to predict hand position, and joint angles in real time. Here, we demonstrate for classifying across the 37 gestures, our decoding algorithm was able to differentiate between sequential movements with 97.3 ± 0.3% accuracy calculated on a 100ms bin-by-bin basis. In a separate mixed dataset

consisting of 19 movements randomly interspersed, decoding performance achieved an average bin-wise accuracy of 92.8 ± 0.8%. When evaluating decoders for use in real-time scenarios, we found that decoders can reliably decode both movements and movement transitions, achieving an average accuracy of 93.3 ± 0.9% on the sequential set and 88.5 ± 0.9% on the mixed set. Furthermore, we estimated continuous joint angles from the HD-EMG sleeve data, achieving a R2 of 0.884 ± 0.003 in the sequential set and 0.750 ± 0.008 in the mixed set. These results demonstrate the HD-EMG sleeve, combined with sophisticated machine learning algorithms, can be a powerful tool for hand gesture recognition and joint angle estimation. This technology holds significant promise for applications in HCI, such as prosthetics, assistive technology, rehabilitation, and human-robot collaboration.

Disclosures: P.T. Putnam: A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **N. Tacca:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **C. Dunlap:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **B. Schlink:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **M. Darrow:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **E. Meyers:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **D. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.05/H19

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01HD106015

Title: Impact of Transcutaneous Spinal Cord Stimulation Parameters on Stroke Survivors: Waveform and Carrier Frequency Analysis

Authors: *C. YANG[1], N. C. VEIT[2], K. A. MCKENZIE[3], S. AALLA[1], A. KISHTA[1], A. JAYARAMAN[1];
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Abstract: Transcutaneous spinal cord stimulation (tSCS) holds promise for enhancing motor recovery, yet its application in stroke survivors presents challenges. The choice between monophasic and biphasic waveforms in tSCS remains underexplored, impacting patient comfort and the overall efficacy of interventions. Additionally, carrier frequency, introduced to alleviate skin discomfort, lacks clarity regarding its influence on resting motor threshold (RMT) in neurological populations. Thirteen participants with chronic stroke completed tolerance and spinal motor evoked responses (sMERs) tests with 12 combinations of 1ms rectangular waveform (biphasic and monophasic) and carrier frequencies (0kHz, 1kHz, 3kHz, 5kHz, 7kHz,

and 10kHz) in randomized order. Cathode electrode was placed at the L1/L2 interspinous process of each participant. RMTs for 8 leg muscles were determined during sMERs tests, as the lowest intensity to elicit a muscle compound action potential greater than 50μV in 3 out of 5 consecutive trials. Tolerance intensity during continuous tSCS was evaluated while participants walked on a treadmill. Additionally, we calculated the tolerance/RMT ratio, the number of activated muscles from sMERs, and the delivered electric charge at RMT intensity for each waveform*carrier frequency combination. Our results demonstrated a significant increase in both RMT ($p<0.001$) and tolerance ($p<0.001$) intensities with higher carrier frequency, while the tolerance/RMT ratio showed no significant difference ($p=0.079$) across carrier frequencies. In addition, there was no significant difference between biphasic and monophasic waveforms in RMT, tolerance, or the ratio. Monophasic waveform (Monophasic: 5.78±3.37 vs. Biphasic: 4.64±2.88; $p=0.003$) and lower carrier frequencies (0kHz: 7.54±0.84 vs. 10kHz: 1.96±2.65; $p=0.007$) tended to activate more leg muscles. The delivered charge at the RMT intensity was greater with higher carrier frequency (10kHz: 109.95±11.46uC compared to 1kHz: 51.26±18.02uC). This study reveals that a higher carrier frequency does not contribute to increased comfort, when considering the increased intensity required to reach RMT. Lower carrier frequencies, combined with a monophasic waveform, exhibit a greater activation of leg muscles. Additionally, higher carrier frequency delivers a greater amount of electric charge to activate the muscles, which presents a higher risk of tissue damage. These findings provide practical guidance for optimizing tSCS parameters, aiming to enhance therapeutic efficacy while minimizing discomfort.

Disclosures: C. Yang: None. **N.C. Veit:** None. **K.A. McKenzie:** None. **S. Aalla:** None. **A. Kishta:** None. **A. jayaraman:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.06/H20

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01HD106015

Title: Impact of Transcutaneous Spinal Cord Stimulation Parameters on Stroke Survivors: Stimulation Location and Electrode Analysis

Authors: *N. C. VEIT[1,2], C. YANG[2], S. AALLA[2], A. KISHTA[2], K. MCKENZIE[2], A. JAYARAMAN[2];
[1]Biomed. Engin., Northwestern Univ., Chicago, IL; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: To translate transcutaneous spinal cord stimulation to different neurological populations such as stroke, it is important to understand how certain stimulation parameters affect the specific population. It has been suggested that varying the placement of electrodes

along the spinal region could have selective activation of different motor pools, which results in targeting specific muscles. In addition to the location, the size of the electrode used can potentially affect the resting motor thresholds (RMT), tolerance to stimulation, and muscle selectivity, which has never been tested before in stroke survivors. Thus, five configurations of electrode placement were tested in 10 participants with stroke: 2.5 cm round electrode over (1) T11-T12 vertebrae, (2) L1-L2, (3) both over T11-T12 and L1-L2 with a splitter cable, (4) both over T11-T12 and L1-L2 as separate channels, and (5) 7.5x13 cm rectangular electrode covering both T11-T12 and L1-L2. A 1 ms biphasic single pulse was delivered at incremental intensities to obtain RMTs of 4 lower limb muscles (HAM, RF, MG, TA) for each configuration. Additionally, the tolerance to stimulation was collected as participants walked in the treadmill with stimulation being delivered at 30 Hz. The percentage of RMT that could be tolerated was measured to determine if a particular configuration improved stimulation tolerance level relative to its RMT. The muscle selectivity was determined by ranking the RMT for each muscle under each configuration to represent the order of muscle recruitment. Overall, the splitter electrodes had the greatest average RMT (96 ± 39 mA) which was significantly different from L1 (83 ± 34 mA, $p=0.04$) and the separate channel electrodes (77 ± 33 mA, $p<0.01$). The highest tolerance was the splitter electrodes (50 ± 30 mA), and lowest tolerance was the separate channel electrodes (28 ± 18 mA, $p<0.01$). Overall, no significant difference existed in tolerance across sites, with participants tolerating on average 53 ± 38% of their RMT. On average for all participants, HAM activated first and TA last when T11 was stimulated. At L1, MG was activated first and RF last. Notable differences in selectivity between proximal and distal muscle were not seen in the other three configurations. In conclusion, different electrode configurations do not change the comfort of stimulation relative to the intensity needed to elicit muscle activation. Instead, electrode configurations appeared to affect muscle selectivity, with T11 and L1 configurations demonstrating different recruitment order between proximal and distal leg muscles. This information can be used when planning spinal stimulation studies in stroke.

Disclosures: N.C. Veit: None. **C. Yang:** None. **S. Aalla:** None. **A. Kishta:** None. **K. McKenzie:** None. **A. jayaraman:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.07/H21

Topic: E.05. Brain-Machine Interface

Support: NIH DP5-OD029571
Meta Reality Labs Award #2990450277899571

Title: Functional Utility and Analysis of Transcutaneous Stimulation at the Wrist for Haptic Feedback from the Hand

Authors: *A. HARRISON[1], A. R. CITTERMAN[2], M. A. TROUT[3], T. N. TULLY[1], K. HANSEN[4], J. A. GEORGE[3];
[1]Biomed. Engin., Univ. of Utah, salt lake city, UT; [2]Gillette Children's Specialty Healthcare, St. Paul, MN; [3]Electrical Engin., Univ. of Utah, Salt Lake City, UT; [4]Utah Neurorobotics Lab/University of Utah, Salt Lake City, UT

Abstract: The long-term goal of this research is to design a non-invasive, wrist-worn interface capable of conveying an artificial sense of touch to users in virtual and artificial environments via transcutaneous stimulation. Our sense of touch plays a critical role in our manual dexterity and ability to explore the world around us. Haptic information in virtual reality is primarily conveyed through sensorized gloves, which tend to use technology like mechanical vibrations or force feedback. These gloves are bulky and limit a user's ability to interact with physical objects, as would be needed for augmented/mixed reality. Here we demonstrate the ability to provide haptic feedback from the hands via transcutaneous electrical nerve stimulation (TENS) at the wrist. We show that wrist TENS can create distally referred sensations at the fingertips or palm of the hand while also allowing unimpeded use of the physical hand for functional tasks. Increasing TENS pulse width (PW), pulse amplitude (PA), or pulse frequency (PF) significantly increased sensation intensity; increasing PW or PA, but not PF, significantly increased sensation perceptive field size; increasing PW, but not PA or PF, significantly increased the spread of the sensations from the centroid (p's < 0.05, 3-way ANOVA). Weber fractions for perceived intensity as a function of activation charge rate were significantly different among PW, PA, and PF ($p < 0.0001$, 1-way ANOVA), suggesting the three parameters cannot be expressed on a single continuum when stimulating transcutaneously. Evoked sensations were rated as possibly natural (2.8/5.0) and mostly comfortable (1.8/5.0). Preliminary data also suggest functional benefits of wrist TENS; participants had significantly lower grip forces ($p < 0.0005$, paired t-test) and fewer "breaks" when transferring virtual fragile objects and could identify different surface textures with up to 100% accuracy using TENS feedback. These findings can help guide the implementation of wrist TENS as an intuitive, non-restrictive haptic feedback interface for augmented and virtual reality interactions in an elegant form factor such as a wristwatch or bracelet.

Disclosures: A. Harrison: None. **A.R. Citterman:** A. Employment/Salary (full or part-time):; Gillette Children's Specialty Healthcare. **M.A. Trout:** None. **T.N. Tully:** None. **K. Hansen:** None. **J.A. George:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.08/H22

Topic: E.05. Brain-Machine Interface

Support: 101092612

Title: Self-optimizing framework for natural sensory feedback through transcutaneous electrical nerve stimulation

Authors: *F. LEONG[1], S. SHOKUR[1,2], S. MICERA[3,2];
[1]Neuro-X, EPFL, Lausanne, Switzerland; [2]BioRobotics Institute, Health Interdisciplinary Center, and Department of Excellence in AI and Robotics, Scuola Superiore Sant'Anna, Pisa, Italy; [3]Bertarelli Fndn. Chair in Translational Neuroengineering, Neuro-X, EPFL, Lausanne, Switzerland

Abstract: Transcutaneous Electrical Nerve Stimulation (TENS) is a promising non-invasive method for providing sensory feedback through peripheral nerve stimulation. Despite its benefits, the conventional application of TENS often results in sensations that users describe as unnatural, primarily characterized by vibrations and tingling. These perceptions can significantly impede the acceptance of TENS, particularly in amputees who rely on sensory feedback for effective prosthetic integration and control. The primary challenge lies in refining TENS parameters to elicit sensations that more closely resemble natural tactile feedback.
This study proposes a framework that redefines the conventional approach to validating sensory encoding strategies in TENS. Traditionally, encoding strategies are pre-defined and assessed by subjectively recording the user's perception. However, our approach reverses this process by adopting a subject-centric methodology. We enable subjects to tailor their sensory experience by manually adjusting stimulation parameters to align with a target sensation. This method not only personalizes the calibration process but also integrates an optimization algorithm that aligns closely with individual sensory perception.
In a three-dimensional space, we track the position of the subject's hand, with each axis representing a variable stimulation parameter that the subject adjusts. This method provides an intuitive means for subjects to explore and optimize the parameter space effectively. Following this 'stimulation search' phase, the optimal parameters are recorded. These parameters are then replayed to the subjects, and their responses are compared against those elicited by conventional methods such as pulse width modulation. To validate the efficacy of this approach, subjects are asked to evaluate which stimulation strategy best replicates the target sensation, providing direct feedback on the perceived naturalness of the sensation. Here we show that our approach elicits a more natural sensation. We also found in some subjects that our approach elicits more frequently the perception of 'tapping' compared to the conventional method.
Given TENS's potential to simulate various haptic modalities, this subject-centric framework could significantly enhance the naturalness of these modalities. Furthermore, the versatility of this framework allows it to be adapted for other stimulation techniques, including invasive methods and ultrasound modulation, broadening its applicability across different sensory restoration technologies.

Disclosures: F. Leong: A. Employment/Salary (full or part-time):; EPFL. 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.; Horizon Europe Research & Innovation Programme under grant 101092612 (Social and hUman ceNtered XR - SUN project). **S. Shokur:** A. Employment/Salary (full or part-time):; EPFL. 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.; Horizon Europe Research & Innovation Programme under grant 101092612 (Social and hUman ceNtered XR - SUN project). **S. Micera:** A. Employment/Salary (full or part-time):; EPFL, SSSA. 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.; Horizon Europe Research & Innovation Programme under grant 101092612 (Social and hUman ceNtered XR - SUN project).

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.09/H23

Topic: I.08. Methods to Modulate Neural Activity

Support: National Research Foundation of Korea 2020M3C1B8081319

Title: Resting-state functional connectivity is modulated by phase-dependent transcranial alternating current stimulation

Authors: J. SEO[1], *B.-K. MIN[2];
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Abstract: Resting-state functional connectivity (rsFC) reflects the intrinsic organization of brain networks and is crucial for understanding cognitive function. Given that, transcranial alternating current stimulation (tACS) offers a potent non-invasive method to manipulate brain oscillations and modulate corresponding brain states. In this study, we investigated the differential effects of 45°- and 180°-phase-lag tACS on rsFC, focusing on the central executive network (CEN), default mode network (DMN), and salience network (SN), which are known to be key networks principally involved in cognitive processing. Twenty-seven participants underwent resting-state fMRI; 6 min for pure resting condition (without tACS treatment) and 30 sec each for 45°- and 180°-phase-lag tACS (in a counterbalanced order across participants) with subsequent 30-s post-tACS condition (3 blocks for each condition). Phase lags in tACS were applied across CEN (bilateral dorsolateral prefrontal cortex [dlPFC] and posterior parietal cortex [PPC]) and DMN (medial prefrontal cortex [mPFC], posterior cingulate cortex [PCC]) nodes. We analyzed seed-based functional connectivity with seed regions of six tACS target areas (bilateral dlPFC, bilateral PPC, mPFC, and PCC) and seven additional key regions for the SN (bilateral rostral PFC, bilateral anterior insula, bilateral supramarginal gyrus [SMG], and dorsal anterior cingulate cortex [dACC]). During the 45°-phase-lag tACS compared to the 180°-phase-lag condition, we observed significantly enhanced rsFC between right dlPFC (a CEN node) and middle temporal gyrus (a DMN node) but reduced rsFC between left PPC (a CEN node) and left premotor cortex. During the 45°-phase-lag tACS compared to the 180°-phase-lag condition, the phase-dependent tACS effects were also observed in the rsFC of the SN nodes, which revealed significantly

higher rsFC between the right anterior insula and the right fusiform; between the left SMG and the left superior parietal cortex; and between the right SMG and the left premotor cortex. Taken together, these findings provide corroborating evidence supporting the importance of phase lags across principal brain networks when administering tACS for effective neuromodulation.

Disclosures: J. Seo: None. **B. Min:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.10/H24

Topic: I.08. Methods to Modulate Neural Activity

Title: Non-invasive vagus nerve stimulation shifts sympatho-vagal balance to the functional optimum

Authors: *S. TUKAEV[1], N. VYSOKOV[2], G. DATKHABAYEVA[3], I. BRAK[4], D. TOLEUKHANOV IV[2];
[1]Natl. Taras Shevchenko Univ. of Kyiv, Educational Scientific Inst. of High Technologies, Kyiv, Ukraine; [2]BrainPatch, London, United Kingdom; [3]Dept. of biophysics, biomedicine and Neurosci., Al-Farabi Kazakh Natl. Univ., Almaty, Kazakhstan; [4]Cognitech Ltd, Astana, Kazakhstan

Abstract: Increased anxiety, severe burnout and depressive disorders are common negative consequences of everyday stress. A modern effective neuromodulation method producing therapeutic effects for treating mental and stress-related disorders is vagus nerve stimulation. The mechanisms of positive affect of the last are not fully understood. Heart rate variability is considered as the most promising indicator of a psychological stress and functional reserves of the organism that modulates is modulating by vagus nerve stimulation a charge-dependent way. We aimed to assess the influence of a novel VNS stimulation protocol on mental and physical health and Heart Rate Variability (HRV) metrics in the workplace environment. 62 healthy volunteers 18-49 years old were recruited for the study. Vagus nerve stimulation was arranged as a 4-day course of 6-minute stimulation sessions with monitoring of Heart Rate Variability. We used the combination of pleasant meditative classical music and a slow bi-polar wave of electrical non-invasive transcutaneous stimulation of auricular area for 5 minutes (BrainPatch platform for non-invasive stimulation). Polar H10 Heart Rate Monitor Chest Strap (Polar Electro, USA) was used to monitor heart rate throughout the study day. Psychological testing (State Anxiety, STAI; psychological stress level, PSM-25; severity of emotional burnout, MBI; depression, IDS) was carried out. Non-invasive stimulation was rated by the participants as a positive experience. We detected beneficial changes in the psychoemotional state of the respondents: improvement of mood, reduction of work stress (PSM-25), emotional exhaustion and professional reduction (MBI), and depression symptoms (IDS). HRV effects of VNS turned out to be short-term and reflected the activation of the parasympathetic nervous system (the

increase of vagally mediated parameter RMSSD and decrease of LF/HF ratio). This reflects a shift towards parasympathetic nervous system dominance. An increase in the spectrum of high-frequency waves indicates a high degree of recovery and readiness for stress.

Disclosures: S. Tukaev: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); BrainPatch. **N. Vysokov:** None. **G. Datkhabayeva:** None. **I. Brak:** None. **D. Toleukhanov:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.11/H25

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R01NS113782-01A1

Title: Non-invasive modulation of brain activity by Transcranial Radio-Frequency Stimulation (TRFS)

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[1]New York Univ. Sch. of Med., New York, NY; [2]Neurosci., New York Univ., Langone Med. Ctr., New York, NY

Abstract: One out of six people worldwide suffer from neurological disorders, many of which deal with drug-resistant conditions. Brain stimulation techniques have proven great merit in the treatment of many of those patients. Among them, non-invasive brain stimulation (NIBS) techniques are of particularly high value as, when effective, they can potentially prevent invasive procedures. Some of the established NIBS methods, e.g. transcranial electrical stimulation, transcranial magnetic stimulation, and transcranial focused ultrasound stimulation, have led to numerous novel insights into brain function and are now widely used in clinical practice, including rehabilitation and treatment of mental disease. Each of these methods has proven some benefits but also comes with limitations. Therefore, the search for novel approaches to non-invasively modulate neural activity, providing a wider range of possible treatments for future clinical applications, is of great interest to the scientific community. Here, we report the application of transcranial radiofrequency stimulation (TRFS) for non-invasive modulation of ongoing neural activity with in-vivo proof-of-concept in mice. Using in-house developed RF circuits, we have been able to induce controlled and safe temperature rises in the brains of mice (with hemispherical preference) without substantially affecting their body temperature. It is well established that temperature changes induce modulation of ongoing neural activity. Our experiments using Ca^{2+} fiber-photometry show that our RF stimulation with thermal effects on the brain can induce significant changes in neural activity in mice. We report suppression of neural activity in TRFS-exposed mice brains in-vivo. In addition, we show that by using this paradigm we can induce reliable changes in animals' behavior. To do so, we show that

hemisphere-specific application of TRFS can induce preference on rotational direction (ipsilateral with reference to the stimulated side) in mice with drug-induced moderate hyperactivity. These preliminary experiments open new possibilities in adding a novel technique to the arsenal of non-invasive brain stimulation paradigms with potential benefits in the treatment of brain disorders.

Disclosures: O. Yaghmazadeh: None. **G. Buzsaki:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.12/H26

Topic: E.05. Brain-Machine Interface

Support: NIH Grant DP5OD029571
ALS Association 1159307

Title: Myoelectric Control of Active Neck Exoskeleton To Restore Head-Neck Motion

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[1]Biomed. Engin., Univ. of Utah, Salt Lake City, UT; [2]Mechanical Engin., Univ. of Utah, Salt Lake City, UT; [3]Electrical Engin., Univ. of Utah, Salt Lake City, UT

Abstract: This work aims to create an effective solution to restore head-neck movements for individuals with dropped head syndrome (DHS). DHS is characterized by weakened cervical paraspinal muscles, most commonly from neuromuscular disorders such as amyotrophic lateral sclerosis (ALS). The resulting chin-on-chest deformity (head drop) causes neck pain, difficulty eating and swallowing, and restricted forward gaze and ambulation. Current management practices typically include stiff braces, which keep the head upright but do not allow any range of motion. Here, we present surface electromyographic (EMG) control of a powered neck brace (Utah neck exoskeleton) to restore intuitive and dexterous head-neck motion. The Utah neck exoskeleton uses a novel linkage mechanism to attain a wider range of physiological motion of the head-neck compared to any static neck collars. Specifically, it allows head flexion-extension (+30°, -15°), lateral bending (+/- 20°), and axial rotation (+/-30°). Joystick control of the device was previously shown to be reliable and dexterous, however, it requires coordinated hand movement to direct head motion, which is unintuitive. Joystick use is also not inclusive to patients with systemic loss of motor control, such as those with ALS. EMG control from neck muscles would utilize the endogenous signals related to head-neck motion to provide more intuitive control. An array of 12 EMG electrodes was placed about the neck over the superficial sternocleidomastoid and splenius capitis muscles. Signals from the 12 electrodes and differential signals between all possible pairs of electrodes (12 choose 2) provided a total of 78 continuous EMG signals sampled at 1 kHz. Healthy participants performed head-neck movements in the three degrees of freedom of the head. EMG signals recorded during these movements were used

to train a modified Kalman filter algorithm that predicted intended head kinematics as input to the exoskeleton. Preliminary studies suggest EMG control from the neck results in similar task performance to other adaptive controllers. EMG control also required significantly less cognitive load, as measured by a detection response task and the NASA Task Load Index survey. Together, these initial results suggest the feasibility of restoring dexterous and intuitive head-neck motion via a myoelectric neck exoskeleton. DHS, to any degree, markedly decreases an individual's quality of life, and affected patients tend to withdraw from any daily activities. Restoration of head-neck motion has the potential to improve quality of life substantially.

Disclosures: M.K. Buczak: None. **J. Brignone:** None. **H. Zhang:** None. **J.A. George:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.13/H27

Topic: I.08. Methods to Modulate Neural Activity

Title: Comparisons of HD-tACS neuromodulatory effects at the M1 hotspot and the conventional C3 site

Authors: *H. MENG[1], M. HOUSTON[2], Y. ZHANG[3], S. LI[4];
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Abstract: Introduction: Transcranial electrical stimulation (tES) often targets the EEG-guided C3/C4 area, which may not accurately represent the M1 for hand muscles. This study aimed to determine whether high-definition transcranial alternating current stimulation (HD-tACS) targeting the M1 hotspot is more effective than targeting the conventionally applied C3 site for neuromodulation.
Methods: A total of 15 healthy subjects received one 20-minute session of HD-tACS intervention (21 Hz at 2 mA) at the M1 hotspot for the flexor digitorum superficialis (FDS) or the C3 sites, randomly, with a 1-week washout period. Subjects performed ball-squeezing exercises with the dominant hand during the HD-tACS intervention.
Results: HD-tACS at the hotspot site significantly increased the MEP amplitude of FDS and the first dorsal interosseous (FDI) muscles but not at the biceps muscle. Additionally, HD-tACS significantly decreased the variability of motor unit firing rates and increased force variability of isometric finger flexion force. However, no significant changes were observed after HD-tACS was applied to the C3 site.
Conclusion: Our results provide strong evidence that targeting the M1 hotspot could optimize outcomes in tES neuromodulation. Additionally, while HD-tACS demonstrated focalized neuromodulation effects, its modulation could extend to adjacent motor areas.

Disclosures: H. Meng: None. **M. Houston:** None. **Y. Zhang:** None. **S. Li:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.14/H28

Topic: I.08. Methods to Modulate Neural Activity

Support: Catalyst Award - Emory University

Title: Non-invasive Spine Stimulation using Temporal Interference (TI) with Applications in Neuromuscular Disorders

Authors: *E. ACERBO[1], R. HOU[2], M. GANTT[2], C.-A. N. GUTEKUNST[3,4];
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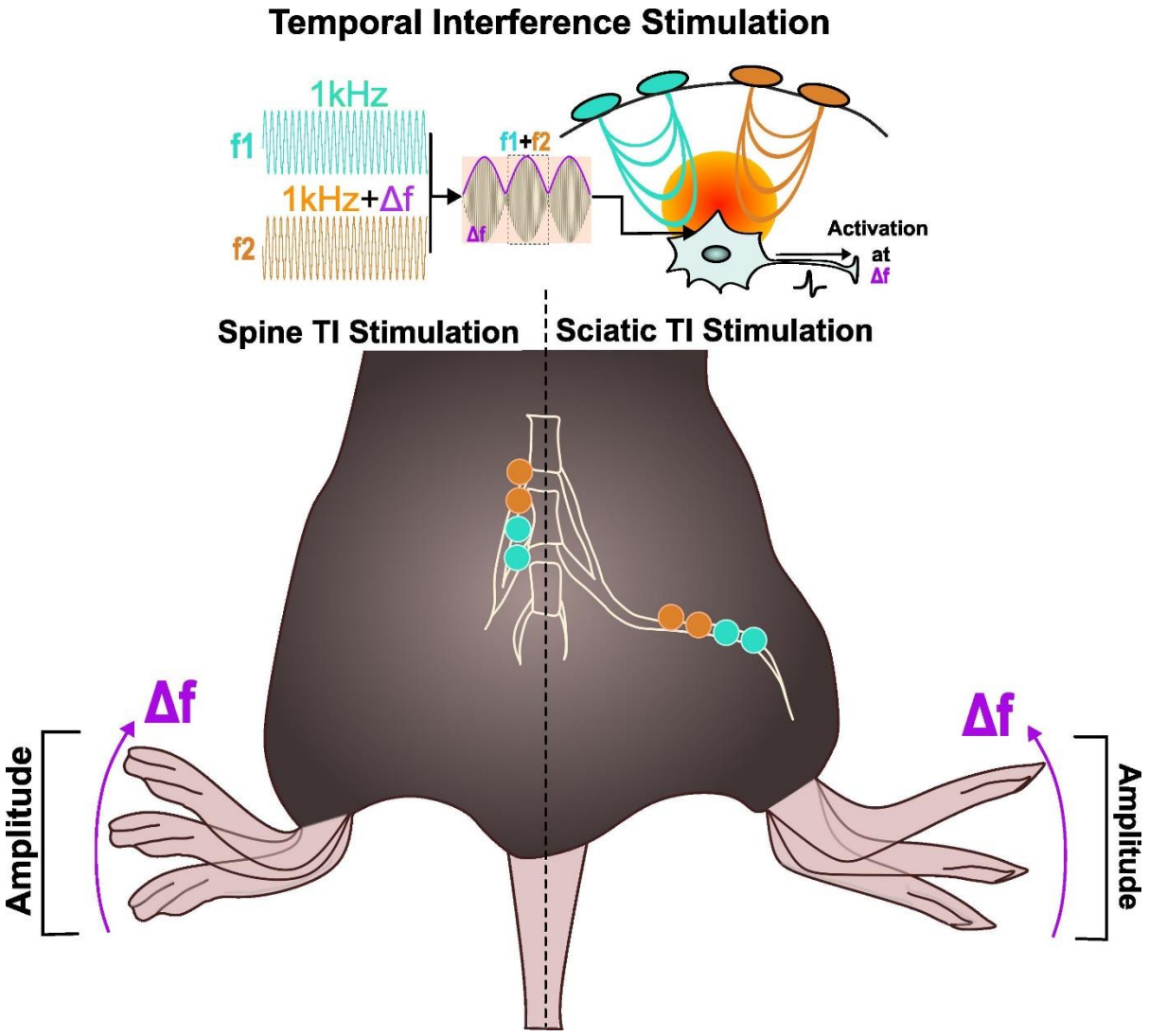
Abstract: Introduction: Temporal Interference (TI) is an innovative, non-invasive technique used to stimulate brain and peripheral nerves (PN) in humans and animal models. This method applies two high-frequency electric fields (>1 kHz) that intersect at a focal point, stimulating tissue at the differential frequency (Δf). Neuronal responses at Δf have been documented in both brain and PN. Despite its potential, TI has not yet been applied to spinal cord, which could be transformative in rehabilitation following traumatic injuries. This study aims to evaluate the efficacy of TI on spinal cord, building on established effects observed in PN stimulation. Methods: We conducted TI stimulation trials (n=7) on anesthetized mice (isoflurane) using two pairs of skin electrodes. Initially, we replicated PN stimulation on the sciatic nerve (n=4) based on previous study, followed by spinal stimulation at the L3-L4 vertebrae (n=3). We gradually increased Δf and assessed the induced leg movements to determine whether spinal cord stimulation responds at the Δf frequency and if the movement amplitudes are comparable to those of PN stimulation. Movement frequency and amplitude were quantified using ImageJ with a motion tracking plugin. Results: Our findings demonstrate that spinal cord stimulation via TI induces movements with frequencies and amplitudes similar to those observed in sciatic nerve stimulation (pvalue= 0.339311), indicating no significant differences between the two stimulation sites. Conclusion: This study is the first to show that spinal stimulation via TI can induce movements, confirming that the effects observed in PN stimulation are due to TI rather than muscular responses to electrode contact. Additionally, to confirm neuronal engagement, future studies will include c-fos staining, providing essential validation of TI's stimulatory effects. Finally, these results have significant implications for rehabilitation therapies for patients with traumatic injuries and may provide a non-invasive alternative to deep brain stimulation systems for Parkinson's disease patients.



Disclosures: E. Acerbo: None. **R. Hou:** None. **M. Gantt:** None. **C.N. Gutekunst:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.15/H29

Topic: E.05. Brain-Machine Interface

Support: NIH 1DP5OD029571-01

Title: Multi-channel functional electrical stimulation to improve specificity and reduce fatigue of evoked motions

Authors: *C. T. STANLEY[1], M. TROUT[2], K. M. SAAD[3], J. A. GEORGE[2], A. NELSON[4]; [1]Electrical & Computer Engin., Univ. of Utah, Salt Lake City, UT; [2]Electrical Engin., Univ. of Utah, Salt Lake City, UT; [3]Physical Med. & Rehabil., Univ. of Utah, Salt Lake City, UT; [4]Biomed. Engin., Univ. of Utah, Salt Lake City, UT

Abstract: The long-term goal of this research is to improve the functionality of non-invasive transcutaneous functional electrical stimulation (FES) by improving specificity and reducing fatigue. Stroke and spinal cord injury result in an estimated 670,000 new cases of upper extremity paralysis yearly. FES can assist and rehabilitate individuals with paralysis by passing current through the skin to activate the paretic muscle and drive neuroplasticity. However, FES often causes rapid muscle fatigue and generates non-specific movements. To address these challenges, we developed a novel multi-channel stimulator for FES with programmatic current steering capable of creating over 6,020 different electrode combinations. A 2x4 array of 22 mm^2 adhesive electrodes were placed longitudinally along the extensor digitorum of one healthy individual with inter-electrode distances of ~20 mm. Software was preprogrammed to automatically sweep through all possible combinations of 2 electrodes (i.e., one cathode and one anode) or 3 electrodes (i.e., two cathodes and one anode or one anode and two cathodes) in 1 mA increments from 6 to 14 mA. At each stimulation current, a total of 191 electrode configurations were explored (8 choose 2 + 8 choose 3) in approximately 191 seconds. Motion capture was used to track the kinematic trajectories of digits 2 through 5, and generated motions were categorized into either no motion or one of the 15 possible single-digit or multi-digit motions (e.g., index, index + middle, all four digits, etc.). Use of 3 active electrodes, instead of 2, significantly increased the total number of stimulation configurations that produced single-digit or multi-digit motions (34 ± 9.29 vs 8.9 ± 3.40 motions, mean ± S.E.M., $p < 0.007$, paired t-test). Using 3 active electrodes trended towards reducing the stimulation current necessary to produce single-digit or multi-digit motions, although this was non-significant (9.75 ± 0.41 vs 10 ± 0.39 mA, $p = 0.136$, paired t-test). Across all stimulation currents, use of 3 active electrodes increased the total number of unique motions generated, out of the 15 possibilities (6.78 ± 1.70 vs 4.33 ± 1.09 unique motions, $p < 0.05$, paired t-test). Across the motions, there was also a significantly greater number of redundant stimulation configurations capable of producing the same motion (56.78 ±15.59 vs 14.78 ± 4.12 configurations, $p < 0.01$, paired t-test). Future work will explore how the additional motions can be leveraged to enhance FES specificity, and if redundant stimulation configurations can be interleaved to reduce FES fatigue. Improvements in FES specificity and duration of use may improve rehabilitation and/or quality of life.

Disclosures: C.T. Stanley: None. **M. Trout:** None. **K.M. Saad:** None. **J.A. George:** None. **A. Nelson:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.16/H30

Topic: E.05. Brain-Machine Interface

Support: NIH 1DP5OD029571-01

Title: Design and Validation of an Adaptive Controller for Functional Electrical Stimulation to Control Grip Force

Authors: *M. TROUT[1], C. T. STANLEY[1], K. M. SAAD[2], J. A. GEORGE[1];
[1]Electrical and Computer Engin., Univ. of Utah, Salt Lake City, UT; [2]Physical Med. & Rehabil., Univ. of Utah, Salt Lake City, UT

Abstract: The long-term goal of this research is to develop a robust, precise controller that is straightforward to set up to improve the translation of upper limb FES to rehabilitation clinics. Functional electrical stimulation (FES) can be used to activate muscles to rehabilitate patients after a cerebrovascular accident (CVA). While FES is regularly used in clinics to rehabilitate patients' lower limb function, FES is rarely leveraged to rehabilitate grasping function as robust, precise control of a hand via FES is difficult to achieve and may require extensive set up time. To address this problem, we developed an adaptive FES controller for controlling grip force that requires minimal setup. The controller uses a first-order model and adaptive model predictive control (aMPC) to control an individual's grip force using the stimulation current amplitude. No tuning is required, and the only setup is finding the individual's twitch threshold and maximum comfort value. We validated the controller design in simulation and with a healthy population. We first tested the control scheme using a modified version of the Xia-Law fatigue model and verified that the adaptive model would appropriately converge, and that the controller could follow a given setpoint. To validate the control scheme in a healthy population, FES was applied to two healthy participants to flex their middle fingers while the resulting grip force was measured. Sine waves with periods of one second and 0.5 seconds were used as setpoints for the controller to track. The amplitude of the setpoint waveforms was set to 15% of the participant's maximum evoked grip force. A proportional-integral (PI) controller was tuned at the beginning of the experiment to serve as a comparison point. When tracking the 0.5 Hz sinusoid, the PI controller resulted in a root mean square error (RMSE) of 0.909 ± 0.159 N, and the aMPC controller resulted in an RMSE of 0.560 ± 0.460 N. When tracking the 1 Hz sinusoid, the PI controller resulted in a root mean square error (RMSE) of 0.879 ± 0.094 N, and the aMPC controller resulted in an RMSE of 0.908 ± 0.117 N. The development of this adaptive control scheme that requires no tuning and performs similarly to a tuned controller constitutes an important step towards more widespread use of FES for rehabilitating prehension during CVA recovery. Future work will leverage this control scheme to apply precise FES to control a paretic hand during hand rehabilitation.

Disclosures: M. Trout: None. **C.T. Stanley:** None. **K.M. Saad:** None. **J.A. George:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.17/H31

Topic: I.08. Methods to Modulate Neural Activity

Support: CIHR 410020048
CRC-2018-00042

Title: Toward personalization of alternating current stimulation (ACS): the dependence of brain response on ACS amplitude and frequency

Authors: *M. BELL VILA[1], P. BAZZIGALUPPI[2], M. KOLETAR[2], A. E. DORR[2], J. G. SLED[3,4], M. GOUBRAN[1,5], B. STEFANOVIC[2,4];
[1]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [2]Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Mouse Imaging Ctr., Hosp. for Sick Children, Toronto, ON, Canada; [4]Medical Biophysics, University of Toronto, Toronto, ON, Canada; [5]Sunnybrook Research Institute, Toronto, ON, Canada

Abstract: Alternating current stimulation (ACS), a noninvasive neuromodulatory method in which low-intensity oscillating currents are delivered to the brain, is particularly promising due to its low cost, portability, and frequency selectivity that may afford targeting of specific neuronal subpopulations. Although its therapeutic potential has been demonstrated in Parkinson's disease, stroke, and Alzheimer's disease, the outcomes have been highly variable across subjects, largely due to the failure to personalize treatment parameters. To elucidate the mechanisms of action underlying ACS, and thus pave the way for treatment optimization within individuals, we have investigated the effects of ACS frequency and amplitude on cortical neuronal response in an adult rat model. Custom made stimulation electrodes were adhered directly on the skull of 22 (11M / 11F), 3-month old propofol anaesthetized Sprague-Dawley rats (44 mg/kg/hr iv), medial and lateral to a 6-by-4.5 mm cranial window (AP - 1.0 to -7.0 mm, ML +1.0 to +5.5 mm). In 9 animals, twenty 1-minute ACS trials were delivered, spanning 4 amplitudes (50, 100, 200, 400 μA) and 5 frequencies (5, 10, 20, 40 Hz) in randomized order. The electrophysiological activity in layer 2/3 was recorded via 4x4 electrode arrays. In 13 rats, 20-minute ACS at 40Hz and 100μA was delivered to investigate whether modulation of neuronal activity could be elicited beyond ACS offset. The LFP (0-300Hz) were used to compute power spectrums and low-frequency to high-frequency phase amplitude coupling (PAC), while the MUA (500-3000Hz) were analyzed to quantify changes in neuronal firing rates and phase locking values, a measure of neuronal entrainment. Results revealed a frequency and amplitude dependence in neuronal responses to ACS. Increasing stimulation frequencies and amplitudes entrained significantly more neurons, and increased their degree of entrainment. On average, firing rates were decreased during the stimulation period, across all stimuli. Increasing the stimulation frequency further attenuated average firing rates, though changes in firing rates remained below 5 Hz. In contrast, the number of neurons showing significantly different firing rates following ACS was not affected by ACS amplitude or frequency. The 20-minute ACS elicited sustained changes in firing rates, entrainment, power, and low-frequency to high-frequency PAC. Our data demonstrate sustained, frequency- and amplitude-dependent effects of alternating current stimulation on the temporal pattern and amount of neuronal network activity, supporting the tailoring of this treatment to individual subjects based on their baseline brain state.

Disclosures: M. Bell Vila: None. **P. Bazzigaluppi:** None. **M. Koletar:** None. **A.E. Dorr:** None. **J.G. Sled:** None. **M. Goubran:** None. **B. Stefanovic:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.18/H32

Topic: I.08. Methods to Modulate Neural Activity

Title: The Effects of Bilateral Anodal tDCS of M1 on Corticomotor Excitability and a Bimanual Videogame Skill Acquisition

Authors: *D. R. GREENWELL[1], J. FEIGH[1], Z. A. RILEY[2], B. J. POSTON[3];
[1]Sch. of Hlth. and Human Sciences: Kinesiology, Indiana Univ. Indianapolis, Indianapolis, IN; [2]Sch. of Hlth. and Human Sciences: Kinesiology, Indiana University- Indianapolis, COLUMBUS, IN; [3]Univ. of Nevada, Las Vegas Neurosci. Grad. Program, Las Vegas, NV

Abstract: Stimulation of the motor cortex (M1) with transcranial direct-current stimulation (tDCS) appears to enhance corticomotor excitability and learning in a variety of simple, unimanual motor tasks. However, the effects of tDCS in more complex bimanual tasks are unclear. The purpose of this study was to investigate how bilateral anodal tDCS of M1 (biM1a-tDCS) affects bimanual motor learning.For this study, 37 healthy, right-handed adults were recruited to complete three visits to the lab (~48 hours between visits) where they would receive either active (n = 21) or sham (n = 16) biM1a-tDCS while playing a bimanual racing video game with a customized dual-joystick control scheme. At the beginning and end of the first visit, transcranial magnetic stimulation (TMS) was delivered to each M1 and TMS-evoked potentials were recorded from the contralateral abductor pollicis brevis muscles using surface electromyography (EMG) to generate a stimulus response curve (SRC). Our results indicate that when subject "gaming experience" is included as a covariate, there is no significant difference between groups in relative improvement ($p = 0.279$). Additionally, while no significant between-groups differences in left hemisphere pre-to-post SRC (SRCΔ) were observed ($p = 0.424$) between-groups differences in right hemisphere SRCΔ between were significant ($p = 0.033$). Interestingly, a significant sex*condition interaction was observed in relative, within-day performance change ($p = 0.05$) and a significant sex*SRCΔ interaction were observed for the right ($p = 0.005$) but not left ($p = 0.14$) hemispheres. Perhaps from stronger corticomotor excitability effects, these results suggest that bilateral anodal tDCS of M1 may improve bimanual motor learning in females but not males. However, it is unclear why this sex-discrepancy exists, and further investigation is warranted.

Disclosures: D.R. Greenwell: None. **J. Feigh:** None. **Z.A. Riley:** None. **B.J. Poston:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.19/H33

Topic: I.08. Methods to Modulate Neural Activity

Support: UL1TR002003

Title: The effect of endogenous theta and alpha rhythms on offset theta-tACS efficacy in an internalizing psychopathology sample

Authors: *J. MCALEER[1], O. A. AJILORE[2];
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Abstract: Internalizing psychopathologies (IPs) affect approximately 30% of individuals worldwide and are characterized by dysregulated emotion regulation. These individuals display higher levels of theta synchrony at rest as well as aberrant theta connectivity during an emotion regulation task. In a pilot study utilizing offset mid-theta transcranial alternating current stimulation (tACS) with individualized electrode placement, participants displayed significant decreases in anxiety and depression metrics after receiving 4 sessions of stimulation (2 sham, 2 verum). In this subsequent analysis, we sought to determine if stimulation efficacy correlated with individuals' endogenous frequencies. EEG data were Fourier transformed and both peak theta (4-8Hz) and peak alpha (8-13Hz) were determined for each electrode site between which participants displayed highest levels of baseline synchrony and at which tACS stimulation was subsequently delivered. The sites were then averaged to produce each individual's peak theta and peak alpha frequencies. Spearman correlational analyses revealed that individuals' peak theta significantly correlated with their overall changes in depression scores (rho=.632, p=.020) and were insignificantly correlated with overall changes in anxiety scores (rho=.436, p=.126), such that peak theta values closest to the stimulation frequency (6Hz) related to greater changes in IP metrics. Additionally, individuals' peak alpha frequencies were inversely correlated with changes in anxiety (rho=-.546, p=.043) and were insignificantly correlated with changes in depression (rho=-.448, p=.108) such that higher alpha frequencies were related to greater changes in metrics. These results add to the growing number of studies that indicate greater tACS efficacy when participants receive stimulation as their peak frequencies. Additionally, we posit that higher individual alpha levels may indicate a greater capacity for changes in anxiety levels due to intervention.

Disclosures: J. McAleer: None. **O.A. Ajilore:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.20/H34

Topic: I.08. Methods to Modulate Neural Activity

Title: Neurofeedback Training to Improve Cognitive Performance in Aging

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Abstract: Processing speed can be defined as the amount of time it takes to extract, synthesize, and respond to presented information. Processing speed, along with other cognitive domains, declines as a function of age. Aging-related decline in cognitive functioning, including working memory, may be explained by a general slowing of this information processing, although it is not fully understood. Specifically, older adults tend to report difficulties in everyday tasks that rely on the ability to suppress irrelevant, or distracting, visual information, which may interfere with processing efficiency. A proposed mechanism by which the brain selectively inhibits irrelevant visual processing is by alpha oscillations (8-12 Hz) in the occipitoparietal cortex. Alpha activity, as measured by electroencephalography (EEG), can be thought of as a “sensory gate” to incoming stimuli, with increases in activity to suppress distractors and decreases in activity to enhance targets in visual attention tasks. Older adults do not modulate alpha as younger adults; however, limited research to date has reported stronger overall alpha modulation when people are better at suppressing irrelevant information. The goal of this study is to evaluate the effects of occipitoparietal alpha band EEG neurofeedback on cognitive performance, including processing speed, across age groups. The first session will consist of cognitive and behavioral assessments in which the primary outcome measure is DSST. The second session will consist of a visual attention assessment utilizing EEG. Participants will return for three subsequent neurofeedback training sessions involving alpha modulation using EEG. Participants will then repeat the behavioral and visual attention assessments at their sixth and final session. Our Study Aim 1 will determine the relationship between alpha modulation and cognitive performance, including processing speed. We hypothesize that participants who better modulate alpha will have better measures of cognitive performance. Further, we hypothesize that younger adults will exhibit better alpha modulation and have better cognitive performance compared to older adults. Our Study Aim 2 will evaluate the effects of alpha modulation neurofeedback training on cognitive performance, including processing speed. We hypothesize that neurofeedback training will improve cognitive performance, including processing speed, and we expect older adults to show greater changes in alpha modulation and cognitive measures compared to younger adults.

Disclosures: A.M. Weber: None. **K.M. Visscher:** None. **K. McGregor:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.21/H35

Topic: E.05. Brain-Machine Interface

Support: Innosuisse Grant 103.116 IP-LS

Title: Upper-limb movement decoding from electrocorticography: stability and reliability

Authors: E. DE MONTALIVET[1], N. MOJTAHEDI[2], I. TOYE[3], V. SPAGNOLO[4], T. COLLIN[5], J. BLOCH[6], G. COURTINE[7], H. LORACH[8], ***K. LEE**[9];
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Abstract: Recent advances in brain-computer interface (BCI) hardware enabled a long-term use of the implantable devices in the human body. This allows a user to interact with the environment in the long term, giving an opportunity to explore different BCI strategies. One important research topic is to achieve a stable decoding performance without minimal recalibration process. As both the brain and electrodes undergo change in their properties, it is important that the decoder also adapt to this change continuously. These challenges are exacerbated by structural and functional differences among individuals' brains as well as variations across multiple sessions within the same subject or across different subjects. Neural signals also exhibit complex temporal dynamics and a nonlinear relationship with the stimuli or actions they represent, demanding advanced decoding strategies that can accommodate such complexities. Deep learning models are promising candidates to deal with these complexities. In this work, we design and present decoding methods that address the problems on ECoG data collected from a period of a few months while performing upper-limb tasks that involve wrist, elbow, and shoulder joints. We present the evolution of the contribution of each electrode and powerband for decoding over the entire period of data and discuss how we solved the problem. We present how the underlying structure of the brain signals shift and how to adapt to this shift for a more reliable decoding by applying manifold alignment methods through self-supervised learning. Specifically, we use a generative model that can identify the shift in the latent space and align the brain data so that the same pretrained decoder can predict with a comparable accuracy as when it was trained. Moreover, we explore the application of foundation models, which are robust pre-trained models developed on extensive ECoG datasets in a self-supervised way. These models are designed to capture the structural representations of neural dynamics which act as feature extractors for downstream tasks, resulting in improved accuracy and generalization.

Disclosures: E. de Montalivet: None. **N. Mojtahedi:** None. **I. Toye:** None. **V. Spagnolo:** None. **T. Collin:** None. **J. Bloch:** None. **G. Courtine:** None. **H. Lorach:** None. **K. Lee:** None.

Poster

PSTR228: Non-Invasive Neuroprosthetics

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR228.22/H36

Topic: E.05. Brain-Machine Interface

Support: Japan Society for the Promotion of Science 22J01084
Buoniconti Foundation
Florida Department of Health
The Miami Project to Cure Paralysis

Title: Brain-controlled neuromodulation for facilitating corticospinal excitability of lower-limb muscles

Authors: *A. SASAKI[1], V. CHARLET[2], **R. FADLI**[1], T. NOMURA[3,4], M. MILOSEVIC[1]; [1]Univ. of Miami Miller Sch. of Med., Miami, FL; [2]Dept. of Biomed. Engin., The Univ. of Strasbourg, Illkirch-Graffenstaden, France; [3]Kyoto Univ., Kyoto, Japan; [4]Grad. Sch. of Engin. Sci., Osaka Univ., Toyonaka, Japan

Abstract: Noninvasive brain-computer interface (BCI)-controlled functional electrical stimulation (FES) has been successfully used for neuromotor recovery after neurological injuries such as stroke and spinal cord injury. While the neurophysiological effects of BCI-FES on upper extremity muscles have been studied, the impacts of lower-limb BCI-FES on neural excitability remain relatively unexplored. This study aimed to investigate the effect of short-term BCI-FES intervention targeting lower-limb muscles on corticospinal excitability. Twelve individuals participated in three different interventions: (1) Synchronized BCI-FES: FES was delivered to the tibialis anterior (TA) muscle when BCI system detected the participant's intention to move; (2) Unsynchronized BCI-FES: FES was delivered before the BCI detect motor intention; (3) Random FES: FES was randomly triggered while the participant remained at rest without performing any tasks. In the Synchronized and Unsynchronized BCI-FES condition, electroencephalography signals were processed online to detect event-related desynchronization of cortical oscillatory activity during ankle dorsiflexion motor imagery. The success rates of BCI detection in synchronized BCI-FES and Unsynchronized BCI-FES conditions were 87.8 ± 4.8 % and 88.3 ± 4.3 %, respectively. Each intervention lasted 25 minutes and was divided into two 12.5 minute sessions to enable assessments before, during, immediately after, and 30 minutes after the interventions. Motor evoked potentials (MEPs) in the TA muscle, elicited through transcranial magnetic stimulation (TMS) of the primary motor cortex, were assessed to evaluate corticospinal excitability. Additionally, F-waves elicited by common peroneal nerve stimulation were assessed to evaluate spinal motoneuronal excitability. Results showed that MEPs, but not F-waves, were facilitated only after the Synchronized BCI intervention and these effects lasted for at least 30 min in follow-up. Such modulations were not observed after the Unsynchronized BCI and Random FES conditions. These findings suggest that short-term (25 min) intention-driven neuromodulation intervention could effectively and efficiently enhance corticospinal excitability, but not motoneuronal excitability, of the lower-limb muscles. The lack of corticospinal facilitation with unsynchronized BCI-FES and FES alone emphasizes the importance of time-synchronized associative cortical and peripheral activation during BCI-FES for effective neuromodulation in lower-limb muscles.

Disclosures: A. Sasaki: None. **V. Charlet:** None. **R. Fadli:** None. **T. Nomura:** None. **M. Milosevic:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.01/H37

Topic: E.07. Rhythmic Motor Pattern Generation

Support: KAKENHI JP19K18951
KAKENHI JP23K15966
KAKENHIJP22K09918

Title: Optogenetic activation of serotonergic neurons in the dorsal raphe nucleus changes masticatory movements in freely moving mice

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Abstract: Mastication is one of the most common rhythmic behaviors in mammals, such as respiration and locomotion. The serotonergic system plays an important role in rhythmic behaviors; however, whether serotonin (5-hydroxytryptamine, 5-HT) is critical for the control of mastication remains unknown. Here, we used optogenetic techniques to investigate the effects of 5-HT on masseter and digastric muscle activties during voluntary mastication in mice. We obtained transgenic mice expressing channelrhodopsin-2 (ChR2) mutant (C128S) only in central 5-HT neurons by crossing tetO-ChR2 (C128S)-EYFP knock-in mice with Tph2-tTA BAC transgenic mice. An optical fiber was inserted into the dorsal raphe nucleus (DRN) or the raphe obscurus nucleus (ROb). Blue light (1 s duration, 1 mW) was applied in freely moving mice to open ChR2. Yellow light (1 s duration, 1 mW) was used as a negative control. Electromyography (EMG) of the masseter and digastric muscles was recorded to measure masticatory frequency, the root mean square (RMS) peak amplitude and EMG burst duration. Optogenetic activation of DRN 5-HT neurons during voluntary mastication significantly increased the masticatory frequency and decreased the RMS peak amplitude in the masseter muscles and EMG burst duration in the masseter and digastric muscles. These changes were blocked by the selective 5-HT_{2A} receptor antagonist MDL 100907 (0.3 mg/kg, s.c.). In contrast, optogenetic activation of ROb 5-HT neurons during voluntary mastication did not affect masticatory frequency and EMG burst in the masseter and digastric muscles. Optogenetic activation of DRN or ROb during the resting state did not induce rhythmic jaw movements. These results suggest that activation of DRN or ROB 5-HT neurons may not be responsible for the initiation of masticatory movement, whereas activation of DRN 5-HT neurons during voluntary mastication induces a fast pattern of masticatory movement, possibly contributing to masticatory pattern changes during a masticatory sequence.

Disclosures: M. Dantsuji: None. **A. Mochizuki:** None. **K. Nakayama:** None. **S. Nakamura:** None. **T. Inoue:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.02/H38

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH RO1NS094176
UMF B-1222-02

Title: Dopaminergic signaling differentially modulates NMDA- and optogenetic-induced spinal locomotor output in larval zebrafish

Authors: *B. MERCIER[1], M. A. MASINO[2];
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Abstract: Spinal locomotor networks have the unique capacity to generate rhythmically patterned outputs. Locomotor output can be shaped by neuromodulation, such as dopaminergic signaling, to refine activity during development (Lambert et al., 2012). The application of N-methyl-D-aspartate (NMDA) has been used to produce locomotor activity in spinal circuits isolated from descending inputs. The use of NMDA to induce locomotion restricts our understanding of the roles of NMDA receptors (NMDARs) in the circuit and any potential interactions between neuromodulators, such as DA. While DA is known to interact with NMDARs in the brain (Wang et al., 2012), we are interested in investigating possible interactions in the spinal locomotor circuits. Thus, an alternative to NMDA-induced locomotion is the use of optogenetics to produce patterned rhythmic locomotor activity in spinalized larval zebrafish (Montgomery et al., 2021). Importantly, this paradigm eliminates the requirement of pharmacological activation of spinal locomotor activity and yields an intact spinal locomotor circuitry that is functionally isolated from the brain. We demonstrated that the locomotor pattern was not dependent on NMDAR signaling (Wahlstrom-Helgren et al., 2019). The use of optogenetic activation of spinal locomotor networks allows us to examine interactions between neuromodulators such as DA and all glutamate receptor subtypes. Due to the role of DA in refining locomotion during development (Lambert et al., 2012), we compared the effects of DA and its agonists on the spinalized locomotor pattern of immature (3 dpf) larval zebrafish when locomotion was induced either optogenetically or with NMDA. In response to optogenetic-induced activity, DA (5µM) led to an increased number of episodes (baseline: mean 3.33 episodes, treatment: mean: 5.83 episodes) and decreased episode durations (Baseline: mean 3.47s, treatment: mean 1.34s) (n=6). Dopamine 4 receptor (D4R) agonist (PD 168 077, 1µM) in optogenetic preparations did not change episode number (Baseline: mean 2.86 episodes, treatment: mean: 2.86 episodes) or duration (baseline: mean 3.07s, treatment: mean: 3.07s) (n=7). In contrast, in response to NDMA-induced activity, DA increased variability of the locomotor pattern (coefficient of variation, episode durations: baseline: 0.58, treatment: 0.83) (n=4), and the addition of a D4R agonist led to an increase in the number of episodes (baseline:

mean 40.00 episodes, treatment: mean: 59.57 episodes) and a decrease in the episode duration (baseline: mean 2.08s, treatment: mean 1.53s) (n=7). We plan to further probe these interactions using optogenetics and pharmacological perturbations.

Disclosures: B. Mercier: None. **M.A. Masino:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

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Program #/Poster #: PSTR229.03/H39

Topic: E.07. Rhythmic Motor Pattern Generation

Support: MarMaRa Institute
NeuroMarseille

Title: Role of Na_v 1.1 and Na_v 1.6 Sodium Channels in Mediating Inspiratory Rhythm Generation and Gasping: Implications for Respiratory Dysfunction in Epilepsy

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Abstract: Epilepsy affects more than 65 million people worldwide, making it one of the most common chronic neurological disorders. Moreover, patients with epilepsy have a higher risk of sudden death (SUDEP). A variety of mechanisms may cause or contribute to SUDEP, such as cardiac arrhythmias, autonomic dysfunction, airway obstruction, and brainstem spreading depolarization. However, a growing body of evidence now strongly indicates that a substantial subset of SUDEP is due to seizure-induced respiratory arrest. The network responsible for the central command of breathing is embedded in the ventral respiratory column (VRC) of the brainstem. The PreBötzinger Complex (preBötC) is both necessary and sufficient for generating the inspiratory rhythm. This network can be isolated in reduced preparation *in vitro*, which has facilitated many insights into the cellular basis of inspiratory rhythm generation. It has been well-documented that the inspiratory rhythm relies on the interplay between synaptic and intrinsic properties. Among the intrinsic properties, the persistent sodium current plays an important role in sustaining the bursting pacemaker activity in preBötC neurons. The persistent sodium current is, in part, mediated by the voltage-gated sodium channel Nav 1.6. However, the preBötC expresses different subunits, within which Na_v 1.1 and Na_v 1.6 are the most expressed in the VRG and the preBötC. Here, we investigated the role of Nav 1.1 and Nav 1.6 using different mice models, including a Dravet Syndrome mouse model. In vivo, Scn1aR1648H/+, Scn1aR1407X mice have a slower respiratory frequency compared to wild-type and Scn8a null mice (Scn8amed/+). *In vitro*, all mouse models can generate inspiratory activity, but the Scn1aR1407X mice have a slower rhythm. We then investigated the generation of the inspiratory rhythm using specific sodium channel blockers for the Nav 1.1 and the Nav 1.6 isoforms.

Although the inspiratory rhythm *in vitro* is not significantly affected after the application of the different sodium channel blockers, subsequent application of FFA 40-80 µM to block ICAN affects the generation of inspiratory activity. Under anoxic conditions, gasping is also affected after the blockade of Nav 1.1 and Nav 1.6 sodium channels. Taken together, Nav 1.1 and Nav 1.6 may mediate the persistent sodium current in the preBötC and contribute to the inspiratory rhythm generation in normoxic and anoxic conditions.

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Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.04/H40

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF Grant 2320895

Title: Long-term regulatory effects of neuromodulators on the ionic currents' parameter space in the oscillatory neurons

Authors: *S. MORE-POTDAR[1], J. P. GOLOWASCH[2], *S. MORE-POTDAR[3];
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Abstract: Neuronal activity in oscillatory neurons results from dynamic interactions between multiple intrinsic and synaptic currents. Neuromodulators are known to regulate neuronal oscillations by exerting instantaneous effects on some ionic currents. For example, IMI is a voltage-dependent, modulator-activated, inward current expressed by pyloric neurons in the stomatogastric ganglion of *Cancer borealis* crabs (Schneider et al., 2021). In *C. borealis*, the experimental removal of neuromodulators (decentralization) inactivates IMI and immediately slows or blocks the pyloric rhythmic activity (Luther et al., 2003). While the short-term effects of neuromodulators on ionic currents are relatively well studied, their long-term effects are not. Our initial results showed that decentralization not only slows down rhythmic activity in pyloric neurons but also changes the parameter space of ionic currents, defined as the multidimensional volume occupied by the maximal currents of a population of identical cells. For example, the pair-wise parameter space composed of two voltage-dependent outward currents ,*i.e*., the high-threshold potassium current (IHTK) and the transient A-type of current (IA), expands significantly ($p < 0.05$) four hours after decentralization. Based on this observation, we hypothesized that over time, neuromodulators constrain the parameter space of ionic currents and enable the generation of stable neuronal activity and that removing neuromodulators should relax those constraints and allow the parameter space to expand. To test this hypothesis, we examined two neurons in the pyloric network: the pyloric dilator (PD) neuron and the lateral pyloric (LP) neuron. We measure eight intrinsic currents (Ileak, IHTK, IA, IH, IMIT, IMI, IKCa, IKd) and

two synaptic currents (ISyn (PD-LP), Isyn (LP-PD)) at two time points: immediately after and at eight hours after decentralization in PD and LP under different conditions. We expected to observe the volume of the multidimensional parameter space to be small before decentralization and to expand eight hours after. We further expected neuromodulator replacement after decentralization to revert the effect on parameter space, and restoration of activity without neuromodulators to be unable to revert the effect. Our results confirm our hypotheses and show that the parameter space expands eight hours after the removal of neuromodulators and that itis restored primarily by neuromodulators. This indicates that off-target effects of neuromodulators slowly regulate the ionic current parameter space of pyloric neurons in addition to the known effects of neuronal activity.

Disclosures: S. More-Potdar: None. **J.P. Golowasch:** None. **S. More-Potdar:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.05/I1

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NS118606

Title: Convergent excitatory effects of small cardioactive peptide on a feeding central pattern generator interneuron may induce a form of arousal that can be maintained during task switching in the mollusk Aplysia

Authors: *C. EVANS[1], C. REAVER[1], M. BARRY[1], M. PERKINS[1], J. JING[2], E. C. CROPPER[1];
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Abstract: Peptides released from neurons in response to different stimuli have been shown to exert convergent as well as divergent effects on the feeding central pattern generator (CPG). The peptides, feeding circuit-activating peptide (FCAP) and cerebral peptide-2, (CP-2), both released by cerebral buccal interneuron-2 (CBI-2) stimulation, and small cardioactive peptide (SCP), released by esophageal nerve (EN) stimulation, exert divergent effects. FCAP/CP-2 and SCP promote ingestive and egestive motor programs respectively. However, the actions of these peptides converge to excite buccal interneuron B63, a cell necessary for the generation of both program types. We are studying these convergent effects on buccal interneuron, B63. In vitro, autonomous, spontaneous subthreshold depolarizations are present in B63, and some convert into plateau potentials and trigger motor programs. Specifically we ask, how is the SCP effect mediated and what is the function of the convergent effect? Previous work using dynamic clamp has shown that subtracting an outward current is an effective way of increasing the B63 excitability and this can impact buccal motor program induction. Voltage clamp experiments revealed that SCP induces a net inward current primarily carried by sodium. We next tested the

effect of exogenously applied SCP on motor program induction. We found that although exogenous SCP increased autonomous activity, i.e., motor program induction, it was irregular with long inter-program intervals. The majority of the programs were classified as intermediate (not ingestive or egestive). Experiments performed by stimulating the EN to cause release of SCP in the buccal ganglia, while monitoring activity in I2N and B8 resulted in similar findings. Although exogenous SCP application and prior EN stimulation modify program induction, neither induces activity that resembles normal feeding behavior. The majority of programs are intermediate with long inter-program intervals. We then asked whether the persistent increase in B63 excitability observed after repeated EN stimulation can impact the subsequent ability of a CPG input (CBI-2) to induce motor programs. We found that when CBI-2 is stimulated to generate a motor program following EN stimulation there is a significant decrease in the latency of ingestive program induction. In conclusion, convergent peptide effects on interneuron B63 promote the induction of both ingestive and egestive responses and may allow arousal to be maintained during task switching.

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Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

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Program #/Poster #: PSTR229.06/I2

Topic: E.07. Rhythmic Motor Pattern Generation

Support: R35 NS097343
BAEF Postdoctoral Fellowship
WBI world

Title: Temperature sensitivity of the gastric muscles is altered by neuromodulators

Authors: *K. JACQUERIE, J. SEDDON, E. MARDER;
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Abstract: Temperature influences all biological processes and presents a challenge in maintaining stereotyped activities within neuronal circuits. Our study focuses on the crab *Cancer borealis*, which experiences daily and seasonal temperature changes. Focusing on its stomatogastric system, comprising the pylorus and the gastric mill responsible for food filtering and grinding, we explore how temperature affects its well-characterized neuronal circuits and muscles. Recent findings show that the pyloric rhythm frequency increases as temperature rises while maintaining its characteristic phase until the rhythm “crashes” at high temperatures. Neuromodulators like oxotremorine (OXO) and proctolin (Proc) enhance rhythm robustness, while serotonin (5-HT) diminishes it. Despite ongoing investigations into the effect of temperature on nerve and neuron activity, little is known about the neuromuscular junction

response to temperature changes. Our project investigates the impact of temperature on the neuromuscular junction across various gastric muscles (gm4b-c, gm5b, gm6, gm8a-b, gm9) innervated by different neurons like DG, LG, and MG. We aim to understand the robustness of muscles innervated by the same or different neurons to temperature and the intricated role of neuromodulators. Initially, we compare muscle fibers through nerve-evoked excitatory junction potentials (EJPs) in response to the 2-second stimulus train whose frequency varies between 1 to 20Hz for 2s every 20s. We observe similar responses to increasing temperature in control saline. All muscles hyperpolarize as the temperature increases and the EJPS drop in amplitude, as the hyperpolarization is associated with a decrease in muscle fiber input resistance. Next, we explore how neuromodulators like dopamine (DA), 5-HT, OXO, and Proc influence muscle temperature sensitivity, finding their actions to be frequency-, temperature-, and muscle identity dependent. The exploratory analysis conducted for DA reveals its potential in mitigating the temperature sensitivity of gastric muscles, indicated by a lesser reduction in EJP amplitude compared to control conditions. Additionally, we quantified the ratio between the last and first EJP amplitude as an indicator of facilitation. Take-home messages: the same motor neuron's postsynaptic actions are muscle target specific, and the effects of neuromodulators are also muscle target specific. We aim to develop a comprehensive understanding of the system by comparing various gastric muscles. Understanding the nervous system response to climate change is crucial, raising awareness about the impact of the current crisis on neurological functions.

Disclosures: K. Jacquerie: None. **J. Seddon:** None. **E. Marder:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.07/I3

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Doherty Charitable Foundation Coastal Studies Research Fellowship

Title: The effects of temperature on neuromodulation in a small motor network

Authors: *D. J. POWELL;
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Abstract: Nervous systems must be able to maintain function despite fluctuations in ambient temperature. Because temperature affects the kinetics of all cellular processes (e.g., ion channel function), it is not immediately clear how temperature compensation is achieved at the level of single neurons or whole circuits. This is particularly true for organisms that do not homeostatically regulate their internal temperature. In some cases, nervous systems are exposed to fluctuations of ten or more degrees Celsius within a given day. The nervous systems of the American lobster (*H. americanus*) can maintain function across a wide range of temperature (~7-30°C). Previous data shows that neuromodulators (e.g., peptides, hormones, and gases) can

influence this temperature range; some modulators truncate this range while others extend it. Here, we further explore the effects of temperature and neuromodulation on the cardiac neuromuscular system of the *H. americanus*. The neuromuscular system includes the cardiac ganglion (CG), a nine-neuron circuit that generates the heartbeat, and the associated cardiac muscles that the CG motor neurons innervate. The heart will continue to beat after being dissected from the animal so long as physiological saline is continuously perfused through it, and similarly, the isolated CG will continue to produce fictive activity *ex vivo*. For these experiments, we perfused the neuromodulatory peptide myosuppressin (dissolved in physiological saline) at four temperature steps (7, 10, 13, and 16°C) to better understand how temperature influences the cardiac response to this modulator. Applying myosuppressin to the whole heart produces an interesting and dynamic response at 10°C (acclimation temperature) whereby the heartbeat frequency and contractile force initially decrease synchronously, followed by a dramatic increase in force and modest increase in frequency. The temperature dependencies, as measure by their Q_{10} values, of heartbeat frequency and force differed between control and myosuppressin conditions. Despite these relatively small changes in temperature (Δ 3°C), we observed temperature dependent responses to myosuppressin application.

Disclosures: D.J. Powell: None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.08/I4

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Bowdoin Paller Neuroscience Academic Year Fellowship

Title: The combinatorial effects of temperature and salinity on the cardiac nervous system of the American lobster, Homarus americanus

Authors: *K. CARRIER[1], J. TIDMORE[1], D. J. SCHULZ[2], D. J. POWELL[3];
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Abstract: The ability of nervous systems to continue to function when exposed to global perturbations such as elevated temperature and altered saline concentration is a non-trivial task. Because each of these perturbations affect channel function and maximal conductance, it is not immediately clear how nervous system can maintain function despite these global perturbations. The nervous system of the American lobster (*H. americanus*), a marine osmoconformer and poikilotherm, must be robust to these stressors as they are exposed to fluctuations in temperature on a seasonal and a daily basis, as well as in salinity as rainfall patterns change and as the lobsters move between areas of the ocean that have varied depths. Using the cardiac nervous system of the American lobster, we characterized the effects of temperature on the output whole

heart and the isolated cardiac ganglion (CG) (*ex vivo*). The CG is a nine-neuron pacemaker circuit that generates the heartbeat. Because the heart will continue to beat after being dissected from the animal, so long as physiological saline is continuously perfused through it, we can dissect the intact organ from the animal and study both muscle and neuron physiology simultaneously. Similarly, the isolated CG will continue to produce fictive activity *ex vivo*. We first established a range of saline concentrations that did not cause the system to crash, and then determined whether combinatorial changes in temperature and salinity concentrations alter the maximum temperature that the system was able to withstand. The CG was able to withstand a wider range of saline concentrations than the whole heart and crashed at higher temperatures than the whole heart in each saline concentration. Interestingly, as observed in another nervous system with the lobster, the whole heart and cardiac ganglion both crashed at higher temperatures in lower saline concentrations and higher temperatures in lower saline concentrations. We then wanted to determine if the CG neurons could sense changes in the extracellular saline concentration and whether they adjust their ion channel expression in response. To do this, we bathed the isolated CG in either 0.75x, 1.5x, or 1x (normal physiological saline) for 24 hours. We then dissected out the individual CG motor neurons, the pacemaker neurons, and sections of axon projects, and using single-cell RT-qPCR, we measured the relative abundance of mRNA for several species of ion channels. We found that some channel correlations in the 1x condition, were lost in the altered saline conditions.

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Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.09/I5

Topic: E.07. Rhythmic Motor Pattern Generation

Title: The effects of neuromodulation on multistability of bursting and silent regimes in single HN and Half-Center Oscillator

Authors: *Y. O. SHAMS[1], T. TRAN[2], *Y. SHAMS[2], G. S. CYMBALYUK[3], A. GIANELLA[2]; [1]Neurosci. Inst., Georgia State Univ., decature, GA; [2]Neurosci. Inst., Georgia state Univ., Atlanta, GA; [3]The Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Neuromodulation adjusts the dynamics of neural circuits to cope with environmental changes. Half-Center Oscillators (HCOs) composed as pairs of mutually inhibitory heart interneurons (HNs) set the leech heartbeat rate. The neuromodulator myomodulin speeds up HCOs and guides their dynamics by decreasing Na^+/K^+ pump current (I_{pump}) and increasing h-current (I_h) constituting a coregulation path between dysfunctional regimes [1,2]. This path was described using a biophysical HCO model matching activity of experimentally recorded HCO under different myomodulin concentrations [2]. Here, we varied leak conductance (g_{leak}) along the coregulation path and investigated HCO activity regimes. We found that there exist range of

g_{leak} supporting multistabilty of bursting and silence. This range appears between the transition from bursting to silence and the transition from silence to bursting. For each point on the path, we defined the propensity index for multistabilty by this range of g_{leak} [3]. This multistability is based on a saddle periodic orbit which appears at Andronov-Hopf bifurcation (AHb) where the silent regime loses stability [4]. We show that, along the coregulation path representing increase of the myomodulin concentration, the propensity index increases by a factor of 2.1 in single HN and 3.4 in HCO.

Within the range of multistability, a short perturbation can switch either a single HN or HCO from bursting to silence. Depending on the phase of the delivery, either depolarizing or hyperpolarizing 30 ms pulse of current (I_{pulse}) can trigger a switch. We tested different amplitudes of I_{pulse} at different phases of a cycle and determined the domains of the amplitude and phase, describing switching pulses in single HN and HCO cases. In both cases, a switch from bursting to silence was only possible when a pulse is delivered during the interburst interval. In a single HN, negative pulses produce larger domains than positive ones. The domains are smaller in HCO. Notably, as the g_{leak} approaches AHb, the domains shrink, along with the size of the saddle orbit tends to zero. Furthermore, along the coregulation path, the diameters of domains decrease in both single HN and HCO. Neuromodulation increases the propensity for multistability of bursting and silence with the increase of myomodulin concentration. These findings also highlight the neuroprotective effects of the HCO motif against switching into a dysfunctional regime.

References

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2. Ellingson, PJ et al. (2021) *J Neurosci* 41: 6468-6483
3. Barnett, WH et al. (2013) *J Neurosci*. *Methods* 220: 179-189
4. Malashchenko, T et al. (2011) *Phys Rev E* 84:041910

Disclosures: Y.O. Shams: None. **T. Tran:** None. **Y. Shams:** None. **G.S. Cymbalyuk:** None. **A. Gianella:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.10/I6

Topic: E.07. Rhythmic Motor Pattern Generation

Support: GSU Brains and Behavior grant

Title: Control of bursting regimes of leech heart interneurons

Authors: Y. SHAMS[1], S. LEE[2], M. FOMENKO[1], R. L. CALABRESE[3], ***G. CYMBALYUK**[2]; [1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Georgia State Univ., Atlanta, GA; [3]Biol., Emory Univ., Atlanta, GA

Abstract: Neuromodulation adjusts neuronal dynamics to suit behavioral aims and external conditions, for example, in central pattern generators (CPGs) governing rhythmic movements, like breathing and locomotion. How neuromodulation coordinates changes of multiple currents to make the CPGs, producing adjustable and yet robust patterns is an open question. The CPG, controlling the leech heartbeat, is driven by two pairs of mutually inhibitory heart interneurons (HNs), forming half-center oscillators (HCO). Neuromodulator myomodulin speeds up the HCO by coordinated decrease of Na^+/K^+ pump current (I_{pump}) and increase of hyperpolarization-activated (h-) current (I_h) and, thus, navigating along a coregulation path between dysfunctional regimes [1,2].
We recently found that in an HCO model a new functional bursting regime emerges which has the spike frequency two times higher than the other, while the cycle periods and burst durations of these regimes are roughly the same. Here, we demonstrate that a single HN model neuron can exhibit co-existence of these two regimes in some domain of parameter space. Our results suggest that the new bursting is engaged by conditions elevating the intracellular Na^+ concentration, like myomodulin neuromodulation which causes a decrease of Ipump enhancing Na^+ efflux and outward current and an increase of I_h increasing Na^+ influx and predominantly inward current. We suggest that the mechanism described in Erazo-Toscano et al. (2023) partakes in generating of the new regime. This mechanism is based on the interaction of the I_{pump} and inward currents contributing to Na^+ influx [3]. This interaction creates relaxation oscillator dynamics with a notable feature: the increase of I_{pump} speeds up the cycle period by simultaneous shortening of the burst duration and interburst interval. This feature contrasts with experimental trends where myomodulin speeds up the cycle period alongside a decrease in I_{pump} [1]. Yet, this feature could resolve the paradox of speeding up the rhythm under the influence of monensin upregulating the pump current by raising the intracellular Na^+ concentration [4]. The co-existence of the two bursting regimes in a single HN neuron allows us to contrast the underlying mechanism and unveil how the transition between the bursting regimes controlled by neuromodulation can enhance the flexibility and robustness of rhythm adjustments.

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1. Tobin, A.-E. & R. L. Calabrese (2005) *J. Neurophys.* 94: 3938-3950.
2. Ellingson, P. J. et al. (2021) *J. Neurosci.* 41: 6468-6483
3. Erazo-Toscano, R. et al. (2023) *eNeuro*, 10: 1-19
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Disclosures: Y. Shams: None. **S. lee:** None. **M. Fomenko:** None. **R.L. Calabrese:** None. **G. Cymbalyuk:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.11/I7

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH R01 MH060605

Title: Convergent modulation of cellular targets increases the similarity of circuit output in the presence of different combinations of comodulators

Authors: *F. NADIM[1], N. DAUR[1], A. C. SCHNEIDER[2], D. M. BUCHER[1];
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Abstract: Neural circuits are always under the influence of multiple neuromodulators, and the prevalent view is that neuromodulation increases the flexibility of circuit output. However, different modulators can have overlapping cellular and subcellular targets, and thus convergence and occlusion may limit the repertoire of possible circuit states. We propose that convergent comodulation may result in consistent circuit activation that, with increasing numbers of modulators, becomes less dependent on the specific identity of the modulators involved. We examine this hypothesis in the pyloric circuit of the crustacean stomatogastric ganglion, where multiple excitatory neuropeptides activate the same ionic current and have similar effects on synapses.

We first used mathematical analysis to examine additive dose-dependent activation of a target ionic current by different (co-)modulators. Increasing numbers of comodulators resulted in increasingly similar activation levels across circuit neurons. With linearly additive activation, this was due to the target ionic channels approaching saturation levels in an increasing number of circuit neurons. However, with sub-linearly additive effects (as suggested by our previous findings), similarity increases even at sub-saturating concentrations. These effects are robust to large variability in dose-response curves across modulators and circuit neurons.

To experimentally measure how similarity in the levels of the target ion channel activation translates to circuit activity output, we applied different combinations of convergent modulators to the decentralized pyloric circuit as a singlet, doublet, and triplet (A, A+B, A+B+C), then washed and applied a second set (D, D+E, D+E+F). In each condition, we quantified activity attributes (cycle frequency, burst phases, spike #s and frequencies). We measured circuit output similarity by calculating the distance between any two circuit outputs in the multidimensional space of all attributes. These distances were decreasing from comparisons between singlet (A vs. D), doublet (A+B vs. D+E), and triplet (A+B+C vs. D+E+F) applications, meaning that circuit output became increasingly similar with increasing numbers of comodulators, independent of their specific identity. This increase in similarity was observed both when applications were additive and when the total concentrations were kept constant, but not when we added modulators with diverging cellular effects to the applications. Our results suggest that convergent comodulation increases the similarity of circuit output, which is complimentary to the modulators' role in increasing flexibility.

Disclosures: F. Nadim: None. **N. Daur:** None. **A.C. Schneider:** None. **D.M. Bucher:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.12/I8

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH060605

Title: High-pass filtering through short-term synaptic facilitation amplifies low-frequency modulation of bursting input

Authors: *D. BUCHER[1], N. DAUR[1], A. C. SCHNEIDER[2], F. NADIM[1];
[1]Dept. of Biol. Sci., New Jersey Inst. of Technol., Newark, NJ; [2]FB 10, Tierphysiologie, Univ. of Kassel, Kassel, Germany

Abstract: Short-term plasticity (STP) lends frequency-filtering properties to synapses, as facilitation amplifies high-frequency inputs whereas depression amplifies low-frequency inputs. Frequency-filtering properties are straightforward to assess across different mean activity rates, but such filtering is less clear for bursting neurons, as the mean firing rate does not fully capture the temporal structure. For example, slow recovery from STP can cause responses to carry a memory of preceding bursts. Consequently, synaptic responses depend not only on intraburst spike frequency and burst duration, but also on burst frequency. Repetitive bursting is also often modulated at a slower time scale, causing periodic changes in burst firing, and thereby adding an additional frequency domain. We observed that two facilitating neuromuscular synapses in the crustacean stomatogastric system amplified a slow-frequency component of motor neuron input, which was ostensibly at odds with the notion that facilitation acts as a high-pass filter. In this input pattern, the fast pyloric rhythm (cycle frequency ~1Hz) was modulated by the slow gastric mill rhythm (cycle frequency ~0.1Hz), so that both the number of spikes per burst and cycle frequency changed over the course of ~10 pyloric-timed bursts. While the number of spikes per burst only doubled over the course of a gastric mill cycle, the postsynaptic muscle response changed severalfold. We explored theoretically how STP may shape responses to slow modulation of bursting. We generated synaptic responses that were amplitude-scaled, depending on prior activity, using the phenomenological model of STP developed by Tsodyks and Markram (1997). We varied release probability and recovery time constants for vesicle depletion and utilization to generate different amounts of depression and facilitation. Facilitation constrained to the time range of single bursts amplified slow modulation because strong bursts facilitated more than weak bursts, thereby enhancing the “contrast” between strong and weak activation within a cycle of slow modulation. Depression reduced contrast. A memory of facilitation or depression across consecutive bursts reduced the magnitude of contrast enhancement or diminishment. Contrast changed in a complex manner across a large parameter space of release probabilities and recovery time constants, and as a function of magnitude and timing of slow modulation. Finally, we found that while synaptic facilitation alone is sufficient to explain amplification of slow burst modulation, the magnitude of the observed biological effect is likely to be also enhanced by postsynaptic voltage-gated conductances.

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Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.13/I9

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH-NINDS 1R15NS128619-01

Title: CPG feedback strength as a switch between network outputs

Authors: M. J. COLEMAN[1], ***D. M. BLITZ**[2];
[1]Keck Sci. Dept., Pitzer and Scripps Colleges, Claremont, CA; [2]Biol. Dept, Ctr. Neurosci & Behav, Miami Univ., Oxford, OH

Abstract: Central pattern generator (CPG) networks underlie rhythmic behaviors such as breathing, walking and chewing. Feedback from CPG networks to their descending projection neuron inputs is common, and its strength is subject to long-lasting (30-60 min) modulation (Blitz, 2023 Front Neurosci). I aimed to determine whether CPG feedback strength is also subject to more rapid changes. I addressed this issue using electrophysiological manipulations of identified CPG, feedback, and projection neurons in small, well-described feeding-related CPG networks in the male Jonah crab, *Cancer borealis*. Two different sensory pathways trigger long-lasting modulatory states via activation of the same projection neurons (MCN1 & CPN2), with different activity patterns and different CPN2 firing rates, to produce different gastric mill (chewing) rhythms (Blitz & Nusbaum, 2008; White & Nusbaum, 2011 J Neurosci). A long-lasting enhancement of a feedback synapse from CPG interneuron Int1 to projection neuron CPN2 occurs in one of these modulatory states (VCN-state), while the Int1 to CPN2 synapse is weak in the other modulatory state (POC-state). The strong Int1 feedback in the VCN state triggers rebound firing and a higher firing rate in CPN2 (Blitz, 2017 J Neurophysiol). The higher CPN2 firing rate is essential for the VCN-motor pattern; experimentally hyperpolarizing CPN2 to decrease its firing rate switches the pattern to POC-like (n=5). To test whether a rapid change in Int1 feedback is sufficient to trigger a switch between patterns, the Int1 firing rate was increased during the POC-state via somatic current injection to increase Int1 feedback strength. This caused a switch in the chewing motor pattern from POC- to VCN-like (n=6). Conversely, starting with a VCN state and decreasing the Int1 firing rate, to weaken the Int1 feedback synapse, switched the motor pattern from VCN- to POC-like (n=4). A physiological rapid, transient switch from the VCN- to POC-motor pattern occurs via activation of a stretch receptor (GPR; White et al., 2017 J Neurophys). In initial CPN2 voltage clamp recordings, starting in the POC modulatory state, the Int1 feedback synaptic currents are transiently enhanced by GPR stimulation (49 to 58 pA, 105 to 168 pA, 7 to 104 pA). In the opposite direction, when the POC pathway was stimulated during a VCN modulatory state, Int1 synaptic current amplitudes decreased (450 to 150 pA, 440 to 130 pA). Collectively, these results suggest that different sensory pathways can toggle a feedback synapse between high and low strengths and support the hypothesis that a feedback synapse can serve as a pivotal switch in determining the modulatory state of a motor system**.**

Disclosures: M.J. Coleman: None. **D.M. Blitz:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.14/I10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS029436

Title: Circuit state-specific tuning by a feeding state-specific hormonal environment

Authors: L. J. FICKLING[1], A. P. COOK[1], W. WU[2], L. LI[3], ***M. P. NUSBAUM**[1];
[1]Dept. of Neurosci., Perelman SOM, Univ. Pennsylvania, Philadelphia, PA; [2]Dept. of Chem., [3]Sch. of Pharmacy, Chem., Univ. of Wisconsin-Madison, Madison, WI

Abstract: We aim to establish how a naturally occurring feeding state-specific hormonal milieu influences the activity pattern of feeding-related circuit states. Thus, we examined the output of four differently modulated pyloric (passage of chewed food) circuits, in saline vs hemolymph from a crab not fed for >24 hr ('unfed hemo'), in the isolated crab *Cancer borealis* stomatogastric ganglion. The circuit states were configured by applying the peptides G-SIFamide, Proctolin or RPCH, or the mACh agonist Oxotremorine (each at 1 µM in saline). These modulator-configured circuit states generate pyloric rhythms with distinct activity patterns (Marder & Thirumalai, 2002). Relative to saline plus modulator, unfed hemo caused modulator-specific changes in the pyloric rhythm. For example, with unfed hemo all four modulators elicited increased #LP neuron spikes/burst (G-SIFamide: p=0.03, n=4; Oxo: p=0.02, n=9; Proct: p=0.008, n=9; RPCH: p<0.001, n=7; Wilcoxon signed-rank test, paired t-test or Welch's t-test), but two were due to an increased LP firing rate (G-SIFamide: p=0.02; Proct: p=0.01) and two to increased LP burst duration (Oxo: p=0.01; RPCH: p=0.01). Phase was only altered for Proct (phase advance: LP onset, p=0.02; IC neuron onset, p=0.006) and G-SIFamide (IC onset advance, p=0.008), while the pyloric cycle period was only altered (increased) by Oxo (p=0.02). Relative to saline alone, unfed hemo alone also altered the pyloric rhythm (e.g. cycle period: +22%, p=0.03; LP firing rate: -16.8%, p=0.01; #IC spikes/burst: +45%, p<0.01, n=15-17). The amount of change evoked by saline plus modulator relative to saline alone was only different between modulators for one of eight studied parameters and for only two modulator pairs (PD burst dur.: Proct vs Oxo, p=0.007, Proct vs G-SIFamide, p=0.01, n=4-5; One-Way ANOVA, Holm-Sidak post-hoc test). For the comparable comparison between unfed hemo plus modulator vs hemo alone, four of eight parameters were different between four modulator pairs (p=0.04 - p<0.001), suggesting a distinct influence of unfed hemo on each circuit state. To test whether unfed hemo altered these modulator actions by increasing the concentration of some or all of the applied modulators, we performed a mass spectrometry analysis of pooled (n=6 crabs) unfed hemo to search for G-SIFamide, Proct, and RPCH. None of these peptides were detected, suggesting the divergent action of unfed hemo on these pyloric circuit states is more complex than a concentration-dependent effect. We next aim to identify the hormone(s) responsible and thereby determine whether the effects of unfed hemo result from the influence of the same or different hormone(s) on each circuit state.

Disclosures: L.J. Fickling: None. **A.P. Cook:** None. **W. Wu:** None. **L. Li:** None. **M.P. Nusbaum:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR229.15/I11

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01HD100544
NYSDOH SCIRP C35594GG
NYSDOH SCIRP C38333GG

Title: Activation of Multisegmental Spinal Neuronal Pathways with Paired Cervical and Lumbosacral Transspinal Stimulation in Humans

Authors: A. SKIADOPOULOS[1], ***M. KNIKOU**[2];
[1]Physical Therapy, [2]City Univ. of New York, Staten Island, NY

Abstract: Transspinal (or transcutaneous spinal cord) stimulation is a promising non-invasive method that may strengthen the intrinsic spinal neural connectivity in upper motor neuron lesions. In this study we assessed the effects of cervical transspinal stimulation on the amplitude of leg transspinal evoked potentials (TEPs), and the effects of lumbosacral transspinal stimulation on the amplitude of arm TEPs and flexor carpi radialis (FCR) H-reflex. Transspinal stimulation was delivered with one cathode electrode placed either on Cervical 3 or Thoracic 10 and associated anodes were placed over the left and right clavicles or iliac crests. The right median nerve was stimulated by a bipolar electrode at an intensity that the FCR H-reflex was half the size of the maximal H-reflex evoked on the ascending portion of the recruitment curve. Cervical transspinal conditioning stimulation produced medium latency inhibition of the net motor output of knee and ankle muscles. Lumbosacral transspinal stimulation produced mixed modulatory effects on TEPs recorded from arm and forearm muscles within a subject. Last, cervical transspinal stimulation produced FCR H-reflex depression in 4 out 10 subjects. This work postulated that in the resting state cervical transspinal stimulation affects the net motor output of knee extensors, but ascending actions from lumbosacral spinal segments onto cervical motoneurons were weak and may require phasic motor activity of arms and legs to manifest. This new protocol warrants further investigation because it may reactivate spinal circuitry after stroke or spinal cord injury through synchronization of upper and lower limb muscle synergies during rhythmic activities like locomotion or cycling.

Disclosures: A. Skiadopoulos: None. **M. Knikou:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01HD100544
NYSDOH SCIRP C35594GG
: NYSDOH SCIRP C38333GG

Title: Effects of Transspinal Stimulation on Gait Patterns and Stability in Healthy Adults

Authors: *A. SKIADOPOULOS, M. KNIKOU;
Physical therapy, City Univ. of New York - Col. of Staten Island, Staten Island, NY

Abstract: Transspinal (or transcutaneous spinal cord) stimulation affects the spinal locomotor networks and is used to improve walking ability in people with spinal cord injury. However, the effects of transspinal stimulation at different frequencies and intensities on intralimb and interlimb coordination and on spatiotemporal gait characteristics has never been systematically investigated before. We hypothesized that transspinal stimulation, regardless of frequency and intensity, promotes physiological step progression and joint movements. Ten healthy young adults undertook a 10-minute baseline walk with no transspinal stimulation at their self-selected comfortable speed on a motorized treadmill, followed by six randomized walking bouts (two intensities × three frequencies) of 10-minute duration each with transspinal stimulation. Stimulation was delivered over the T10-L1 spinal process with interconnected anodes placed bilaterally on the iliac crest at either 15, 30, or 50 Hz (1-ms pulse; 10 kHz carrier frequency) at sub-threshold or supra-threshold intensities. Kinematics data collected using reflective markers attached to participants and tracked using an eight-camera motion analysis system. Static trials were used to define a seven-segment mechanical model of lower limbs. EMG was recorded bilaterally from leg muscles (soleus, tibialis anterior, medial gastrocnemius, vastus medialis, biceps femoris, hip adductor gracilis, gluteus medius, peroneus longus) using surface electrodes and linear envelopes were derived from the recordings. The step length and step width time series were evaluated with detrended fluctuation analysis to identify changes in the temporal structure of gait variability, while the largest Lyapunov exponent was used to establish the extent to which transspinal stimulation affected the dynamic stability of joint movements over stride-to-stride during walking. Additionally, the continuous relative phase was analyzed to determine the impact of transspinal stimulation on interlimb and intralimb coordination. Linear mixed-effects models followed by a generalized linear hypothesis test for planned contrasts were used to compare transspinal stimulation groups with the control group. Transspinal stimulation promoted a greater dynamic stability compared to control walking, a more stable intralimb and interlimb coordination, and a more persistent step length variability-characteristics of healthy gait. These results support that transspinal stimulation is an important neuromodulatory strategy that directly affects spinal locomotor centers.

Disclosures: A. Skiadopoulos: None. **M. Knikou:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

Location: MCP Hall A

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Program #/Poster #: PSTR229.17/I13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Canadian Institutes of Health Research (CIHR)
Natural Sciences and Engineering Research Council (NSERC), Canada
Fonds de Recherche du Québec - Nature et Technologies (FRQNT)
Healthy Brains, Healthy Lives - McGill University

Title: The efficacy of intermittent theta burst (iTBS) stimulation in neural facilitation and motor adaptation learning

Authors: *N. JOHN[1], D. J. OSTRY[1,2];
[1]Psychology, McGill Univ., Montréal, QC, Canada; [2]Yale School of Medicine, New Haven, CT

Abstract: Intermittent theta burst stimulation (iTBS), a non-invasive brain stimulation (NIBS) technique based on the principles of theta-gamma coupling in the hippocampus, was introduced as a method to focally facilitate learning, memory, and cortical excitability in diverse brain regions. In the standard practice of using a single block of iTBS for facilitatory brain stimulation protocols, evidence shows that substantial iTBS response variability arises both between and within subjects, thereby contributing to inconsistent findings in the existing literature. Therefore, following the double-block methodology of continuous theta burst stimulation (cTBS), a NIBS technique that more reliably produces disruption of cortical activity, this work explores the effects of two rounds of iTBS on learning and retention in a force-field adaptation task, as well as the effects of double iTBS on motor (MEPs) and somatosensory evoked potentials (SEPs). Two studies were conducted. In the first, an upper-limb motor learning study, participants received two blocks (iTBSx2) of iTBS to either the primary motor cortex (M1), primary somatosensory cortex (S1), or medial occipital lobe (Control) before training in a force-field adaptation task. To assess the consolidation of motor learning, participants returned 24 hours later for a retention test. No significant differences in either learning or retention were observed following iTBSx2. In study two, changes in MEPs and SEPs were assessed post double iTBS. In the MEP experiment, participants received brain stimulation to either M1 or S1. There were no significant differences in MEP amplitudes across conditions. However, in an SEP protocol combining EEG and iTBS to assess differences in evoked potentials following iTBSx2 to S1, a significant decrease in SEPs was observed. In both the MEP and SEP results, high post-stimulation response variability remained evident. The findings from both the motor learning and event-related potential (ERP) study show that an increased number of iTBS blocks may not induce greater facilitatory effects on cortical activity. As the observed variability in responses suggests a need for a more nuanced understanding of iTBS mechanisms, the findings demonstrate that the complex effects of iTBS on motor learning and neural excitability necessitate a more refined approach for future research.

Disclosures: N. John: None. **D.J. Ostry:** None.

Poster

PSTR229: Neuromodulation of Neuronal and Synaptic Properties

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Program #/Poster #: PSTR229.18/I14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Simons Foundation Junior Fellow
NIH Grant K99NS118052

Title: Serotonergic modulation of spinal circuits drives long-lasting changes in locomotor behavior

Authors: *S. J. FENSTERMACHER[1], A. VONASEK[2], H. GATTUSO[3], C. CHAIMOWITZ[1], S. M. DYMECKI[4], T. M. JESSELL[5], J. DASEN[6];
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Abstract: Neuromodulators act throughout the central nervous system potently altering the dynamics of neural circuits. Neuromodulation by serotonin (5-HT) is intimately linked with control of mood and cognition, but its reach extends beyond these functions in the brain. The spinal cord is densely innervated by serotonergic fibers and the activity of spinal circuits is strongly influenced by 5-HT, yet the role of serotonergic modulation in motor control remains unclear. Interestingly, the 5-HT neurons that innervate the spinal cord are distinct from those targeting the forebrain, providing a separate and dedicated pathway for descending modulation of spinal motor circuits. Thus, my work seeks to understand the role of descending genetically-defined 5-HT neurons in control of movement. Using viral and genetic tracing strategies in mice, we find that a distinct population of brainstem 5-HT neurons selectively targets the spinal ventral horn, which contains the motor neurons and interneurons that drive motor output. Using *in vivo* calcium imaging with fiber photometry, we observe that the activity of ventrally-projecting 5-HT neurons correlates directly with locomotor activity, whereby neural activity is increased during running bouts, a pattern distinct from other 5-HT populations. Furthermore, we find that the ventrally-projecting 5-HT neurons receive input from upstream locomotor control regions, suggesting that this descending system may be recruited to influence locomotor behavior. Indeed, phasic optogenetic activation of ventrally-projecting 5-HT neurons leads to long-lasting increases in speed and duration of locomotion over minute timescales. These studies suggest that 5-HT modulates spinal motor circuits to influence motor behavior over long time scales.

Disclosures: S.J. Fenstermacher: None. **A. Vonasek:** None. **H. Gattuso:** None. **C. Chaimowitz:** None. **S.M. Dymecki:** None. **T.M. Jessell:** None. **J. Dasen:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.01/I15

Topic: E.09. Motor Neurons and Muscle

Support: DFG Grant - 233886668/ GRK1960

Title: Deciphering how the premotor network of nonspiking interneurons processing load feedback contributes to the motor output of forward and backward stepping of an insect leg

Authors: *A. RUTHE[1], P. ROSENBAUM[1], A. ÖZYER[1], S. DAUN[2,1], A. BUSCHGES[1];
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Abstract: Animals rapidly change their walking direction from forward to backward when needed, a behavior that is tightly controlled and adjusted by the interaction between premotor networks and sensory feedback. Neural mechanisms underlying both of these tasks are still merely understood. Stick insects (Medauroidea extradentata) constitute a suitable model of study given their size and speed of walking in combination with their well-organized nervous system structure, allowing for the simultaneous application of a variety of electrophysiological approaches. In stick insect middle legs, the main difference between forward and backward stepping is the timing in which the thorax-coxa (ThC) joint motor neurons (MN) and muscles protractor and retractor coxae that move the leg back and forth are activated, while the activity of the other main muscles remains nearly unchanged. During forward stepping, protractor coxae serve swing and they serve stance during backward stepping, with the reverse being true for retractor coxae. Here, we aim to unravel how local premotor nonspiking interneurons (NSIs) as well as load feedback from leg load sensors, i.e. campaniform sensilla, contribute to the generation of forward and backward stepping. We performed intra- and extracellular recordings of MNs and NSIs combined with stimulation of campaniform sensilla, the analog to 1b afferents in vertebrates, during forward and backward stepping of the middle leg in a semi-intact preparation. While NSIs supplying MNs of the distal leg joints exhibit qualitatively similar activity patterns during forward and backward stepping, NSIs supplying MNs of the ThC joint fall into two groups: NSIs changing their activity pattern and those that show the same activity pattern for both forward and backward stepping. Walking direction specific effects of stimulating campaniform sensilla during forward and backward stepping on premotor NSIs of ThC-MNs suggest a role of load feedback for establishing the motor output for forward and backward stepping.

Disclosures: A. Ruthe: None. **P. Rosenbaum:** None. **A. Özyer:** None. **S. Daun:** None. **A. Buschges:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.02/I16

Topic: E.09. Motor Neurons and Muscle

Support: KAKENHI JP 22K06435

Title: Corticomotoneuronal direct synapses and their regression during development in rodents

Authors: *S. FUKUDA, M. SAKURAI;
Teikyo Univ. Sch. of Med. Dept. of Physiol., Tokyo, Japan

Abstract: Cortico-motoneuronal (CM) synapses, connecting the motor cortex directly to spinal motoneurons (MNs), were previously thought to be exclusive to higher primates. However, our studies have revealed the presence of such direct connections in juvenile rats innervating forearm muscles (J Physiol, 2016). Additionally, we demonstrated these CM connections in juvenile mice, which were subsequently eliminated by postnatal day 22 (P22) using retrograde trans-synaptic labeling with genetically-modified rabies virus (Sci Rep, 2018). We investigated corticospinal (CS) synapses on MNs using electrophysiological methods in spinal cord slices. Forearm MNs were identified either by retrograde labeling with CTB-alexa488 injected into the forearm muscles or by ChAT-GCaMP6f fluorescence within the forearm MN pool. We stimulated the CS axons and recorded EPSCs from the forearm MNs using whole-cell recording. The proportion of CM-EPSCs-positive MNs remained relatively constant at 70% from P8 to P13 (plateau phase), but began to decrease from P14 and disappeared by P21 (regression phase). For the quantal study of CS-MN synapses, we replaced the 2.5 mM Ca^{2+} in the perfusate with 5 mM Sr^{2+} and fractionated the recorded CM-EPSCs. We also conducted morphological analyses of these synapses. ChR2-EYFP-AAV was injected into the sensori-motor cortices of P1 mice to optogenetically stimulate and label CS axons with ChR2-YFP. After stimulating the CS axons and recording CM-EPSCs from MNs, neurobiotin was electrically injected into the MNs via recording pipettes. CS presynaptic terminals were visualized using vGluT1 (vesicular glutamate transporter-1) immunostaining. Contacts between vGluT1-positive varicosities in corticospinal axons and MN dendrites were identified as CM synapses using confocal microscopy at >1000x magnification. The amplitudes of fractionated CM-EPSCs remained relatively constant from P8 to P19, with distribution peak ranging from 7.0 to 8.0 pA. This suggests that postsynaptic sensitivity remained stable in the most of the regression phase. Additionally, from P14 to P19, the time to peak of fractionated CM-EPSCs tended to decrease, suggesting that synaptic sites were predominantly located closer to the soma during the regression phase. During the plateau phase (P11-12), the number of CM synaptic contacts averaged 12.0 ± 1.8 per MN, covering nearly the entire dendritic length. However, the number of CM synapses decreased to less than one-third (2.8 ± 1.2) during the regression phase (P18-19), with a shift in their distribution towards the somata and proximal dendrites.

Disclosures: S. Fukuda: None. **M. Sakurai:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

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Program #/Poster #: PSTR230.03/I17

Topic: E.09. Motor Neurons and Muscle

Support: NIH R25 Bridges to the Biomedical Doctorate (#2R25GM107754-06A1)
NSF CREST Center for Biological Signatures & Sensing (#HRD2112556)

Title: Investigating the intersection of the dopaminergic circuit with the blue-light sensing circuit in C. elegans

Authors: *C. DAVIS[1], N. GAYLUAK[2], B. NELMS[1];
[2]Biol., [1]Fisk Univ., Nashville, TN

Abstract: Dopamine is an important neurotransmitter conserved across the animal kingdom. It is important for motor control and cognitive function and is associated with feelings of pleasure, motivation, and a sense of reward. Dysregulation of dopamine levels in humans is associated with disorders including Parkinson's disease, schizophrenia, and attention-deficit/hyperactivity (ADHD) disorder. The various systems controlled by dopamine signaling have multiple inputs that may influence function, and in the present study, we investigate the intersection of the dopaminergic circuit with the blue-light sensing circuit in the model organism *C. elegans.* Previous studies have shown that loss of dopamine transporter function in *C. elegans* can lead to swimming-induced paralysis (SWIP) due to the accumulation of extrasynaptic dopamine. We discovered that exposure of paralyzed *C. elegans* to blue light can "reanimate" or rescue paralysis during SWIP assays. Further studies have shown that this reanimation is independent of the temperature of water and is observed exclusively to the blue light wavelength. Subsequently, we found that worms lacking the LITE-1 receptor do not reanimate upon blue-light exposure. We hypothesize the rescue of accelerated SWIP paralysis by blue light is contingent on the LITE-1 photoreceptor and directly impacting the dopaminergic circuit. Utilizing 4 strains of C. *elegans* (wildtype, *dat-1, lite-1, and dat-1/lite-1* double mutants), swimming-induced paralysis (SWIP) assays were conducted (both in the presence and absence of the D2 receptor antagonist azaperone, and we measured the time for C. *elegans* to paralyze. The time to paralysis likely reflects changes in extra-synaptic dopamine with shorter non-paralyzing times indicating an increase in extra-synaptic dopamine levels. We are also interested in establishing the specific neuronal intersections between the two neuronal circuits to determine the exact cells that are actively involved in the circuit overriding paralysis.

Disclosures: C. Davis: 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 R25 Bridges to the Biomedical Doctorate (#2R25GM107754-06A1), NSF CREST Center for Biological Signatures & Sensing (#HRD2112556). **N. Gayluak:** 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 R25 Bridges to the Biomedical Doctorate (#2R25GM107754-06A1), NSF CREST Center for Biological Signatures & Sensing (#HRD2112556). **B. Nelms:** A. Employment/Salary (full or part-time):; Fisk University.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.04/Web Only

Topic: E.09. Motor Neurons and Muscle

Title: Modulation effect of discrete lower extremity movement on the amplitude of soleus and gastrocnemius h-reflex in humans

Authors: *E. KOLOSOVA[1,2], D. I. SHUSHUIEV[3], A. V. GORKOVENKO[3], A. MAZNYCHENKO[3,4], A. KOSTYUKOV[3];
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Abstract: Muscle stretching is performed in rehabilitation and sport to increase the joint range of motion. It was reported that there were certain specificities of the H-reflex indices in states with different muscle length under static conditions. At the same time, involvement of neural control in gradual movement of lower extremity is still a matter of debate. The objective of the research was to assess peculiarities of H-reflex indices in different lower leg joint angles during discrete movement in humans. Nine healthy individuals without musculoskeletal disorders (6 men and 3 women, 17-27 years old, M_{age}=23.6, SD=4.6) took part in this study. The method of H (Hoffmann) reflex of the soleus (Sol), gastrocnemius lateralis (GL) and gastrocnemius medialis (GM) muscles was performed using the electromyographic stimulator A365 (World Precision Instruments, Inc.), system of bioamplifiers (AC-916, CWE, Inc., USA), input-output device CED 1401plus and the program Spike2 (Cambridge Electronic Design Limited, UK). The foot of a seated person was moved passively by a mechanotronic system at a constant speed of 1 cm/s from 120 to 70 degrees in the knee joint (i.e. knee flexion and ankle dorsiflexion) and after that in the opposite direction (knee extension and ankle plantar flexion). Two-factor analysis of variance with repeated measures considering two within-subjects factors (knee joint angle with 6 levels (110, 90, 80 degrees of knee flexion and the same angles for knee extension) and muscle activity with 3 levels (no force, active plantar flexion, active dorsiflexion) was carried out in the IBM SPSS Statistics 26.0. It was found that the joint angle factor had significant effect on values of the H-reflex amplitude for Sol, GL and GM (F=3.809, P=0.006; F=3.041, P=0.020; F=5.536, P=0.001, respectively), which were lower at less ankle joint degrees perhaps due to presynaptic inhibition of Ia terminals while muscle stretching. The muscle activity factor also had effect on values of the H-reflex amplitude for all three muscles (F=6.798, P=0.007; F=5.509, P=0.015; F=5.441, P=0.016, respectively), which was higher at rest or during active plantar flexion than during active dorsiflexion probably because of reciprocal inhibition of soleus muscle motoneurons. It should be noted that H-reflex amplitude at the same angle, but during movement in opposite directions was higher during extension in knee joint in comparison with flexion. It

might be due to the phenomenon of hysteresis properties of the muscle spindle activity system. Obtained data demonstrate significant influences of joint angles, kind of muscle activity and movement direction on H-reflex of calf muscles in humans.

Disclosures: E. Kolosova: None. **D.I. Shushuiev:** None. **A.V. Gorkovenko:** None. **A. Maznychenko:** None. **A. Kostyukov:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.05/I18

Topic: E.09. Motor Neurons and Muscle

Support: R01AG078129
R01AG067758

Title: Establishing and Validating an H-Reflex Protocol in a Mouse Model

Authors: *A. KETABFOROUSH[1], M. WANG[1], F. B. DARVISHI[2], A. ROSHANI DASHTMIAN[3], W. ARNOLD[4];
[1]Univ. of Missouri, Columbia, MO; [2]NextGen precision Hlth., Columbia, MO; [3]Univ. of Missouri, Columbia, Columbia, MO; [4]NextGen Precision Hlth., Univ. of Missouri, Columbia, Columbia, MO

Abstract: The Hoffman (H)-reflex, an electrical analog of the monosynaptic stretch reflex, is utilized to evaluate the excitability of sensory-motor loops and motoneuron pools. During nerve conduction studies, the compound muscle action potential (M-wave) is evoked by stimulating a nerve which triggers action potential propagation distally to directly activate the muscle being recorded. Conversely, the H-wave is recorded after action potential propagation proximally to the spinal cord which activates the monosynaptic reflex arc. This study aimed to: 1) establish a protocol for recording M-wave and H-reflex responses following tibial nerve stimulation in a mouse model, and 2) validate this protocol by inducing a dorsal root ganglia lesion to eliminate the H-reflex while preserving the motor evoked response after spinal cord stimulation and the M-wave after tibial nerve stimulation. For recordings, a pair of 28-gauge monopolar needle electrodes were placed at the distal leg to serve as the anode and cathode for nerve stimulation. Fine wire ring electrodes were positioned at the proximal and distal foot as active and reference electrodes, respectively. An adhesive surface electrode was used as the common reference/ground electrode on the tail. Prior to lesioning the dorsal root ganglia, the H-reflex showed a peak-to-peak amplitude of 3.2 ± 1.1 mV and a latency of 7.3 ± 1.0 ms. The lesioning procedure involved making an incision along the dorsal midline from L3 to L5 in four C57BL/6J mice, retracting skeletal muscles to expose the vertebral column, and performing a partial laminotomy to access each dorsal root ganglion (DRG) without damaging the dura or spinal cord. The dorsal nerve root was severed on both sides of the ganglion, and the ganglion was

completely removed. Following the ganglionectomy, the H-reflex was absent, but the M-wave and motor evoked potentials following spinal cord stimulation were preserved. Additional uninjured male and female C57BL/6J mice (n=8, 50% female) underwent M-wave and H-reflex testing at four months of age. The mean M-wave was 28.3 ± 6.9 mV, the mean H-reflex was 8.4 ± 3.0 mV, and the mean M/H ratio was 0.29 ± 0.07. Through this study, we have developed and validated a minimally invasive approach to assess the H-reflex as a means to study neural excitability and plasticity in mouse models. Consequently, the H-reflex can be applied across model systems and clinical studies to enhance the translation of research findings and potentially serve as a biomarker to assess the efficacy of treatment interventions.

Disclosures: A. Ketabforoush: A. Employment/Salary (full or part-time):; University of Missouri. 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.; Missourian Spinal Cord Injury/Disease Research Program. **M. Wang:** A. Employment/Salary (full or part-time):; University of Missouri. **F. B. Darvishi:** A. Employment/Salary (full or part-time):; University of Missouri. **A. Roshani Dashtmian:** None. **W. Arnold:** A. Employment/Salary (full or part-time):; University of Missouri. 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.; NMD Pharma, Avidity Biosciences. F. Consulting Fees (e.g., advisory boards); Novartis, Avidity Biosciences, Dyne Therapeutics, NMD Pharma, Catalyst Pharmaceuticals.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.06/I19

Topic: E.09. Motor Neurons and Muscle

Support: NSERC Discovery Grant (LRB)
NSERC Discovery Grant (GAP)

Title: Foot sole stimulation can influence early phases of voluntary rate of torque development in fatigued plantar flexors

Authors: *L. C. MARRELLI, T. SHARMA, G. A. POWER, L. R. BENT;
Human Hlth. and Nutritional Sci., Univ. of Guelph, Guelph, ON, Canada

Abstract: Rate of torque development (RTD), how quickly one can generate torque, is important in balance, posture, and athletics. Reduced RTD, due to factors such as fatigue or aging, is associated with an increased fall risk and decreased performance. Thus, enhancing RTD through training or other means may prevent injury and improve motor outcomes. RTD is often measured in time-based epochs. Literature has suggested that the early phase of RTD (contraction onset to

100ms) is primarily influenced by neural factors, such as speed of motor unit recruitment and motor unit discharge rate. Thus, altering RTD via these neural factors would likely affect early epochs most. Foot sole cutaneous stimulation generates location dependent muscle responses in the plantar flexors of the leg: heel stimulation generates excitation, while metatarsal stimulation elicits inhibition. Further, cutaneous stimulation has been shown to preferentially increase the excitability of high threshold motor units (MUs), potentially facilitating faster force production. Thus, cutaneous stimulation of the foot sole may alter RTD in a location dependent manner during voluntary contractions. This study aimed to investigate if cutaneous stimulation could mitigate the fatigue-induced decline in voluntary RTD. Seven young, healthy males performed isometric plantarflexions *"as fast and hard as possible"* in the rested and fatigued state, under three conditions: no stimulation (CON), heel stimulation (HEEL), and metatarsal stimulation (MET). RTD was measured as the slope of torque over time from contraction onset to 100ms, in continuous 25ms epochs. Fatigue was induced via a sustained maximum voluntary plantarflexion. All cutaneous electrical stimulation was set to 2x perceptual threshold. We calculated the percent decrease in RTD from rest to fatigue in HEEL, MET and CON, and compared across conditions. On average, at 100ms HEEL reduced the effects of fatigue on RTD. Conversely, MET was shown to exacerbate the effects of fatigue on RTD at 100ms, further reducing the rate at which force can be generated. Our findings suggest cutaneous stimulation at the heel may attenuate fatigue-induced reductions in early RTD, while stimulation at the metatarsal may exacerbate these effects. We speculate these changes may be due to alterations in MU recruitment gain induced by the cutaneous stimulation. Maintenance of RTD during fatigue with heel stimulation may inform clinical interventions aimed to improve motor outcomes. Additionally, exploring the ability of metatarsal stimulation to reduce RTD is important to fully understand potential applications of cutaneous stimulation.

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Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

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Program #/Poster #: PSTR230.07/I20

Topic: E.09. Motor Neurons and Muscle

Support: NINDS R01 NS104436
Advance Clinical Translational Network AWD12047
URI College of Pharmacy Seed Grant
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URI Proposal Development Grant
NINDS RF1135580

Title: Primary afferent depolarization and hyperreflexia in cerebral palsy

Authors: E. MENA-AVILA[1], D. J. BENNETT[2], M. GORASSINI[3], ***K. A. QUINLAN**[4]; [1]Biomed. and Pharmaceut. Sci., Univ. of Rhode Island, Kingston, RI; [2]Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada; [3]Neurosci., Univ. Alberta, Edmonton, AB, Canada; [4]Univ. of Rhode Island, Kingston, RI

Abstract: Perinatal injuries can result in lifelong motor impairments known as cerebral palsy (CP). Clinical hallmarks of CP include spasticity, muscle stiffness / hypertonia, and hyperreflexia (both increased amplitude of reflexes and the spread of reflex activity to unrelated muscle groups). Few treatments are available to reduce the impact of developmental injuries once they occur, and rodent models of CP do not display prominent motor deficits after developmental injuries, making the disorder difficult to study. In contrast to rodents, New Zealand White rabbits have a developmental pattern more closely aligned to that of humans and display prominent hypertonia and hyperreflexia after exposure to prenatal hypoxia-ischemia (HI). Altered sensorimotor processing in reflex circuits is present in humans and in the rabbit model and could provide an avenue for prevention / treatment of motor dysfunction. In this context we investigated primary afferent depolarization (PAD) in the rabbit HI model of CP. Briefly, we performed HI or sham surgery (where the pregnant dam was anesthetized but fetuses did not experience HI) at 70-80% gestation. At birth, we performed a battery of behavioral tests to assess motor disfunction including modified Ashworth, righting reflex, and joint torque. Roughly half of the HI kits have motor deficits (HI affected), and the other half were classified as HI unaffected. At postnatal days 1-5, we isolated hemi-spinal cords from kits and stimulated a single dorsal root to evoke PAD from several adjacent dorsal roots while perfusing drugs. In all rabbits tested, short duration (200ms) evoked phasic PAD was dependent on $GABA_A$ and glutamate transmission, and the duration of phasic PAD was unchanged between sham and HI rabbits. However, intersegmental spread of dorsal root potentials was increased in HI motor-affected spinal cords compared to both sham and HI unaffected cords but only when high threshold afferents in the dorsal roots were stimulated using 5 and 10 x threshold stimulation parameters. These results suggest there is a similar involvement of synaptic $GABA_A$ and glutamate PAD in sham and HI rabbits, and high threshold afferents in kits with motor deficits are driving at least one component of hyperreflexia. Future studies will examine the mechanisms of hyperreflexia particularly the role of nociceptor-driven activation of GAD2 interneurons mediating PAD.

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Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.08/I21

Topic: E.09. Motor Neurons and Muscle

Support: NIH NS132728

Title: Motor unit development in a rabbit model of cerebral palsy

Authors: *E. REEDICH, E. MENA-AVILA, D. SANDERS, C. M. HAMILTON, Jr., E. GONZALEZ, H. MCCANN, L. GENRY, K. A. QUINLAN, M. MANUEL;
Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral palsy (CP) is the most common motor disability in children, occurring in 1:500 live births. Symptoms of CP include hyperreflexia, hypertonia, muscle weakness, and fatigue. The mechanisms through which CP-causative injuries like hypoxia-ischemia (HI) cause motor deficits remain unresolved. However, motor unit (MU) development occurs in the perinatal period when CP-causative injuries occur, and depends on spinal motoneuron (MN) activity, which is increased in the HI rabbit model of CP partly through enhanced serotonergic neuromodulation. We are testing the hypothesis that prenatal HI injury alters serotonin receptor composition in MNs, dysregulates neuromuscular junction (NMJ) maturation, and disrupts the development of MU physiological types (S, slow; FR, fast fatigue-resistant; and FF, fast fatigable). To test our hypothesis, we are using immunofluorescence to quantify serotonin receptor expression in MNs and to track the emergence of mono-innervation at the NMJ, an anatomical hallmark of maturity. We are recording single MUs *in vivo* in anesthetized sham-operated control and HI rabbits using the split ventral root method, and characterizing MU contractile properties throughout the early postnatal period. 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 sham-operated control and HI rabbit muscles. We find that serotonin receptor expression is different in MNs from neonatal HI rabbits compared to age-matched sham-operated controls and our preliminary data suggests that NMJs undergo delayed maturation in HI rabbit skeletal muscle. The impact of prenatal HI injury on MU electrophysiology will be presented, and our preliminary analysis indicates that in the third postnatal week when poly-neuronal innervation is eliminated, HI skeletal muscle has a slower, weaker, and less fatigable fiber type profile than that of typically developing rabbits. A slower muscle fiber type profile in HI muscle may reflect chronic, low frequency MU activity consistent with CP. Overall, this project elucidates whether aberrant MU development and electrophysiology after prenatal HI injury contribute to motor dysfunction in the rabbit model of CP.

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Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

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NIH Grant R01NS132728
NIH Grant RF1135580

Title: Prenatal hypoxia ischemia results in persistent enhanced nociceptive behavior in a rabbit model of CP

Authors: *L. T. GENRY[1], E. REEDICH[2], E. MENA-AVILA[3], G. GIDDINGS[4], M. SINGER[5], P. MCGINNIS[6], M. R. DETLOFF[7], K. A. QUINLAN[8];
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Abstract: Cerebral Palsy (CP) is the most common motor disability in children. It affects about 1 in every 500 live births. Many factors can lead to an increased risk of a child developing CP, but these risk factors are closely associated with an injury to the developing central nervous system (CNS) which can lead to CP. Motor dysfunction, including spasticity and dystonia, is a defining sign of CP. Recently, it has been shown these motor deficits are most often accompanied by pain, but the cause of this pain is unclear. To study CP and the associated pain, we use a rabbit model of CP. We hypothesized that rabbit kits used to model CP will have changes in pain-like behavior and this will be associated with histological changes within the spinal cord. We performed a hypoxia-ischemia (HI) surgery on pregnant dams to occlude blood flow to the uterus for 40 minutes (a model of CP) or a sham surgery at 70-80% gestation to control for the effect of anesthesia on the developing kits. Rabbit kits were born normally, and we assessed motor function and pain-like behavior at several developmental time points. Then, at postnatal day (P) 18, we tested cognitive function to aid in determining the pain phenotype and harvested spinal cords from kits to examine changes in C-fiber distribution within the spinal cord. We found HI rabbit kits were more sensitive to mechanical stimuli (von Frey) at P1, P11, and P18 compared to sham kits. Previously, we showed an increased sensitivity to thermal stimuli (Hargreaves') in HI kits at P5, but there were no differences between sham and HI kits' sensitivity to thermal stimuli at P1 and P11. The increase in pain-like behavior in HI kits is associated with an increase in anxiety-like behavior. HI kits spend less time in the center of an open field compared to sham kits at P18 despite no obvious differences in motor function. This increase in anxiety-like behavior in HI kits continues to at least P45. Using immunofluorescence, we examined changes in peptidergic and nonpeptidergic C-fibers in the dorsal horn of the spinal cord. At P18, the proportional area of nonpeptidergic C-fibers in the dorsal horn of the cervical spinal cord is greater in HI kits than it is in sham kits. Overall, our results show that HI rabbit kits display an increase in pain-like behavior and changes in cognitive function that is supported by changes in nonpeptidergic C-fiber (those associated with mechanical nociception) distribution within the spinal cord. This suggests the rabbit model of CP could be a model used to study potential therapeutics to treat pain in people with CP and, more broadly, people with chronic pain.

Disclosures: L.T. Genry: None. **E. Reedich:** None. **E. Mena-Avila:** None. **G. Giddings:** None. **M. Singer:** None. **P. McGinnis:** None. **M.R. Detloff:** None. **K.A. Quinlan:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.10/I23

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant AI093504
NIH Grant AI173059
NIH Grant AI180532
DTRA BAA CB11178
DOD OTA MCDC2212-003

Title: Development of a mechanism-based combination treatment for botulism

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Abstract: Botulinum neurotoxin (BoNT) is a potent microbial dichain toxin that specifically cleaves neuronal SNARE proteins required for synaptic vesicle fusion, causing prolonged muscle paralysis and death by asphyxiation. BoNT has an extraordinary affinity for presynaptic receptors and a long latent period between neuronal internalization and development of neuromuscular weakness. By the time that clinical signs of botulism emerge, sufficient BoNT has already internalized into respiratory motor neurons to cause asphyxiation. Although prolonged intensive care support has reduced botulism mortality from 60% to 5%, intensive care facilities have limited spare capacity and thus even a moderate-scale botulism outbreak would be expected to rapidly overwhelm regional healthcare systems. The lack of botulism therapies and the immense resource burden to maintain survival has resulted in the classification of BoNT as a Tier 1 select agent with high risk of use as a mass casualty bioweapon. Here we test novel combinatorial treatment strategies for botulism using three therapies selected based on extensive mechanistic studies. The first component (antitoxin) is FDA-approved and has been shown to reduce the duration of paralysis, but not proven respiratory arrest, when administered to symptomatic patients. The second component (intracellular antidote) is an improved version of an experimental biologic that blocks catalytic activity within the nerve terminal. The final component is a repurposed drug (3,4-diaminopyridine; 3,4-DAP) that increases acetylcholine release by broadening the duration of the action potential, thus enhancing neuromuscular transmission from intoxicated motor nerve terminals. Here we compare the benefits of multimodal treatments to single therapies when administered to symptomatic rats after challenge

with BoNT at LD_{99} doses. Compared to single therapies, bimodal combinations consisting of 3,4-DAP plus either antitoxin or antidote were highly effective, producing high levels of survival and significantly shortening the duration of botulism symptoms. Due to the rapid progression of disease once toxic signs emerge, it was difficult to determine whether trimodal treatment produced superior outcomes to bimodal treatments, although additional studies are underway. Collectively, these studies illustrate the first mechanism-based, combination therapies for botulism that rapidly reverse disease and sustain respiratory function while accelerating full recovery.

Disclosures: S. O'Brien: None. **T. Wentz:** None. **K. Ichtchenko:** None. **R. Jin:** None. **P. McNutt:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.11/I24

Topic: E.09. Motor Neurons and Muscle

Support: NSW Health Spinal Injuries Grant
NHMRC

Title: Effect of acute intermittent hypercapnic-hypoxia on voluntary activation of the thumb adductors in humans

Authors: *C. PRAJNADEWIE[1,2], A. J. MATHEW[3,2], H. T. FINN[3,2], B. L. LUU[3], S. GANDEVIA[3,2], J. E. BUTLER[3,2];
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Abstract: Acute intermittent hypoxia (AIH) is reported to induce spinal plasticity and facilitate motor output in humans with incomplete spinal cord injury. However, there is variable efficacy across studies. Combining AIH with hypercapnia (AIHH) has improved respiratory outcomes in situations where AIH has not. In this study, we investigated the effects of AIHH on limb muscle strength and voluntary activation (VA) in humans.
Fifteen able-bodied adults (6 females, age 21-46 years) performed maximal voluntary contractions (MVCs) of thumb adduction before and after 30 minutes of an AIHH or sham intervention. Each intervention was performed on a separate day and ≥1 week apart (randomised crossover design). AIHH included 15 cycles of alternating between 1 minute breathing 0.09 FiO_2 with 0.05 $FiCO_2$ and 1 minute breathing 0.21 FiO_2 with 0.004 $FiCO_2$ (room air). The sham condition involved continuously breathing room air. Participants performed 4 sets of 3 MVCs: (i) baseline 1, (ii) baseline 2, (iii) 30 minutes post-intervention (P30), and (iv) 60 minutes post-intervention (P60). Force and surface electromyographic activity were recorded from the adductor pollicis muscle. During each MVC, a pair of supramaximal electrical stimuli (200 μs

pulse width, 10 ms apart) was delivered to the ulnar nerve to produce a superimposed twitch and, 2-5 seconds later, a resting twitch, from which VA was calculated using the twitch interpolation method. Baseline 1 and 2 were combined and compared to the measures at P30 and P60 with a mixed linear model with time, intervention and their interaction as fixed factors.
There was a significant interaction between intervention and time for VA of thumb adduction ($p=0.02$) but no overall effect of intervention ($p=0.16$) and time ($p=0.41$). Post-hoc comparisons showed no difference in VA between the AIHH and sham interventions at baseline ($p=0.10$), P30 ($p=0.05$) and P60 ($p=0.15$). However, during the MVCs there was a significant overall effect of intervention ($p<0.001$) and time ($p<0.001$) on peak force, and a significant interaction between intervention and time ($p=0.004$). The estimated marginal mean (EMM) of MVC force at P60 after the AIHH was 67.5 N [95% CI (55.4, 79.6)], which was significantly lower than for MVC force after the sham, 73.0 N [95% CI (60.9, 85.1)] ($p<0.001$). However, there was no significant difference between the EMM of peak force between AIHH and sham at baseline ($p=0.22$) and P30 ($p=0.36$).
AIHH did not facilitate voluntary activation of thumb adduction. In contrast, the reduction of MVC force one hour after AIHH suggests that AIHH may reduce muscle recruitment and maximal strength.

Disclosures: C. Prajnadewie: None. **A.J. Mathew:** None. **H.T. Finn:** None. **B.L. Luu:** None. **S. Gandevia:** None. **J.E. Butler:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.12/I25

Topic: E.09. Motor Neurons and Muscle

Support: NIA/NIH R01AG078129

Title: Age-related neuromuscular hypoexcitability in mice is accompanied by cortical motor neuron hyperexcitability and compensation

Authors: *J. VITERI[1], N. KERR[2], F. B. DARVISHI[4], A. ROSHANI DASHTMIAN[2], S. AYYAGARI[2], J. M. SANTIN[5], W. ARNOLD[3];
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Abstract: Sarcopenia, or pathological age-related muscle wasting and weakness, limits the independence of older adults and is a risk factor of all-cause mortality. Since growing evidence suggests that age-related changes at the level of the primary motor cortex (PMC) may accelerate the development of age-related weakness, we quantified cortical motor neuron (CMN) and

neuromuscular excitability in young (2-3 months) and old (23-24 months) C57/BL6 mice. First, measures of strength showed that old mice had decreased grip strength (5.4 g/bw vs 9.7g/bw; $p<0.0001$) and max-weight pulled (10 g/bw vs 16.2 g/bw; $p<0.0001$). We then quantified sciatic nerve transmission to the gastrocnemius. Old mice had decreases in compound muscle action potentials (CMAP) (72.7 mV vs 88 mV; $p=0.0083$), motor unit number (394 motor units vs 467 motor units; $p=0.0412$), and twitch (0.07 mN-m/bw vs 0.09 mN-m/bw; $p=0.0015$) and tetanic muscle contractility (0.3 mN-m/bw vs 0.4 mN-m/bw; $p<0.0001$). Overall, old mice are weak and exhibit diminished nerve-muscle transmission.
Next, by cervically stimulating corticospinal tracts and recording gastrocnemius excitability, we quantified motor-evoked potentials (MEPs) which serve as a proxy for PMC connectivity to the neuromuscular system. Old mice had decreased MEPs (0.05 mV/CMAP vs 0.09 mV/CMAP; $p=0.0004$). However, patch-clamp experiments on single CMNs revealed that old CMNs had a higher mean firing frequency at either small (50 pA; 17.3 Hz vs 10 Hz, $p=0.0012$) and large stimulation steps (300 pA; 40.7 Hz vs 30.4 Hz, $p=0.0004$). Given the critical role of the persistent inward current (PIC) in regulating spinal motor neuron activity, we measured the PIC in CMNs. PICs of old CMNs were larger (-1722 pA vs -1402 pA, $p=0.0161$), had an earlier onset voltage (-69.67 mV vs -65.38 mV, $p=0.0162$), had a more delayed offset voltage (-73.91 mV vs -66.52 mV, $p=0.0008$), and had a bigger total voltage range (143.6 mV vs 131.9 mV, $p=0.0016$). We also measured the paired-pulse ratio (PPR) of post-synaptic currents. Old CMNs had an increased excitatory PPR (1.2 vs 0.8744, $p=0.0203$) and a decreased inhibitory PPR (0.45 vs 0.7, $p=0.0038$). Overall, old CMNs are hyperexcitable, have increased PICs, and an imbalance of synaptic inputs.
Taken together, our results suggest that aging leads to decrements in nerve-muscle connectivity. Similarly, our results also suggest decrements in connectivity from the PMC to the neuromuscular system. However, we found that single CMNs are hyperexcitable. Thus, it remains unclear whether CMN hyperexcitability represents a compensatory and detrimental phenotype conducive to aberrant motor outputs in aged animals.

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Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.13/I26

Topic: E.09. Motor Neurons and Muscle

Support: Tehran University of Medical Sciences (TUMS) with grant no. 40221345003.

Title: Computation of Cortico-Muscular Global Delay using the EEG and sEMG signals during Static and Dynamic Muscle Contraction Tasks by CMCTL Method

Authors: S. SAJADI[1], A. KEIHANI[2], S. NAFISSI[3], A. JAFARI[4], *M. RAZA[5];
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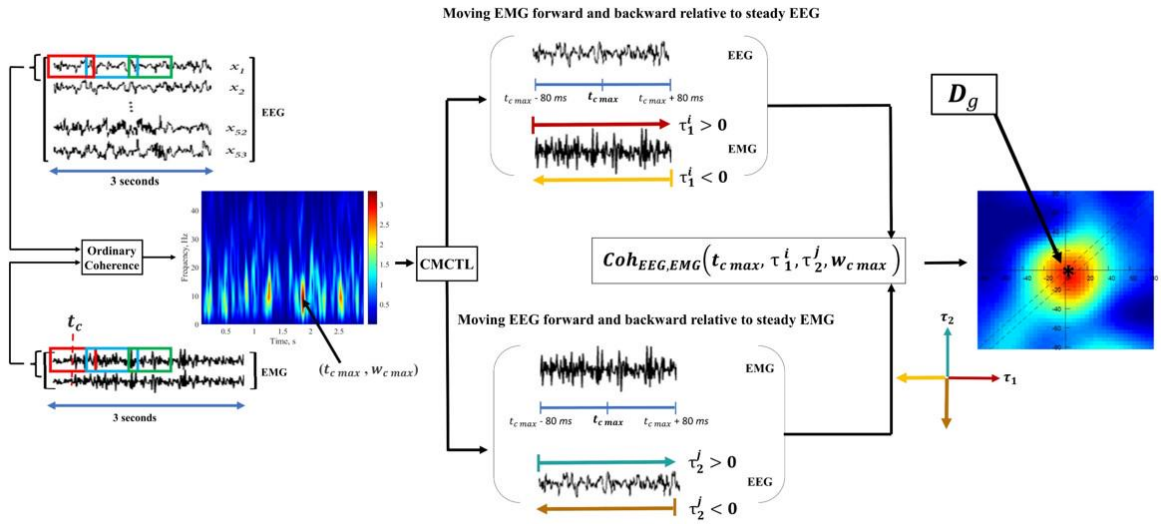
Abstract: Cortico-muscular coupling (CMC) occurs with a global time delay and if it is not accurately calculated, it may lead to decreased coupling values and misalignment bias between the synchronized events of brain and muscles. In addition, knowledge of the global time delay between the periphery and motor cortex can provide crucial insights into the interaction and communication between the motor cortex and peripheral muscles in terms of direction and propagation of oscillations. In this study we used Cortico-muscular coherence with time lag (CMCTL) method to compute global delay values. Global time delay between the EEG and sEMG signals was measured to distinguish task-related sEMG channels from non-task channels. For this purpose, simultaneous high-density EEG-sEMG data (53 EEG channels, 4 sEMG bipolar channels) was collected from 15 (30.26 ± 4.96 years) normal subjects during four specific hand and foot static (Task 1 and 3) and dynamic (Task 2 and 4) contraction. Our results show that non-task related sEMG channels had significantly longer time delay values compared to the task-related signals in both static and dynamic tasks, as seen in Task 1 (Z = -2.616, p = 0.009), Task 3 (Z = -2.731, p = 0.006), Task 2 (Z = -2.412, p = 0.016) and Task 4 (Z = -2.709, p = 0.007). Using CMCTL method, the transition of signals between the EEG and task-related sEMG channels was found faster during muscle contraction. Accurate global delay measurement during CMC could have potential applications for rehabilitation, prosthesis control and human computer interaction systems.



Disclosures: S. Sajadi: None. **A. Keihani:** None. **S. nafissi:** None. **A. Jafari:** None. **M. Raza:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.14/I27

Topic: E.09. Motor Neurons and Muscle

Support: Carol & Gene Ludwig Family Foundation
Knight Initiative for Brain Resilience

Title: Multi-plane two-photon imaging studies of motor cortical neural activity patterns in normal and parkinsonian mice

Authors: *Y. B. WENG[1,2], G. CHATTREE[3], L. WANG[4], J. J. LI[3], Y. ZHANG[3,2,5], R. T. CHRAPKIEWICZ[3,2], M. J. SCHNITZER[1,6,2,5];
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Abstract: How motor cortical neurons of the different cortical layers support the control of movement remains poorly understood. Neurons in different cortical layers seem to encode distinct movement features and may be differentially affected in pathologic brain states in which motor control is impaired. To reveal how motor cortical neurons in different laminae represent movement-related variables in normal and parkinsonian brain states, we developed a simple method for simultaneous, multi-plane two-photon Ca^{2+} imaging of neural activity in behaving mice. To a conventional, single-beam laser-scanning two-photon microscope, we added a module comprising a series of polarization beam splitters that allow multi-plane imaging using multiple beams, each focused to a different depth in brain tissue. To correct optical aberrations in beams focused far from the focal plane of the infinity-corrected microscope objective lens, we performed adaptive optical corrections using a deformable mirror. We achieved simultaneous imaging of neural Ca^{2+} activity in up to 8 different axial planes at frame acquisition rates of 30 Hz, across ~3000 cells lying in a 1.0 x 1.0 x 0.3 mm^3 tissue volume. To estimate the dynamics of individual neurons in the multi-plane movies, we used the cell extraction algorithm, EXTRACT*, which is based on the mathematical framework of robust estimation. To assess the detectability of neural activity using multi-plane two-photon imaging and EXTRACT, we characterized the estimated neural activity traces by using the metric d' from signal detection theory to quantify the fidelity with which individual Ca^{2+} transients could be detected. We found that d' declined approximately inversely with the square root of the number of imaging planes, as expected if fluorescence activity from planes that are axially displaced from a neuron effectively increase the baseline fluctuations in the neuron's estimated activity trace. Using multi-plane imaging, we recorded the simultaneous Ca^{2+} activity patterns of pyramidal neurons in layers 2/3 and 5 of the primary motor cortex in mice performing a pellet-grasping task. We compared the evoked activity patterns in the same individual mice before and after ablation of dopamine cells in the substantia nigra pars compacta, which induces a parkinsonian brain state. This revealed layer-specific changes in cortical activity after the loss of dopamine signaling, highlighting the

utility of monitoring neural activity across multiple cortical layers simultaneously. *https://github.com/schnitzer-lab/EXTRACT-public

Disclosures: Y.B. Weng: None. **G. Chattree:** None. **L. Wang:** None. **J.J. Li:** None. **Y. Zhang:** None. **R.T. Chrapkiewicz:** None. **M.J. Schnitzer:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.15/I28

Topic: E.09. Motor Neurons and Muscle

Support: R01NS124820

Title: Activation of intermediate zone interneurons in response to hindlimb peripheral nerve stimulation following chronic spinal cord injury

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Abstract: Spinal reflex activity is mediated by extensive interneuronal networks spanning multiple levels of the spinal cord, making it challenging to understand how these networks ultimately shape motor output. Here, we record the activity from a large number of muscles in combination with lumbar intermediate zone interneurons in response to hindlimb peripheral nerve stimulation. In five female chronic spinal cats (T10 complete transection; 6 weeks post-injury, untrained), two 64-channel microelectrode arrays were placed into lamina VII of the spinal cord on the right side at multiple segments spanning L3-S1 at depths between 1500-3000 µm. Bifilar EMG was recorded bilaterally from multiple ankle (soleus, medial gastrocnemius, tibialis anterior), knee (vastus lateralis, biceps femoris posterior), and hip (biceps femoris anterior, sartorius) flexor and extensor muscles. The right tibial nerve was isolated and stimulated at 2 Hz from a bipolar cuff electrode at 2, 5, and 10x sciatic nerve threshold. Interneuron and muscle activity were quantified as short- (8-40 ms; SLR) and long- (50-450 ms; LLR) latency responses from the spinal multi-unit and EMG recordings, respectively. At all intensities, the EMG SLR following tibial nerve stimulation demonstrated a focal ipsilateral response in hip, knee, and ankle flexors. A robust EMG LLR became evident only at higher intensities and was more distributed across all muscles, both ipsilateral and contralateral. Spinal interneurons demonstrated a robust SLR, which was larger at more caudal recording sites. The averaged interneuronal LLR was very small and of similar amplitude across recording sites. This suggests only a small number of spinal interneurons contribute to the EMG LLR and/or the EMG LLR is largely maintained by currents intrinsic to the spinal motoneuron. The decomposition of these spinal interneuron data into the spike times of single units will allow us to assess for subpopulations of spinal interneuronal networks that contribute to sensorimotor function

following spinal lesions. Understanding how these circuits contribute to motor output will allow for targeted biomarkers and therapies to improve motor impairments following spinal lesions.

Disclosures: M. Zaback: None. **J. Paz Amaya:** None. **C.K. Thompson:** None. **M.A. Lemay:** None.

Poster

PSTR230: Motor Neurons: Activity, Sensory, Central Control, and Disease

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR230.16/I29

Topic: E.09. Motor Neurons and Muscle

Support: NIH Grant AG44615
NIH Grant HL146114

Title: Chemogenetic Inhibition of BDNF/TrkB Signaling Reduces Mitochondrial Biogenesis in Rat Cervical Ventral Horn

Authors: *D. DASGUPTA[1], A. DAVIS[1], M. J. FOGARTY[2], C. B. MANTILLA[3], G. C. SIECK[4];
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Abstract: Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, signals through its high-affinity full-length tropomyosin receptor kinase B (TrkB.FL). BDNF/TrkB.FL signaling promotes motor neuron survival during embryonic and early postnatal development, and disruption of this signaling play a role in aging and neurodegenerative diseases. Previous studies from our lab demonstrated that BDNF/TrkB.FL signaling activates $pCREB^{S133}$ phosphorylation in rat cervical ventral horn. In other cell types, we demonstrated that $pCREB^{S133}$ transcriptionally activates PGC1α, with downstream upregulation of mitochondrial transcription factor A (TFAM) and mitochondrial biogenesis. We developed a novel chemogenetic rat model ($TrkB^{F616}$ rats) that expresses a knock-in allele sensitive to the selective kinase inhibitor 1NMPP1; permitting rapid and reversible inhibition of TrkB.FL kinase activity. 14-day inhibition of BDNF/TrkB.FL leads to phrenic motor neuron death in adult rats, although the mechanism has not been characterized yet. In the present study, we hypothesized that inhibition of TrKB.FL kinase activity reduces BDNF-induced $pCREB^{S133}$ phosphorylation, with downstream inhibition of PGC1α, TFAM mediated mitochondrial biogenesis. Adult female and male $TrkB^{F616}$ rats were intrathecally treated with either 25 μM 1NMPP1 or DMSO as vehicle. All rats were pre-exposed to BDNF to eliminate endogenous differences in BDNF availability. We showed that intrathecal BDNF-induced $pCREB^{S133}$ phosphorylation was blocked by 1NMPP1 treatment in $TrkB^{F616}$ rats. Using bioinformatic analysis, we identified a putative binding site for $pCREB^{S133}$ in the *PGC1α* promoter in rats and

confirmed using chromatin immunoprecipitation (ChIP) assay. PGC1α and TFAM mRNA and protein expressions were compared in cervical ventral horn lysates from vehicle and 1NMPP1-treated $TrkB^{F616}$ rats by quantitative realtime PCR (qPCR) and Western Blot, respectively. Following 1NMPP1-induced inhibition of TrkB.FL kinase, $pCREB^{S133}$ phosphorylation was reduced with downstream reduction in mRNA and protein levels of PGC1α and TFAM. Relative mtDNA copy number was quantified by qPCR, normalized to nuclear DNA to assess the extent of mitochondrial biogenesis. mtDNA copy number was decreased following TrkB.FL kinase inhibition by 1NMPP1. We conclude that inhibition of BDNF/TrkB.FL kinase signaling inhibits $pCREB^{S133}$ phosphorylation, which further reduces transcriptional activation of PGC1α and TFAM-mediated mitochondrial biogenesis in rat cervical ventral horn promoting mitochondrial dysfunction, that is associated with the death of larger PhMNs.

Disclosures: D. Dasgupta: None. **A. Davis:** None. **M.J. Fogarty:** None. **C.B. Mantilla:** None. **G.C. Sieck:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.01/I30

Topic: F.01. Neuroethology

Support: R01 NS113071

Title: Large-scale volumetric map of cortical dynamics in vocal communication of the singing mouse (S. teguina)

Authors: *K. KOCSIS[1], L. FLIPTS[2], H. GOLDMAN[3], Y. Z. WADGHIRI[3], M. A. LONG[2]; [1]NYU Neurosci. Inst., New York, NY; [2]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY; [3]Preclinical Imaging Core, NYU Grossman Sch. of Med., New York, NY

Abstract: Coordinated and dynamic exchange of acoustic information is an essential part of social interactions in humans and numerous other animal species. This process, called 'turn-taking' is exceptionally fast (< 1 s), which indicates that responses are actively planned while listening to the conspecific. Despite the social impact, the neural control mechanisms engaging the vocal motor circuitry for dynamic vocal interactions such as turn-taking are poorly understood. Here, we studied large-scale neocortical activation in a turn-taking animal, Alston's singing mouse (*Scotinomys teguina*), which exhibits rapid note sequences with dynamics resembling human conversation. To uncover activity underlying rapid counter-singing, we used high-density surface electrode arrays in freely behaving singing mice. The arrays conform to nonplanar surfaces, which allowed us to simultaneously record from dorsal and temporal (auditory) cortical regions. Using a reference singing mouse MRI brain volume and fluorescent anatomical landmarks for each recorded electrode grid, we created a volumetric activity map for multiple vocal conditions. Sensory and motor responses were isolated by examining cortical

high-gamma (70-150 Hz) activities associated with non-reactive/listening song trials ('sensory only') and songs performed in the absence of a partner ('motor only'). Sensory responses to the partner songs show phasic and note-tracking activity in the auditory cortical areas, as well as positive or negative modulation in frontoparietal areas. Motor activity (song production) is characterized by a widespread drop in ongoing activity, initiating from the frontal cortical areas to parietal regions, sparing the central primary motor area and auditory responsive areas. In trials in which counter-singing was elicited by the partner song ('sensorimotor'), we observed a recovery from suppression in frontoparietal areas in the motor phase as well as an increased parietal suppression in the sensory phase. This large-scale activity map will enable us to identify crucial network dynamics so that the underlying mechanisms can be further explored.

Disclosures: K. Kocsis: None. **L. Flipts:** None. **H. Goldman:** None. **Y.Z. Wadghiri:** None. **M.A. Long:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.02/I31

Topic: F.01. Neuroethology

Support: A.C. is the incumbent of the Vera and John Schwartz Family Professorial Chair in Neurobiology at the Weizmann Institute of Science.
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the Adelis Foundation (to A.C.)
Sonia T. Marschak (to A.C.)
European Research Council (ERC) Consolidator Grant 819496 to Ofer Yizhar

Title: Prefrontal representations of social information and complex group dynamics

Authors: *A. BENJAMIN, S. ANPILOV, O. IZHAKI, I. SARAF-SINIK, T. BIAGINI, Y. SHEMESH, A. RUBIN, A. CHEN, O. YIZHAR;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Processing and using social signals to guide appropriate social interactions is essential for survival and reproduction. The medial prefrontal cortex (mPFC) is one of the primary mammalian brain regions linked with such functions. However, many crucial questions remain regarding how mPFC neurons process and represent social information and coordinate complex social interactions. We used wireless head-mounted neural loggers and wireless optogenetics, to

simultaneously silence and electrophysiologically record mPFC activity, with single-unit resolution in 17 groups of three or four outbred male mice freely interacting in an enriched environment. We used a battery of complementary, ethologically-relevant experimental paradigms, which allowed us to study diverse types of correlative and causal relationships between neural activity and behavioral or external variables. Overall, we recorded 5,516 neurons from 59 mice and ~500h of simultaneous neural-behavioral data, which allowed us to investigate the neural-behavioral relationships with great richness and scale. Single-unit tuning curves with diverse tuning properties showed that the vast majority of recorded mPFC neurons represent different aspects of social and nonsocial information and behavior, including both the subjects' and the other group members' location in the arena, 3D posture and movement kinematics, not just in the present, but also in the past and future. Interestingly, different neurons represented this information in different reference frames (e.g., cartesian vs polar, allocentric vs egocentric). Using multivariate models and feature attribution methods for explainable machine learning suggested that these neurons represent social variables per se, beyond what could be explained by the subjects' own behavior. Moreover, these neurons represent the *identity* of the other group members, beyond what could be explained by the behavioral content of the social interactions. Furthermore, the mice exhibit prominent and stable social dominance hierarchies, and a large fraction of mPFC neurons represent the social rank of both the subject and the other group members. Finally, presenting the subjects with odor stimuli that represent individual mice in a highly controlled, pseudo-randomized design demonstrates directly that these neurons represent the familiarity, social rank, and identity of the other group members per se. Taken together, this study demonstrates that mPFC neurons continuously represent the behaviors of self and others in the group, providing a constantly updating representation of social context.

Disclosures: A. Benjamin: None. **S. Anpilov:** None. **O. Izhaki:** None. **I. Saraf-Sinik:** None. **T. Biagini:** None. **Y. Shemesh:** None. **A. Rubin:** None. **A. Chen:** None. **O. Yizhar:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.03/I32

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Social and vocal behaviors of olfaction-impaired mice

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[1]Psychological and Brain Sci., Univ. of Delaware, Newark, DE; [2]Univ. of Delaware, Newark, DE

Abstract: During social interactions, the behaviors of an individual are modulated by the environment and actions of others from multisensory cues, including olfaction and audition

(Chen & Hong, 2018). Mice, as a social species, emit specific ultrasonic vocalizations (USVs) during distinct social behaviors, which influence the behavior of social partners (Sangiamo et al., 2020). Olfactory signals are essential in modulating mouse social (Barabas et al., 2021) and vocal (Wilson et al., 2022) behaviors. To understand how olfactory cues influence the interplay between mouse social and vocal behaviors, we sought to record USVs and social behaviors of freely interacting mice with impaired olfactory function in a naturalistic environment. We performed binasal irrigation on adult (PD49) C57BL/6J mice with either 0.7% Triton X-100 or saline. Mice irrigated with Triton X-100 took significantly longer time (n = 14, median = 300 seconds) to find buried food compared to mice irrigated with mice (n = 13, median = 15 seconds; Wilcoxon, W = 182, $p < 0.0001$), suggesting that Triton X-100 nasal irrigation effectively impaired mouse olfactory function. Using a sound source localization system (Warren et al., 2018), we continuously recorded 5 hours of audio and trajectory data with mixed-sex groups (2 males and 2 females per group). We found that in the first hour of recordings, Triton X-100 irrigated mice (n = 7 groups, median = 2 vocalizations) produced significantly fewer USVs compared to saline irrigated mice (n = 7 groups, median = 1009 vocalizations; Wilcoxon, W = 48, $p < 0.01$). Moreover, we observed and defined an atypical social behavior exhibited by Triton X-100 treated mice: group huddling, which is when at least 2 mice stayed within 5 cm for at least 1 min. Compared to saline irrigated mice (n = 7 groups, mean = 0.15 min), Triton X-100 irrigated mice spent more time closer together huddling (n = 7 groups, mean = 21.82 min; $t = 3.74$, $p < 0.01$) in the first hour of the recordings. The findings suggest that mice with impaired olfactory function drastically decrease vocal emission and huddle more often. Further analysis is needed to extract distinct social behaviors to untangle how olfaction modulates vocal and social behaviors.

Disclosures: K. Chen: None. **Z. Shteyn:** None. **J.P. Neunuebel:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.04/I33

Topic: F.01. Neuroethology

Title: Fronto-amygdala circuit dysfunction leads to abnormal social behavior in MeCP2 mild overexpression mice

Authors: *J. QI[1], Y. CHENG[2], P. LAU[3], G.-Q. BI[3];
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Abstract: Fronto-amygdala circuit dysfunction leads to abnormal social behavior in MeCP2 mild overexpression mice
Qi Jing, Yuxiao Cheng, Guo-Qiang Bi and Pak-Ming Lau
Abstract: Methyl-CpG binding protein 2 (MeCP2) is a gene associated with DNA methylation

and plays a crucial role in maintaining brain function. As a high-risk autism gene, overexpression of MeCP2 can lead to a severe developmental disorder with dysfunctional sociability. However, the underlying pathogenic neural-network mechanisms remain largely unknown. In this study, we used a high-speed Volumetric Imaging with Synchronized on-the-fly-scan and Readout (VISoR) technique to map brain activation in order to probe how specific social behaviors are represented in the brain. Our findings revealed distinct activity patterns between MeCP2 overexpression (MeCP2-OE) and wild type (WT) mice. The medial prefrontal cortex (mPFC) and the basolateral amygdala (BLA) have been implicated in modulating social behavior, however it remains unclear how the projection from mPFC to BLA functions in social behavior and its dysfunction in autism. To address this question, we utilized fiber photometry, optogenetic and chemogenetic method to investigate. Our results demonstrated different calcium signal level between MeCP2-OE and WT mice, and manipulation of the circuit affects social behavior. Additionally, employing a sparse labeling strategy and VISoR technique, we present reconstructed single-neuron whole-brain projections of BLA neurons. We also performed single-cell RNA sequencing and presented a cell-type taxonomy of the BLA. Our findings suggest that MeCP2 overexpression induces gene-level changes and impact neuronal morphological structure, leading to aberrant neuronal activity and social disorders. These results provide valuable insights for clinical research.

Disclosures: J. Qi: None. **Y. Cheng:** None. **P. Lau:** None. **G. Bi:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.05/I34

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Investigating the activity of CA2 hippocampal neurons in freely interacting mice

Authors: *D. PANDE[1,2], R. S. CLEIN[3,2], J. P. NEUNUEBEL[4,5];
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Abstract: Dorsal CA2, a hippocampal subregion, is critical for representing social experiences (Hitti & Siegelbaum, 2014; Alexander et al., 2016). There is, however, a lack of knowledge about the firing properties of CA2 neurons as multiple mice freely interact. To capture the dynamic nature of naturalistic social behavior, we combined a sound source localization system (Warren et al., 2018) and wireless neural recordings to simultaneously track the vocalizations and actions of adult mice (C57BL/6J) in conjunction with CA2 neural activity. Male mice (n = 2)

implanted with a 32-channel silicon probe were recorded during interactions with social partners or objects over four days. Each day of recording consisted of two 15-minute solo sessions, a 60-minute social interaction session with three social partners (one male and two female mice) or three objects, followed by a third 15-minute solo session. The order of social and object days was pseudorandomized (2 social and 2 object days interleaved). We recorded 33 and 39 well-isolated putative dorsal CA2 neurons on social and object days, respectively. The average firing rates of CA2 neurons recorded during social interaction sessions were significantly higher than the preceding solo sessions (Friedman ANOVA; post hoc Dunn's test; p values < 0.05). The firing rates (average and peak) of CA2 neurons recorded during social interaction sessions were significantly higher than those recorded during object interaction sessions (Mann-Whitney test; p values < 0.05). The firing rates (average and peak) and the spatial information scores of neurons recorded during solo sessions that followed a social interaction session were significantly different from those recorded during solo sessions that followed an object interaction session (Mann-Whitney test; p values < 0.05). The spatial information scores of CA2 neurons recorded during solo sessions that followed the social interaction sessions were significantly lower than the solo sessions preceding the social interaction sessions (Friedman ANOVA; post hoc Dunn's test; p values < 0.05). Further, the spatial information scores of CA2 neurons recorded during object interaction sessions were significantly lower than the first and last solo sessions (Friedman ANOVA; post hoc Dunn's test; p values < 0.01). Overall, our results suggest that social interactions may modulate the firing and spatial properties of CA2 neurons. Future analyses probing activity during specific social behaviors and vocalizations may shed light on how CA2 encodes sensory cues related to social experiences.

Disclosures: D. Pande: None. **R.S. Clein:** None. **J.P. Neunuebel:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.06/I35

Topic: F.01. Neuroethology

Title: Subgroup-specific patterns of nesting behavior in African Naked Mole-rats

Authors: A. GUADAGNINO[1], S. TIRADO[2], *D. MCCLOSKEY[3];
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Abstract: Nesting, congregation among members of a group, has evolved independently in diverse taxa, reflecting the adaptability and resilience of cooperative strategies as a result of environmental pressures. In the eusocial animals, the evolutionary advantages of cooperative nesting may include enhanced predator defense, social immunity, thermoregulation, comfort, and enhanced parental care. It is thought that *Heterocephalus glaber*, the African Naked Mole-rat (NM-R), forms congregations with colony mates (i.e. nests), to capitalize on these effects, but

also to serve physiological needs. We have previously shown that elements of the nest environment, particularly carbon dioxide, help to mask the diminished GABAergic tone in this species. Here, we demonstrate differential nesting patterns of two main subgroups, large workers (LW) and small workers (SW) using an approach to model nesting behavior in an open field type environment. Subgroup differences in latency to nest and patterns of nesting were assessed using machine-learning aided visual observation in this environment and responses to different external (environmental gases) and internal (pharmacological manipulation) were assessed to determine the physiological benefits to nesting in each subgroup.

Disclosures: A. Guadagnino: None. **S. Tirado:** None. **D. McCloskey:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.07/I36

Topic: F.01. Neuroethology

Support: CIHR

Title: The effect of vision loss on several common mouse behavioral tests

Authors: *N. ARNOLD[1], S. TRENHOLM[2];
[1]IPN, McGill Univ., Montréal, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Background: Mice are a leading animal model in behavioral neuroscience. However, it remains unclear how much they use their visual system in common behavioral tests that are not explicitly designed to evaluate visual capabilities. To address this issue, we tested mice in a varied set of common behavioral paradigms that span the scope of 'natural' behaviors for a lab mouse. We then performed detailed analyses of their behaviors using machine learning models and compared results between sighted and blind mice. **Methods**: The behavioral paradigms we tested included open-field and novel object exploration tests, mating and aggression tests, and a test of maternal behavior - the pup-retrieval test. We performed experiments in mice of both sexes, and compared between sighted and blind animals. For WT mice we used C57Bl/6J mice. For blind animals, we use $Gnat^{1/2}$ double mutants, who have non-functioning rod and cone photoreceptors, and are on a C57Bl/6J background. For some behavioral paradigms, we also tested sighted mice in darkness, to allow a comparison between adaptive and non-adaptive changes following vision loss. All behaviors were recorded with an overhead camera. Mice were tracked using DeepLabCut and their behaviors classified using simBA. **Results and Conclusions**: Our results reveal that vision loss leads to widescale changes in behavioral performance in each behavioral paradigm that we tested. These results indicate the importance of vision in every aspect of a lab mouse's daily life, and that mice rely on vision during the performance of behavioral paradigms that were not classically designed to test the visual system. Our results thus provide insights into novel 'naturalistic' visual behavioral paradigms that could

be further investigated at the cellular, circuits and systems levels to study the mouse's visual system.

Disclosures: N. Arnold: None. **S. Trenholm:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.08/I37

Topic: F.01. Neuroethology

Title: Environmentally relevant exposure to bisphenol-A in the perinatal and juvenile period impacts morphology and reproductive behavior of rats

Authors: N. JAMES, S. LONG, A. WISENBACH, ***G. M. LANGE**;
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Abstract: In classic work by William C. Young's research laboratory, it was established that organization of mammalian brain morphology is guided by gonadally expressed hormones both in-utero and continuing into the perinatal environment. This suggests, while the genetic sex of an organism may drive development of sexual morphology and the brain, the environment also can and shape phenotypic expression. Bisphenol-A is a chemical agent found in many plastics used in everyday life. Bisphenol-A can and does leach from plastics and is inadvertently ingested by organisms. Bisphenol-A has been identified as an endocrine disruptor and impacts the neuroendocrine system and can shape development. We theorize that perinatal and juvenile exposure to bisphenol-A may influence phenotype by reshaping organization of the mammalian central nervous system during perinatal and juvenile development to alter phenotypic expression of morphology and reproductive behavior. Exposure to bisphenol- A occurred during post parturition days 1-21. At maturity, neuromuscular development was assessed. Additionally, the presence and timings of reproductive behaviors of mounts, intromissions, inter-intromission intervals, post-ejaculatory intervals, and lordosis quotients were examined.

Disclosures: N. James: None. **S. Long:** None. **A. Wisenbach:** None. **G.M. Lange:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

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Topic: F.01. Neuroethology

Support: Grass Foundation
Kavli Foundation
Marine Biological Laboratory, Woods Hole, MA, U.S.A.
Centre of Neural Computation 223262
Centre for Algorithms in the Cortex 332640
Ministry of Science and Education, Norway

Title: Behaviors during social encounters in the cuttlefish Sepia Bandensis

Authors: Z. H. DALVA-BAIRD[1], *H. A. OBENHAUS[2];
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Abstract: Effective communication between individuals of a group is crucial to survival, especially in the context of predator avoidance, mating, and the establishment of dominance hierarchies. Some species of coleoid cephalopods (octopuses, cuttlefish, and squid), which are marine invertebrates that exhibit a wide behavioral repertoire, have been observed to exchange rich communication cues when interacting with conspecifics and predators. These cues are visually more distinct than in most other species due to the involvement of a neurally controlled skin patterning system, which is highly active during social interactions. The stumpy-spined or dwarf cuttlefish, Sepia Bandensis, displays particularly diverse and colorful skin patterns during encounters with other individuals of the same species. Quantification of these rapidly changing skin dynamics allows for indirect readout of central neuronal activity during unrestrained social communication, which is hard to obtain in any other model organism. Here we show data from video recordings of five S. Bandensis over several weeks. During this time, we ran interaction trials between all pairs of individuals in a behavioral apparatus that allows for exposure and isolation of cuttlefish without invasive manipulation of their behavioral space. During interaction, S. Bandensis females and males displayed skin patterns, which varied in content and dynamics from baseline camouflage. Using custom image analysis routines we are extracting a library of cuttlefish mantle and head region patterns from social interaction trials. We noticed that older, possibly sexually mature, females exhibited increased locomotor activity compared to younger animals, and we are currently exploring how skin displays in S. Bandensis are used for social communication in different age groups. The methods employed here open the door to rich quantification of behavior in conjunction with brain state dynamics during social encounters in cephalopods.

Disclosures: Z.H. Dalva-Baird: None. **H.A. Obenhaus:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Program #/Poster #: PSTR231.10/I39

Topic: F.01. Neuroethology

Support: NIMH R15MH124042

Title: Unsupervised clustering of mouse pup ultrasonic vocalizations did not identify any distinct call types relevant for efficient pup retrieval by female adult mice

Authors: *J. ELROD[1], B. Y. LAU[2], K. KRISHNAN[3];
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Abstract: During pup retrieval, female mice gather scattered pups back to the nest. Early studies in the field showed that rat dams use a combination of olfactory, tactile, and auditory information to locate, identify and retrieve pups. Isolated pups emit ultrasonic vocalizations (USVs), which are thought to prompt retrieval from adults. However, in our studies with female CBA and C57/Bl6J inbred strains, we noticed that retrievals are very fast, and can occur without USVs. This prompted us to inquire if pup USVs communicate socially relevant information useful for retrieval. Though pup calls in isolation have been reported for decades, fewer studies have clustered calls to determine if there is a call type that prompts retrieval in early age pup, when retrieval to the nest is essential for survival. Using Deepsqueak, we analyzed nearly 59,000 calls emitted by CBA pups aged postnatal days 0-5 across different phases of the retrieval task: (1) pup isolation, (2) during pup retrieval, and (3) habituation phase. 8 distinct call clusters were identified, with two major groups, high frequency clusters and low frequency clusters. We found no significant differences in how these clusters were used during isolation, retrieval, or habituation phases. We identified that the cluster prevalence changes across days, potentially due to development of vocal repertoire as pups grow. UMAP analysis reveals that while distinct, these clusters do not cluster separately spatially, as seen in other vocal species such as birds. Additionally, pup vocalizations did not differ in the presence or retrieval efficiency of wild-type or Rett syndrome female mice, a model with known retrieval deficits. These findings show that pups do not emit specific call types to prompt efficient retrieval. However, the developmental progression of call types suggests that pups hone their repertoire, which could have ethological relevance.

Disclosures: J. Elrod: None. **B.Y. Lau:** None. **K. Krishnan:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Topic: F.01. Neuroethology

Support: Cold Spring Harbor Laboratory
NIH Brain Initiative RF1-NS132046-01
International Society for Neuroethology Konishi Neuroethology Research

Award
Searle Scholars Program
Pershing Square Innovator Fund
Esther A. & Joseph Klingenstein Fund

Title: Evolutionary diversification of vocal behavior requires the midbrain periaqueductal gray

Authors: *C. E. HARPOLE[1], X. ZHENG[1,2], M. B. DAVIS[1], **A. BANERJEE**[3];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Cold Spring Harbor Laboratory School of Biological Sciences, Cold Spring Harbor, NY; [3]Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Using sounds to communicate is widespread across the animal kingdom. Despite recent advances, our understanding of how mammalian brains function and evolve to enable vocal communication remains limited. To address this, we investigate the neural circuits for vocalizations in the singing mouse (*Scotinomys teguina*). To probe the vocalizations of individuals during social interactions, we developed a behavioral assay where two mice interact across a perforated divider. Using a custom analysis pipeline, we were able to assign a vast majority of vocalizations to their sources. We found that vocalizations are organized into two distinct modes - calls and songs. Songs are comprised of a series of progressively longer notes that evolve predictably over many seconds. In contrast, calls are much less stereotyped and much quieter. During social interactions, calls become more frequent and songs more variable, and individuals rapidly switch between the two modes. Despite their differences, songs and calls largely share the same peripheral mechanism. Since calls resemble the ultrasonic vocalizations (USVs) in other rodents, we they may share neural control by the periaqueductal gray (PAG). To test this, we asked whether activation of the PAG is sufficient for generating calls. Unilateral optogenetic activation of ChR2-expressing PAG_{CAMKII} neurons was sufficient to elicit calls throughout the stimulation period. The same stimulation in lab mice elicited USVs. Based on acoustic properties, vocal usage, production mechanism, and neural control, we posit that singing mouse calls are homologous to USVs and represent an ancestral behavioral mode shared across many rodents, while the songs represent a behavioral novelty. Furthermore, synaptic blocking of PAG_{CAMKII} neurons with tetanus toxin light chain (TeLC) disrupted the temporal patterning of songs and eventually rendered animals mute. Together, our results demonstrate that the midbrain PAG controls both vocal modes and is a critical circuit locus for behavioral innovation.

Disclosures: C.E. Harpole: None. **X. Zheng:** None. **M.B. Davis:** None. **A. Banerjee:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Program #/Poster #: PSTR231.12/J1

Topic: F.01. Neuroethology

Support: Cold Spring Harbor Laboratory
NIH Brain Initiative RF1-NS132046-01
Searle Scholars Program
Pershing Square Innovator fund
Klingenstein - Simons Fellowship

Title: Computational and neural insights into the temporal patterning of songs in the singing mice

Authors: *Y. THAPA, X. ZHENG, M. B. DAVIS, C. E. HARPOLE, B. R. COWLEY, A. BANERJEE;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Communication is essential for any social interaction, and a primary way animals communicate is through vocalization. From the chirps of songbirds to conversational human speech, vocalization requires temporal precision. Here, we study the unique vocalizations of Alston's singing mice, which modulate their song length and rate during social interaction—a hallmark of communication. However, even when alone, these mice will also produce dozens of songs throughout the day. The temporal patterning of such solo songs over multiple timescales (e.g., minutes to hours), how it changes during social interactions, and the neural mechanisms of this behavior remain unclear. Based on weeks-long recordings, we observed time periods where solo mice produced songs in equally spaced intervals resembling a clock. This clock-like singing behavior is modified in the social context to facilitate turn-taking among individuals. To better understand the internal states of the animal, we model this clock-like behavior, as well as social interactions, with a latent variable model. We then analyze our model to generate hypotheses about possible neural mechanisms underpinning an internally generated rhythmic behavior and how it changes in the context of social interaction.

Disclosures: Y. Thapa: None. **X. Zheng:** None. **M.B. Davis:** None. **C.E. Harpole:** None. **B.R. Cowley:** None. **A. Banerjee:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Topic: F.01. Neuroethology

Support: NSF GRFP
Cold Spring Harbor Laboratory
NIH Brain Initiative RF1-NS132046-01
Searle Scholars Program
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Klingenstein Philanthropies Simons Fellowship

Title: Modification of motor cortical circuitry underlies the evolution of behavioral novelty in the singing mouse

Authors: *E. ISKO[1], M. DAVIS[1], H. ZHAN[2], C. HARPOLE[1], X. ZHENG[1], A. M. ZADOR[3], A. BANERJEE[4];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]MAPseq/BARseq Core Facility, Cold Spring Harbor Lab., Cold Spring Harbor, NY; [3]Zador Lab., Cold Spring Harbor Lab., Cold Spring Harbor, NY; [4]Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: A fundamental goal of neuroscience is to understand how neural circuits evolve to enable novel behavior. Since behaviors do not fossilize, our strategy is to identify neural circuit modifications among closely-related species with large behavioral divergences. One such divergence can be observed in the vocalization behaviors of *Mus musculus* (lab mice) and *Scotinomys teguina* (singing mice). While most rodents, including lab and singing mice, produce short, ultra sonic vocalizations (USVs), singing mice display a novel vocalization type that is human-audible, stereotyped, and many seconds long. Manipulation and recording experiments indicated a role of the orofacial motor cortex (OMC) in this behavior leading us to hypothesize that singing arises from modifications in OMC. Here, we investigated 3 possible modifications that OMC could evolve to acquire novel behavior: (1) OMC neurons form novel projections, (2) OMC neurons form increased projections, or (3) more OMC neurons project to downstream targets.To test for novel projections, we identified bulk projection targets of OMC neurons using viral tracing and serial two-photon tomography. In both species, OMC neurons project to identical downstream brain areas including the contralateral cortex, striatum, thalamus, superior colliculus, periaqueductal grey (PAG), and others (N=3 mice per species). In the absence of novel projections, we wanted to determine whether there were any changes in projection strength requiring the quantification of many neural projection patterns at single-cell resolution. This feat is made possible with MAPseq, a high-throughput RNA barcoding technique. We found no difference between species in the number of projections individual neurons had to each target region. But in the singing mice, a larger proportion of OMC neurons project to the PAG and a temporal cortical area (singing mice: n=5114 neurons, 7 animals; lab mice: n=71704 neurons, 5animals). No other target region (11 total) showed significant species-specific differences. We next found that this increased projection strength from OMC was driven by neurons with direct projections to temporal cortex and PAG with no/few collaterals. In summary, we found evidence for expansion of existing vocal motor circuits in the singing mice compared to lab mice, which may explain their species-typical vocal behaviors. Ongoing experiments examine the function of these projections with neural circuit perturbations. Our work demonstrates how comparing related species can garner insight into the neural mechanisms of behavior as well as reveal details of neural circuit evolution.

Disclosures: E. Isko: None. **M. Davis:** None. **H. Zhan:** None. **C. Harpole:** None. **X. Zheng:** None. **A.M. Zador:** None. **A. Banerjee:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.14/J3

Topic: F.01. Neuroethology

Support: NIH Brain Initiative Grant U01-NS128612

Title: Bred for affection: The canine anterior ectosylvian gyrus responds selectively to social reinforcement

Authors: ***K. J. MILLER**[1], F. LAMPERT[1], F. MIVALT[2], I. KIM[2,3], N. F. INCE[4], J. KIM[2], V. KREMEN[2], M. BAKER[1], M. A. VAN DEN BOOM[4], D. HERMES[4], V. A. COENEN[5], G. SCHALK[6], P. BRUNNER[7], G. A. WORRELL[2];
[1]Dept. of Neurosurg., [2]Dept. of Neurol., [4]Dept. of Biomed. Engin., [3]Mayo Clin., Rochester, MN; [5]Dept. of Stereotactic and Functional Neurosur, Freiburg Im Breisgau, ; [6]Fudan University/Huashan Hosp., Shanghai, China; [7]Washington Univ., St. Louis, MO

Abstract: Studying mammalian brain function aids our understanding of human brain evolution. A beagle was implanted with a prototype human neuromodulation platform that measures activity from the brain surface. One year later, a set of simple sensory tasks were performed, finding visual and somatosensory representation in the canine homologs of the expected areas in humans. Surprisingly, the canine anterior ectosylvian gyrus, which is anatomically homologous to human receptive speech areas, was selectively active during independent social reinforcement tasks. This suggests that speech understanding may have evolved from more general mammalian brain structures that are specialized for social reinforcement.

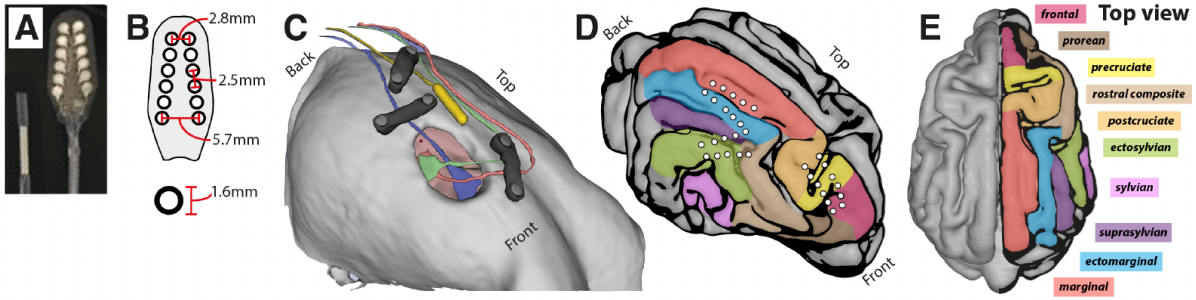

Figure 1. Right hemisphere implant and anatomic segmentation **(A)** Electrode grid and ground electrode. **(B)** Schematic with dimensions of electrode grid. **(C)** Rendering of skull and craniotomy with electrodes in situ, ground electrode in yellow. Plates that hold lead in place are shown in black. **(D)** Brain rendering showing three grids in situ, extracted from pre-implant MRI and registration to post-implant CT, with gyri color-coded. **(E)** Top view of the canine cortex, with gyri and labels color-coded.

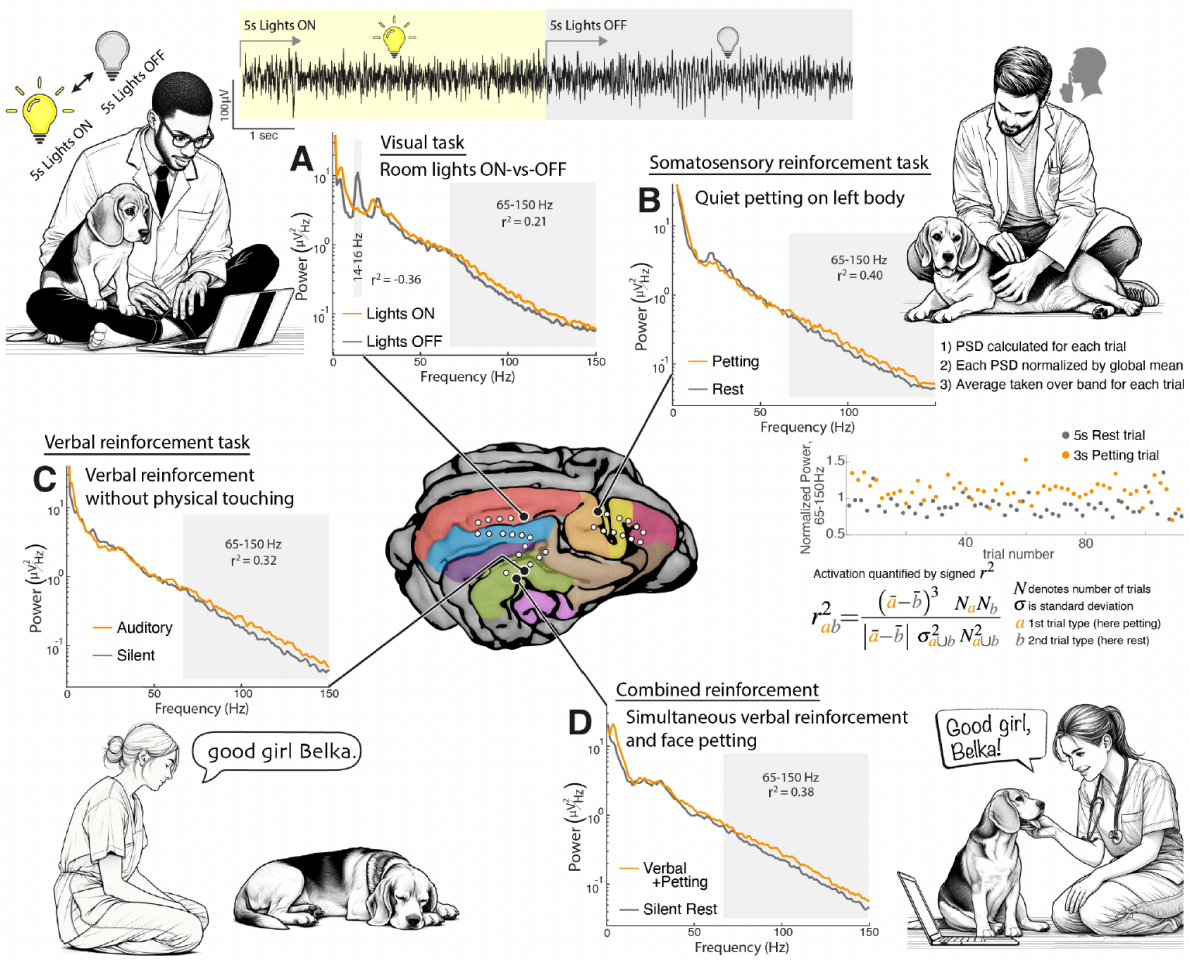

Figure 2. Brain electrophysiology during sensory tasks. Power spectral densities (PSDs) are shown for four different sensory tasks, comparing active (orange) and inactive (gray) behavioral states. **(A) Visual task** - Blocks of 5 seconds with the room lights on ("Lights ON") were interleaved with 5 seconds in the dark ("Lights OFF"). Signal changes are seen in the raw voltage trace from the marginal gyrus, both in low frequency oscillation ranges (here at 14-16Hz) and also in broadband spectral changes (here captured at 65-150Hz), which have been shown in humans to correlate with local neuronal population activity[7,8]. **(B) Somatosensory reinforcement task** - 3s blocks of tactile stimulation (petting left whiskers, front and hind limbs, and torso) were interleaved with 5s blocks of rest, with no verbal inputs. PSD from the post-cruciate gyrus is shown. **(C) Verbal reinforcement task** - 3s blocks of verbal reinforcement, saying "Good girl Belka" once each block, were interleaved with 5s quiet blocks. There was no physical contact. PSD is shown from the anterior ectosylvian gyrus. **(D) Combined verbal & somatosensory reinforcement** 5s simultaneous reinforcement blocks where the examiner provided simultaneous praise ("Good girl Belka!") & gently touching the left side of the face with eye contact, were interleaved with 5s rest periods. PSD from the anterior ectosylvian gyrus is shown. For analysis, PSDs for each task block were normalized by the average PSD over the whole experiment, and averaged normalized power was quantified for each frequency range. Task-associated changes were quantified using a signed r^2 metric (which can range from -1 to 1), as illustrated in the right-middle. All reported r^2 are significant at $p < 10^{-5}$ (unpaired t-test, Bonferroni corrected for number of channels). Note that data are common average re-referenced for generation of these PDSs.

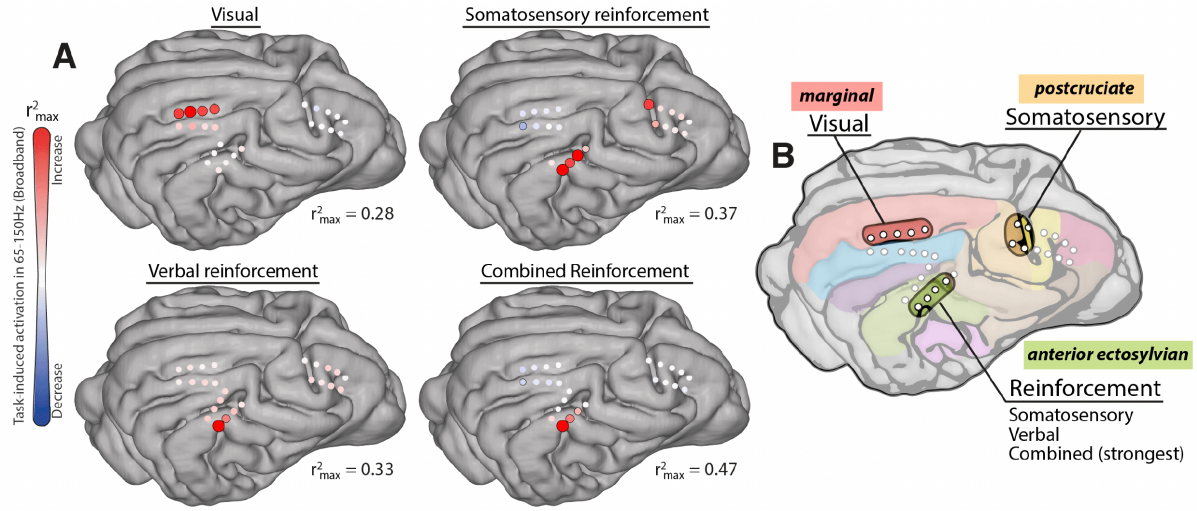

Figure 3. Functional maps across across sensory tasks. (A) Maps of function in the canine brain showing distinct representation during each of four tasks. The electrode diameter and color intensity reflect task-associated revealed by broadband power changes in the PSD (65-150 Hz), quantified by r^2 value. Each task map is independently scaled for each task to r^2_{max}, which is noted to the bottom right of each brain. A black circle around the channel denotes statistical significance at $p < 0.05$ by unpaired t-test, after Bonferroni correction for number of channels. Data were bipolar re-referenced for these maps. Note that processing of a simple tone did not activate any area covered by the 3 grids (Not shown here). **(B)** Summary plot showing functional representation. Note that the anterior ectosylvian gyrus, which is the canine homolog of human Wernicke's area, is active for social reinforcement, whether it is verbal or somatosensory (petting), and is most active for both combined.

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Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.15/J4

Topic: F.01. Neuroethology

Title: Vocalization-correlated neural responses in the marmoset brainstem

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of Tübingen, Med. Ctr., Tübingen, Germany; [2]Neurobio., Duke Univ., Sch. of Med., Durham, NC

Abstract: The execution of vocalizations relies on the coordination of cortical and brainstem neural networks. In monkeys, cortical regions are primarily involved in the initiation of call production, while the complex acoustic features of vocalizations are generated at the brainstem level. The vocal pattern generator in the brainstem generates the neural patterns necessary to drive all the motoneuron pools involved in call production. Using a semi-chronic electrophysiological recording approach, we investigated the role of the vocal motor network in the brainstem of marmoset monkeys producing volitional vocalizations within a visual detection task. We found single neurons with vocalization-related activity in all the recorded motoneuron pools involved in vocal output as well as in the putative vocal pattern generator. The neural activity patterns reveal a complex brainstem network involved in vocal motor control.

Disclosures: E. Cavani: None. **T. Pomberger:** None. **S.R. Hage:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.16/J5

Topic: F.01. Neuroethology

Support: HFSP RGP0019

Title: Neurobiology of vocal production in pinnipeds

Authors: *A. A. ROUSE, P. COOK;
New Col. of Florida, Sarasota, FL

Abstract: Parallel neurobiological adaptations supporting vocal production learning have been elucidated in humans and psittacine birds. In addition, graded brain adaptations have been mapped corresponding to varied vocal learning capability across primate and bird species. To date, there are limited data on relevant brain circuits in any nonprimate mammal. We present the first tractography study of auditory-motor and brainstem circuits covarying with vocal learning capability across pinniped species. Following rapid speciation on moving to an amphibious lifestyle, extant pinnipeds demonstrate a spectrum of vocal production learning capabilities. Otarridae are vocally stereotypic. Phocids show more vocal flexibility, with elephant seals demonstrating critical period vocal learning sensitivity, and harbor seals showing some signs of florid vocal production learning throughout the lifespan. Using post-mortem diffusion imaging and tractography, we found evidence of graded vocal motor and auditory connectivity and altered brainstem circuits matching putative vocal learning behavior across California sea lions, Northern elephant seals, and Pacific harbor seals. These findings solidify the pinnipeds as a valid large mammal model for studying the evolution of vocal learning.

Disclosures: A.A. Rouse: None. **P. Cook:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.17/J6

Topic: F.01. Neuroethology

Title: Marmoset monkeys use different avoidance strategies to cope with ambient noise during vocal behavior

Authors: *J. LÖSCHNER[1], T. POMBERGER[2], S. R. HAGE[1];
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Abstract: Multiple strategies have evolved to compensate for masking noise, leading to changes in call features. One call adjustment is the Lombard effect, an increase in call amplitude in response to noise. Another strategy involves call production in periods where noise is absent. While mechanisms underlying vocal adjustments have been well studied, mechanisms underlying noise avoidance strategies remain largely unclear. We systematically perturbed ongoing phee calls of marmosets to investigate noise avoidance strategies. Marmosets canceled their calls after noise onset and produced longer calls after noise-phases ended. Additionally, the number of uttered syllables decreased during noise perturbation. This behavior persisted beyond the noise-phase. Using machine learning techniques, we found that a fraction of single phees were initially planned as double phees and became interrupted after the first syllable. Our findings indicate that marmosets use different noise avoidance strategies and suggest vocal flexibility at different complexity levels in the marmoset brain.

Disclosures: J. Löschner: None. **T. Pomberger:** None. **S.R. Hage:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.18/J7

Topic: F.01. Neuroethology

Support: NSF IOS 1934386

Title: Mechanisms underlying testosterone-induced vocal masculinization in African clawed frogs

Authors: L. KLAR, A. YALAVARTY, ***A. YAMAGUCHI**;
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Abstract: Male and female African clawed frogs, X. laevis, emit sexually distinct calls to coordinate reproduction. Both sexes produce calls consisting of a series of clicks repeated at different rates: the male advertisement call comprises clicks repeated at 30 or 70Hz, while the female release calls consist of clicks repeated at approximately 6Hz. Previously, we found that administering testosterone to sexually mature adult female X. laevis resulted in the masculinization of their vocalizations in as little as two weeks, indicating that the central vocal pathways initially producing slow motor rhythms are reconfigured to produce rapid motor rhythms within a short amount of time. Testosterone-induced vocal masculinization thus provides a unique opportunity to explore the neural basis of behavioral modification in adult vertebrates. Perineuronal nets (PNNs) are specialized extracellular matrices in the brain known to inversely correlate with neural plasticity. During development, PNNs progressively increase as neural circuitry becomes more stable. Here, we hypothesized that androgen-induced vocal masculinization is accompanied by a decrease in PNNs in the vocal nuclei that facilitate neural plasticity. Our results, however, showed that the quantity of PNNs in testosterone-treated, fully masculinized female brains was similar to those of sham-operated female brains. We suggest that the loss of PNNs may not be a part of the mechanisms regulating the neural plasticity in the motor systems in adulthood.

Disclosures: L. Klar: None. **A. Yalavarty:** None. **A. Yamaguchi:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.19/J8

Topic: F.01. Neuroethology

Support: Illinois Computes project which is supported by the University of Illinois Urbana-Champaign and the University of Illinois System
Student Sustainability Committee of the University of Illinois Urbana-Champaign and the Illinois Green Fund
This research was funded [in part] by the Beckman Institute for Advanced Science and Technology Graduate Fellows Program with support from the Arnold and Mabel Beckman Foundation.

Title: Establishing multiscale measures of myelin in the mouse auditory cortex

Authors: *S. SINGH[1], Z. LI[2], L. SUTKUS[3], B. SUTTON[4], D. LLANO[5], D. J. MILLER[6];
[1]Evolution, Ecology, and Behavior, Univ. of Illinois, Urbana-Campaign, Champaign, IL;

[2]Neurosci., Univ. of Illinois Urbana-Champaign, Urbana, IL; [3]Neurosci., UIUC, Urbana, IL; [4]Bioengineering Dept., Univ. of Illinois Urbana Champaign, Urbana, IL; [5]Univ. of Illinois at Urbana-Champaign, Urbana, IL, ; [6]Evolution, Ecology and Behavior & Beckman Inst. for Advanced Sci. and Technol., Univ. of Illinois, Urbana, IL

Abstract: Histological myelin content has historically been used to map the brain, yet translating myelin density patterns found in histology to neuroimaging datasets to assess individual variability remains an outstanding challenge for noninvasive clinical applications. Magnetic resonance imaging (MRI), for example, is sensitive to the diffusion of water, however we do not know how sensitive and specific the signal is to myelin versus other cellular processes. Histological myelin maps in cats and primates suggests auditory cortical subfields can be identified by differential myelin content. Therefore, we contribute the first direct multiscale quantitative volumetric measures of myelin as estimated in MRI and histology in the mouse to determine the MRI modality most sensitive to myelin content and use that to measure individual variability. Specifically, we hypothesize that using 9.4T small animal bore, quantitative R1 (1/T1) will be most reflective of myelin content relative to longitudinal relaxation time (T1), transverse relaxation time (T2), and T2* MRI data. In line with previous work, our preliminary data shows differences in columnar myelin content across auditory cortical subfields. Specifically, using our modified Gallyas silver staining method, we show in histology that primary auditory cortex (ACp) is slightly more heavily myelinated than dorsal auditory cortex (ACd), and that both ACp and ACd exhibit more myelin in the upper cortical layers than ventral auditory cortex (ACv). We will compare this to multimodal MRI to determine which modality is most reflective of the myelin patterns shown in histology and define the ventral boundary of auditory cortex. Next, we assess individual variability in terms of the size of auditory cortical subfields using T1 and we observed that across mice, ACp was the largest (mean = 2.40mm^3, standard deviation = 0.26) followed by ACv (mean = 2.06 mm^3, standard deviation = 0.22) and ACd (mean = 1.22 mm^3, standard deviation = 0.13). The average coefficient of variance for the size of each auditory cortical subfield across individuals was 10.8%. Ultimately, we aim to improve our interpretation of neuroimaging data by determining which MRI modality is most representative of histological myelin content to map auditory cortex and assess individual variability and its significance for therapeutic interventions, like cochlear implants.

Disclosures: S. Singh: None. **Z. Li:** None. **L. Sutkus:** None. **B. Sutton:** None. **D.J. Miller:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.20/J9

Topic: F.01. Neuroethology

Support: Fulbright Postdoctoral Fellowship ED481B-2023-091
Simons Society of Fellows Junior Fellowship 855220

Vision Sciences Training Grant 305 T32EY013933
NIH K99GM151689
Searle Scholarship
Sloan Fellowship in Neuroscience
NIH Grant R34NS116734
NIH Grant R35GM14305

Title: Probing the neural basis of visually-evoked aggression in male and female Siamese fighting fish

Authors: *P. RODRIGUEZ VILLAMAYOR[1], A. NOROVICH[2], P.-Y. SHIH[3], C. EVERETT[4], M. R. WHITEWAY[5], A. BENDESKY[6];
[1]Zuckerman Inst., Columbia Univ., New York, NY; [2]Simons Fndn., New York, NY; [3]Columbia Univ., Pasadena, CA; [4]Zuckerman Inst., New York, NY; [5]Neurosci., Columbia Univ., Brooklyn, NY; [6]Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY

Abstract: Probing the neural basis of visually-evoked aggression in male and female Siamese fighting fish
Paula R Villamayor[1*], Amy Norovich[1], Pei Yin-Shih[1,2], Claire Everett[1], Matthew R. Whiteway[1], Andrés Bendesky[1,2]
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Aggression is a fundamental aspect of social behavior across species, influencing the dynamics of human and animal societies. While visual cues prominently trigger aggression in humans and primates, rodent and fly models, commonly used in behavioral studies, primarily rely on olfactory cues. Consequently, the impact of visual stimuli on aggression, and social behavior more broadly, remains relatively underexplored. Siamese fighting fish (Betta splendens), selectively bred for robust aggressive behavior driven by visual cues, offers a promising new model species to investigate the neurobiological underpinnings of visually-evoked aggression. This study aimed to (i) develop behavioral assays that elicit aggressive display in adult male and female and (ii) identify the neural circuits underlying visually-evoked aggression. Leveraging recent advances in machine learning, we employed markerless tracking methods to capture behavioral hallmarks of aggression (i.e. flaring). Additionally, tracing, phosphorylation of ribosomal subunit-S6 (pS6) labeling, and RNA sequencing (RNAseq) were utilized to map the neural representation of aggressive behavior. Our findings revealed that males exhibit significantly higher levels of aggression than females when visually exposed to a male conspecific. PS6 immunolabelling revealed an increase of activated neurons in the dorsomedial-pallium (Dm), and other telencephalic regions during aggressive display to a conspecific, compared to exposure to an empty tank. Furthermore, RNAseq analysis identified several differentially expressed genes (DEGs) in these brain regions between males and females, potentially contributing to sex differences in aggressive behavior. Finally, we identified distinct projections of retinal ganglion cells to lateral thalamic nucleus and optic tectum, but not to Dm, suggesting a complex neural pathway involved in processing visual stimuli triggering aggression. Our study provides valuable insights into the neurobiological mechanisms underlying sex differences in visually-evoked aggression in Siamese fighting fish. Future directions involve genetic manipulation of DEGs between males and females and elucidating the intersection of identified brain regions with the visual system.

Disclosures: P. Rodriguez Villamayor: None. **A. Norovich:** None. **P. Shih:** None. **C. Everett:** None. **M.R. Whiteway:** None. **A. Bendesky:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.21/J10

Topic: F.01. Neuroethology

Title: Exposure to a receptive female rat produces changes in the proportion of dendritic spines in the medial prefrontal cortex and nucleus accumbens of male rats

Authors: *F. BARRERA COBOS[1], P. CORTES ESPARZA[2], M. N. VÁZQUEZ HERNÁNDEZ[3], E. HERNANDEZ-ARTEAGA[4], A. C. MEDINA[5], M. HERNANDEZ[6]; [1]Univ. de Guadalajara, Zapopan, Jalisco., Mexico; [2]Technol. Monterrey, Mexico, Mexico; [3]Ciencias de la Salud, Univ. Tecnológica de México, Tlaquepaque, Mexico; [4]Human Developmental Sci., Autonomous Univ. of Tlaxcala, Tlaxcala, Mexico; [5]Neurobiología Conductual y Cognitiva. Inst. de Neurobiología-UNAM, Inst. de Neurobiología, UNAM, Queretaro, Mexico; [6]Inst. de Neurociencias, Univ. de Guadalajara, Guadalajara, Mexico

Abstract: Sexual experience facilitates copulation in male rats and has been associated with morphologic changes in the neurons of the medial prefrontal cortex (mPFC) and nucleus accumbens (NAcc), structures involved in detecting and processing sexual stimuli. Dendritic spines represent the main sites of excitatory inputs to neurons and their morphology is associated with the post-synaptic processing of information. Although male sexual experience has previously been linked to dendritic spine density in the brain, the flexibility of these spines concerning the detection and processing of female stimuli remains unknown. Hence, this study aims to evaluate the effect of female stimuli on the density of dendritic spines in the mPFC and NAcc of sexually naive and experienced male rats. 60 Wistar adult male rats were used, sexually-experienced (n=30) and naive (n=30). One day after the last copulatory test 10 subjects from each group were exposed to one of the following three types of stimuli: without any stimulus, in front of an ovariectomized female rat, and in front of a sexually receptive female rat. The brain tissue was then removed and fixed. The density of dendritic spines in the mPFC and NAcc was then quantified and classified according to their morphology using the modified Golgi method. The results indicate that regardless of sexual experience, male rats present a lower proportional density of mushroom spines in the mPFC in the receptive female condition regarding other conditions [FB (2,54) = 11.07; p (F) <0.05]. In the receptive female condition, the sexually-experienced rats show a lower proportional density of mushroom spines in the NAcc, compared to the naive group [(FAxB (2,54) = 4.14; p (F) <0.05]. Regardless of sexual experience, male rats, after being exposed to a receptive female rat, present a greater proportional density of stubby spines in both the mPFC [FB (2,54) = 4.86; p (F) <0.05] and NAcc [FB (2,54) = 5.25; p (F) <0.05]. These findings show that the presence of an inaccessible receptive female rat induces mainly plastic changes of mushroom and stubby spines in the mPFC

and NAcc of sexually naive and experienced male rats. In sexually male rats, it could be associated with processing relevant stimuli to generate sexual motivation. In contrast, in naive rats, it could facilitate the processing of novel stimuli at a distance. Together, these results suggest that the stimuli from a receptive female rat, not only modulate synaptic morphology in brain areas related to sexual motivation but may also elicit modifications in neuronal excitability, thus showing the complexity of the neural mechanisms underlying sexual behavior in rats.

Disclosures: F. Barrera Cobos: None. **P. Cortes Esparza:** None. **M.N. Vázquez Hernández:** None. **E. Hernandez-Arteaga:** None. **A.C. Medina:** None. **M. Hernandez:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.22/J11

Topic: F.01. Neuroethology

Support: NSFC Grant 32271083

Title: Distinct neural dynamics underlying context-dependent vocal production in marmoset prefrontal cortex

Authors: *Y. XU, J. TSUNADA;
Chinese Inst. for Brain Res., Beijing, Beijing, China

Abstract: A key aspect of human vocal communication is our ability to flexibly modify and control our speech depending upon social contexts as well as environmental conditions (context-dependent vocal production). Whereas recent progress in human and monkey neurophysiological studies has identified differential roles within the frontal cortex in vocal control, spanning planning, initiation, and articulation of vocalizations, the specific neural computations underlying context-dependent vocal control, particularly at the single neuron level, remain unknown. To address this gap, we recorded the local field potentials (LFPs) and single-unit activity from the prefrontal cortex of marmoset monkeys, a species known for their sophisticated vocal control abilities, while marmosets voluntarily produced vocalizations in different social and environmental contexts. The social context manipulations varied the distance from a partner monkey (close: 1m, far: 3.5m), and the environmental manipulations changed the ambient noise level (standard: 25 dB, noise: 70 dB). Consistent with human findings, monkeys altered their vocal behavior, including the use of different call types and acoustics, in response to the contextual manipulations. The theta-band and high-gamma bands of LFPs showed increases immediately before vocal production (pre-vocal activity), which further encoded call type information. Interestingly, single-unit activity exhibited similar modulations, with a subset of neurons encoding call types only in a specific context. To further understand whether and how neural populations integrate call types with contextual information, we analyzed low-dimensional neural trajectories of population spiking activity. This analysis revealed distinct pre-

vocal trajectories that varied based on the interplay between call types and contexts. Our findings suggest that unique neural population dynamics link vocal production with specific contexts, offering insights into context-dependent vocal behavior.

Disclosures: Y. Xu: None. **J. Tsunada:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.23/J12

Topic: F.01. Neuroethology

Support: NINDS 2 R01 NS021135
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NIH/NINDS (U24-NS109103, U01-NS108916)
NIH R21 Hd116195-01

Title: Intracranial EEG insights into inter-individual communication

Authors: *S. L. ALNES[1,2], J. WEYERMANN[3], O. KIM-MCMANUS[4,5], A. STOLK[6,7], I. SAEZ[8], J. T. WILLIE[9,10], P. BRUNNER[9,11,10], R. T. KNIGHT[12,13], A. LLORENS[14,15], A. TZOVARA[3,2,16];
[1]Univ. of Bern, Bern, Switzerland; [2]Zentrum für Experimentelle Neurologie, Department of Neurology, Inselspital University Hospital Bern, Bern, Switzerland; [3]Inst. for Computer Sci., Univ. of Bern, Bern, Switzerland; [4]Dept. of Neurosci., UCSD, San Diego, CA; [5]Division of Neurology, Rady Children's Hospital, San Diego, CA; [6]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [7]Donders Institute for Brain, Cognition, and Behaviour, Radboud University, Nijmegen, Netherlands; [8]Icahn Sch. of Med., Mount Sinai, New York, NY; [9]Dept. of Neurosurg., Washington Univ. Sch. of Med., Saint Louis, MO; [10]National Center for Adaptive Neurotechnologies, Albany, NY; [11]Department of Neurology, Albany Medical College, Albany, NY; [12]Dept. of Psychology, UC Berkeley, Berkeley, CA; [13]Helen Wills Neuroscience Institute, UC Berkeley, Berkeley, CA; [14]SUPMICROTECH, CNRS, Inst. FEMTO-ST, Univ. de Franche-Comté, Paris, France; [15]Institut de Psychiatrie et Neurosciences de Paris-IPNP, INSERM, Université de Paris, Paris, France; [16]Sleep Wake Epilepsy Center | NeuroTec, Department of Neurology, Inselspital University Hospital Bern, University Bern, Bern, Switzerland

Abstract: Interpersonal communication allows knowledge exchange and the creation of new concepts by forming predictions about the intentions of others. Studying the neural basis of human interpersonal interaction is challenging. Here, we introduce a novel experimental approach based on hyperscanning intracranial EEG (iEEG). We developed a novel task assessing

how categorical representations are refined through interaction. We hypothesized that cognitive alignment would result in interbrain correlations in a hierarchy of brain regions involved in decision-making and social interaction. We further hypothesized successful and unsuccessful communications would modulate interbrain synchrony in distinct brain networks.
We simultaneously recorded iEEG data from pairs of patients (PoP) who were undergoing presurgical evaluation for medication-refractory epilepsy at the same time in the same hospital. We created a communication task where both patients assumed the roles of sender/receiver in alternating order. The sender aims to communicate features about an item (animal or object) by adjusting two axes (e.g., size and texture). The receiver tries to guess which of two superimposed items (an animal and an object) the sender describes. Feedback is provided at the end of each trial. We recorded 569 electrodes across four PoP (from 47 to 141 per patient) for 317 trials across PoP. To study synchrony, we extracted local field potentials (LFP; 1-20Hz), evaluated responsive electrodes, and tested the correlation between electrode pairs across PoP. We compared correct versus incorrect trials at the onset of communication and at the feedback presentation.
Our results show that all PoP performed the task successfully (~81% of correct trials). We found that the number of electrodes, including electrodes in frontal and temporal lobes, with strong inter-patient synchrony decreased in all four PoP between the onset of the communication ($\bar{x}$ = .075) and its end ($\bar{x}$ = .055; $p < .05$). At trial onset, the pairs of electrodes neurally coupled across the patient pairs were different for trials that were later correct, or incorrect. These results show that during communication, 1) brain synchrony manifests across multiple brain regions, 2) decreases as communication ends, and 3) neural alignment differs between successful vs. unsuccessful communication.

Disclosures: S.L. Alnes: None. **J. Weyermann:** None. **O. Kim-McManus:** None. **A. Stolk:** None. **I. Saez:** None. **J.T. Willie:** None. **P. Brunner:** None. **R.T. Knight:** None. **A. Llorens:** None. **A. Tzovara:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.24/J13

Topic: F.01. Neuroethology

Support: R21DC019992

Title: Coordinating breathing, laryngeal and orofacial movements in a mouse model

Authors: *T. RIEDE;
Physiol., Midwestern Univ., Glendale, AZ

Abstract: We investigated the coordination of orofacial and laryngeal movements for vocal production in the California mouse (*Peromyscus californicus*). This mouse generates a

stereotypic vocal pattern by airflow-induced vocal fold vibrations. Pups and adults produce calls that consist of 1 to 6 syllables which are150 ms in duration and have an average fundamental frequency of 18 to 24 kHz. This vocal behavior is associated with prominent orofacial movements in neonates and adult animals. We investigated the coordination between laryngeal sound production and orofacial movements during the first 3 weeks of life. The aim was to to investigate whether mouth movements are coordinated with laryngeal sound production. Mouse pups begin to call immediately when separated from their home cage. We collected video and audio recordings at different ages. Then we performed frame-by-frame video analysis to measure the on- and offset of mandibular movement, and we performed an acoustic analysis to measure the on- and offset of vocal utterances. We found that laryngeal sound production and mandibular movement are highly correlated, i.e., the durations of both movements are strongly associated. Furthermore, mandible movement onset precedes laryngeal sound production onset, and mouth opening is maintained beyond the duration of each syllable. Interestingly, the alignment of syllable on-/offset and mouth movement on-/offset, respectively, improved with age. The findings suggest that the coordination of orofacial and laryngeal movements for vocal production is present in many mammalian lineages, including rodents.

Disclosures: T. Riede: None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.25/J14

Topic: F.01. Neuroethology

Support: Hertz Foundation

Title: A zebra finch brain-computer interface for studying motor learning

Authors: *J. SCHERRER[1], J. WHITE[1], M. S. FEE[2];
[1]MIT, Cambridge, MA; [2]Brain & Cog Sci. / McGovern Inst., Massachusetts Inst. Technol., Cambridge, MA

Abstract: The songbird Zebra Finch (Taeniopygia castanotis) learns a complex motor sequence through a trial-and-error process suggestive of reinforcement learning. This learning process requires a basal ganglia-thalamocortical loop called the anterior forebrain pathway (AFP). Existing evidence suggests that the AFP learns a time-dependent bias signal that steers the motor pathway to avoid vocal errors. This bias signal is known to be dependent on the cortical output of the AFP known as LMAN (lateral magnocellular nucleus of the anterior nidopallium). However, little is known about the neural code in LMAN that underlies this bias signal, or how this neural code is learned and generated. We seek to address these questions through a variant of the conditional auditory feedback (CAF) paradigm in which noise bursts are played to the bird during song to disrupt its performance evaluation conditional on parameters of the song. In our

experiments, these noise bursts are instead played contingent on the activity of individual neurons in LMAN recorded in real time using a lightweight chronic Neuropixels 2.0 implant (neuron-contingent CAF or nCAF). Using nCAF, we can drive up and down the activity of specific LMAN neurons at precise points in time in song with response widths and jitter on the order of 10 ms. We can also use nCAF to measure the eligibility trace associated with learning in the AFP and compare it to observed premotor latencies. By targeting individual neurons, we can measure the degree of independent versus collective dynamics within single motor channels. These observations allow us to test specific circuit models for how the AFP drives the learning process.

Disclosures: J. Scherrer: None. **J. White:** None. **M.S. Fee:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.26/J15

Topic: F.01. Neuroethology

Support: American Australian Association Graduate Education Fund
Quad Fellowship
Simons Collaboration on the Global Brain Research Award Project: Neural Circuit Dynamics Underlying Sequence and Variability

Title: Closed loop neurofeedback for studying motor memory consolidation in the songbird

Authors: *J. M. WHITE[1], J. R. SCHERRER[2], M. S. FEE[2,3];
[1]Hlth. Sci. and Technol., [2]Brain and Cognitive Sci., [3]McGovern Inst., MIT, Cambridge, MA

Abstract: Motor skill consolidation is the process by which new, fragile motor skills are transformed into stable motor memories. It is well documented but poorly understood. Avian song learning presents a promising model system for understanding motor skill consolidation. Juvenile male zebra finches (*Taeniopygia guttata*) learn to imitate a tutor bird's song through a trial-and-error process of reinforcement learning. Learned changes to song are initially mediated by a basal-ganglia-thalamocortical loop known as the anterior forebrain pathway (AFP); however, over time they are consolidated into the downstream songbird motor cortex analog, the robust nucleus of the arcopallium (RA), becoming independent of the AFP. To date, this consolidation process has been studied by measuring the regression of recent spectral changes to the song following chemical inactivation of the AFP. Although these experiments have provided strong evidence for the existence of this motor skill consolidation, they have presented conflicting accounts of the timescale over which it occurs and the role of sleep in the process. Moreover, these behavioral experiments are unable to provide insights into the neural mechanisms underlying motor skill consolidation in the songbird. To overcome these limitations, we introduce a closed loop neurofeedback paradigm we refer to as neuron-contingent auditory

feedback (nCAF). In this paradigm, we record from RA in freely moving songbirds with chronically implanted Neuropixels 2.0 probes. Using a custom library, this data is streamed into a Bonsai-RX workflow in real-time, where we perform online spike inference using a Bayes optimal template matching method. Then, at a precise point in time during each rendition of the bird's song, a white noise burst is played (or withheld) contingent on the spiking activity of a given neuron at that time. We show that using this technique we are able to cause the bird to learn to drastically alter the activity of individual neurons at precise timepoints in the song. Using female-directed song as a readout of consolidated song features, we then track the consolidation of these learned changes over time and compare the results to those from earlier, behavioral measures of consolidation. By doing so, we demonstrate nCAF as a powerful tool for studying song learning and further our understanding of motor learning and motor skill consolidation in the songbird.

Disclosures: J.M. White: None. **J.R. Scherrer:** None. **M.S. Fee:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.27/J16

Topic: F.01. Neuroethology

Support: APVV-20-0344
VEGA 2/0150/24

Title: Song variability is associated with neurogenesis in songbirds

Authors: *L. NIEDEROVA-KUBIKOVA, J. POLOMOVA, B. BILCIK, V. HODOVA; Ctr. of Biosci., Slovak Acad. of Sci., Bratislava, Slovakia

Abstract: Neurogenesis in the adult brain has been associated with enabling the perception of novel sensory signals and/or mediating behavioral variability. The new neurons in songbirds are born in the neurogenic ventricular zone (VZ), similarly as in mammals. Then they migrate to the whole telencephalon including the areas involved in control of the learned song, such as HVC (proper name) and Area X. A higher number of new neurons in HVC in the songbird Bengalese finch (*Lonchura striata var. domestica*) is associated with higher variability of song sequence. The aim of this work was to determine whether this relationship is valid also in general when comparing several songbird species. We used males from six species from the *Estrildidae* family, Bengalese finch, zebra finch (*Taeniopygia guttata*), Java sparrow (*Lonchura oryzivora*), strawberry finch (*Amandava amandava*), common waxbill (*Estrilda astrild*), and star finch (*Neochmia ruficauda*). We compared their song variability, the cell proliferation in the VZ (using a cell division marker 5-ethynyl-2'-deoxyuridine [EdU]), and incorporation into the song control nuclei HVC and Area X (using a cell division marker 5-bromo-2′-deoxyuridine [BrdU], neuronal marker NeuN, and marker of young neurons doublecortin [DCX]). We found that the

songs of Bengalese finches and Java sparrows were the most variable and they also showed the highest rates of cell proliferation. The variability in song syllable order correlated with cell proliferation in VZ within a species in zebra finches and Java sparrows and with the number of new neurons incorporated to HVC or Area X in Java sparrows and Bengalese finches. Moreover, the higher song sequence variability was associated with the number of newborn cells in the neurogenic VZ as well as the number of new neurons recruited to HVC and Area X also in the analyses encompassing all the species. These results suggest that the neurogenesis and the newly recruited neurons in the vocal brain nuclei may facilitate the vocal variability in songbirds.

Disclosures: L. Niederova-Kubikova: None. **J. Polomova:** None. **B. Bilcik:** None. **V. Hodova:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.28/J17

Topic: F.01. Neuroethology

Support: Farouk Jabre Foundation

Title: Neural network mechanisms underlying the combination sensitivity property in the HVC of songbirds

Authors: *R. DEBIAN[1], Z. MERAABE[2], A. DAOU[2];
[1]American Univ. of Beirut, Beirut, Lebanon; [2]Biomed. Engin. Program, American Univ. of Beirut, Beirut, Lebanon

Abstract: Temporal order of information processing in the brain is an important code in many acoustic signals including speech, music, and animal vocalizations. Despite its significance, surprisingly little is known about its underlying cellular mechanisms and network manifestations. In the songbird telencephalic nucleus HVC, a subset of neurons show temporal combination sensitivity (TCS). These neurons respond in a facilitatory or inhibitory manner to patterns of distinct spectral elements in a signal, when they occur in a specific temporal order. HVC neuron types include basal-ganglia-projecting HVC_X, forebrain-projecting HVC_{RA}, and interneurons (HVC_{INT}), each exhibiting distinct cellular, electrophysiological and functional properties. In this work, we develop conductance-based neural network models connecting the different classes of HVC neurons via different network architecture patterns with the aim of unveiling the intrinsic and synaptic mechanisms that orchestrate the combination sensitivity properties exhibited presumptively by HVC_X, as well as replicating *in vivo* firing patterns observed when TCS neurons are presented with various auditory stimuli. The model neurons in each class are designed to express pharmacologically identified ionic currents and the neurons are connected via pharmacologically identified synaptic currents, rendering our networks biologically plausible. We present for the first time several realistic scenarios in which the different types of

HVC neurons can interact to produce this behavior. The different networks highlight intrinsic and synaptic mechanisms that could help to explain combination sensitivity, including 1) interplay between inhibitory interneurons' activity and the postinhibitory firing of the HVC_X neurons enabled by T-type Ca^{2+} and H currents, 2) temporal summation of synaptic inputs at the TCS site of opposing signals that are time-and frequency- dependent, and 3) reciprocal inhibitory and excitatory loops as a potent mechanism to encode information over many milliseconds. The result is a plausible network model characterizing auditory processing in HVC. Our next step is to test the predictions of the model.

Disclosures: R. Debian: None. **Z. Meraabe:** None. **A. Daou:** None.

Poster

PSTR231: Vocal/Social Communication: Physiology and Behavior I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR231.30/J18

Topic: F.01. Neuroethology

Support: Farouk Jabre Foundation
University Research Board

Title: Biophysical network models underlying the generation of neural sequences in the HVC

Authors: *Z. BOU DIAB[1], A. DAOU[2];
[1]American Univ. of Beirut, Beirut, Lebanon; [2]Biomed. Engin., American Univ. of Beirut, Beirut, Lebanon

Abstract: The neural mechanisms of sequential behaviors are intensively studied, with songbirds a focus for learned vocal production. We are studying the premotor nucleus HVC at a nexus of multiple pathways contributing to song learning and production. The HVC consists of multiple classes of neuronal populations, each that has its own cellular, electrophysiological and functional properties. During singing, a large subset of motor cortex analog-projecting HVC_{RA} neurons emit a single 6-10 ms burst of spikes at the same time during each rendition of song, a large subset of basal ganglia-projecting HVC_X neurons fire 1 to 4 bursts that are similarly time locked to vocalizations, while HVC_{INT} neurons fire tonically at average high frequency throughout song with prominent modulations whose timing in relation to song remains unresolved. This opens the opportunity to define models relating explicit HVC circuitry to how these neurons work cooperatively to control learning and singing. We developed conductance-based Hodgkin Huxley models for the three classes of HVC neurons (based on the ion channels previously identified pharmacologically) and connected them in several physiologically realistic networks (based on pharmacologically identified glutaminergic and gabaergic connectivity) via different architecture patterning scenarios with the aim to replicate the *in vivo* firing patterning behaviors. We are able through these networks to reproduce the *in vivo* behavior of each class of HVC neurons as shown by the experimental recordings. The different networks unveiled key

intrinsic and synaptic processes that modulate the sequential propagation of neural activity (continuous or punctate) in the HVC by highlighting important roles for the T-type Ca^{2+} current, Ca^{2+}-dependent K^+ current, A-type K^+ current, hyperpolarization activated inward current, as well as $GABA_A$ and AMPA synaptic currents in governing important neural mechanisms for sequence propagation, like post-inhibitory rebound bursting in HVC_X, mono-synaptic HVC_X to HVC_{RA} excitatory connectivity, and different classes of interneurons, among others. The result is an improved characterization of the HVC network responsible for song production in the songbird.

Disclosures: Z. Bou Diab: None. **A. Daou:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.01/J19

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: A NEW OUTLOOK ON THE REASONS OF DYSLEXIA

Authors: *O. LEVASHOV;
Brain & Body Develop. Ctr., Ho Chi Minh City, Viet Nam

Abstract: It is believed that Dyslexia results from phonological reasons, in other words, it is a deficit of visually recognized grapheme transforming in phoneme. However, the first mention of reading disability ("word blindness") was made by W. Morgan, who had noted that the syndrome was purely optical and not associated with speech disorder (A Case of Congenital Word Blindness, W. Pringle Morgan, 1897, M.B., Seaford, Sussex). Further, Stein, J., & Walsh, V. (1997) and Vidyasagar, T. R., & Pammer, K. (2010) proposed that visual dorsal channel control visual scanning of letters in words in reading and Dyslexia can be explained by its deficit. In this report, we attempted to summarize a lot of data about visual system deficits from the level of the retina to the level of interhemispheric relation to explain any reading disorders, including Dyslexia. 1. Our optic system is not perfect. There can be disorders of refraction (spheric aberration and astigmatism) which result in letter blurring and reading disturbance. 2. Some signs of strabismus can destroy the binocular fusion which leads to diplopia, then reading can be destroyed. 3. A small working memory can also cause a reading deficit due to difficulty in combining several letters into a word to recognize it. 4. A deficit of the dorsal visual channel results in visual masking disorder and difficulties in erasing the retinal image after each saccade (J. Stein, 2010). It leads to overlapping the former and the latter text fragment images. 5. In addition, a deficit of the dorsal visual channel can result in a disturbance of the letter scanning from the left to the right. 6. Our visual cortex in the right hemisphere realizes a global vision and the visual cortex in the left hemisphere realizes picture fragment processing (E. Kok, 1967, O. Levashov, 1996, 2009). Both these processes are necessary in reading. At first global visual perception (the right hemisphere) finds a large piece of the text (the next paragraph, line, or

word) and then starts consecutive scanning from the left to the right along the line (the left hemisphere). However, the dominance of the right hemisphere cortex in the early sensitive period of childhood can slow down the development of the neuron structure in the inferior occipital/temporal area which is responsible for a visual reading (J. Stein, 2010). As a result reading person can use only global vision and can't use the consecutive scanning of the text elements (O. Levashov, 2009, 2018, 2021). An explicit dominance of the right hemisphere we can see in such great dyslexics as A. Einstein, W. Disney, S.Spilberg, T. Croze, S. Eisenstein, etc.

Disclosures: O. Levashov: None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.02/J21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Indiana University/Purdue University, Signature Center Initiative – Center for Spinal Cord and Brain Injury Research

Title: Dendritic and somal morphology of motoneurons innervating the external urethral sphincter in rats is sensitive to gonadal steroids

Authors: *E. HIBBARD, D. R. SENGELAUB;
Indiana Univ., Bloomington, IN

Abstract: A variety of sex differences are present in the anatomy and physiology of lower urinary tract, including differences in the bladder, urethra, and striated muscle of the external urethral sphincter (EUS) muscle. As in humans, the EUS muscle in rats is significantly larger in males than in females. The number of motoneurons that innervate the EUS muscle does not differ between male and female rats, but males have larger EUS motoneuron somata; furthermore, we have previously shown that EUS motoneurons in males also have longer dendrites. In this study, we examined whether the larger somata and longer dendrites of EUS motoneurons in male rats were a consequence of their potential sensitivity to gonadal steroids. Such sensitivity has been suggested previously, wherein we reported that treatment with gonadal steroids prevented spinal cord injury-induced regressions in EUS motoneuron dendrites and protects urinary function. Young adult male rats were either sham castrated or castrated and then implanted with Silastic capsules filled with testosterone (4-androsten-17β-ol-3-one) or left blank. These testosterone implants produce plasma titers of testosterone in the normal physiological range. After 4 weeks of treatment, a period sufficient for producing castration-induced changes in the morphology of steroid-sensitive motoneuron populations, the EUS muscle was exposed and bilaterally injected with cholera toxin-conjugated horseradish peroxidase (BHRP; 1 µL/side, 0.2%) to retrogradely label EUS motoneurons. Stereological assessments of EUS motoneuron

soma size (Nucleator method) were made with Stereo Investigator. Dendritic arbors of EUS motoneurons were reconstructed in three dimensions using Neurolucida. Somal areas of EUS motoneurons in castrated male rats were reduced by almost 20% compared to those of sham castrated males, and treatment with testosterone prevented this decline. In all groups, the dendritic arbors of EUS motoneurons displayed dense bundling dorsally along the lateral edge of the gray matter extending into lamina VI and VII as well as deep into the lateral funiculus; a second, medially-projecting array of dendrites coursed along the ventral margin of the gray matter and into lamina X. Although the distribution of dendrites was similar across groups, total dendritic lengths in castrated males were reduced by over 47% compared to those of sham castrated males, and treatment with testosterone prevented this decline. Thus, EUS motoneuron morphology appears to be sensitive to gonadal steroids. This sensitivity could explain the protection of urinary function and EUS morphology by steroid treatment we observed after spinal cord injury.

Disclosures: E. Hibbard: None. **D.R. Sengelaub:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.03/J22

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01 HD109248

Title: Evidence that steroid negative feedback during undernutrition inhibits ovine arcuate kisspeptin neurons differently in male and female sheep

Authors: S. SHUPING[1], J. PRADO BALCAZAR[1], C. ATWATER[1], J. SOMMER[1], R. MCCOSH[2], ***C. NESTOR**[2];
[1]Animal Sci., North Carolina State Univ., Raleigh, NC; [2]Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Proper energy intake is essential for reproduction. Recent work in gonadectomized sheep has demonstrated that arcuate kisspeptin neurons, which also contain neurokinin B (NKB), are part of the central mechanism whereby undernutrition impairs GnRH/LH secretion. However, the regulation of these key reproductive neurons in the presence of gonadal sex steroids during undernutrition in sheep remains to be fully elucidated. Given the presumptive GnRH pulse generator role of arcuate kisspeptin neurons, we hypothesized feed restriction would decrease arcuate kisspeptin and NKB protein expression in male and female sheep as well as reduce the total number of activated arcuate kisspeptin cells, as determined by cFos coexpression. We utilized sixteen wethers that were each given a 1 cm subcutaneous estradiol implant and sixteen gonadally-intact ewe lambs for this study. Animals in each sex were evenly divided into either a fed-to-maintain body weight (FM) group or a feed restricted to lose 20% of

pre-study body weight (FR) group. Weekly body weights were recorded and feed amounts were adjusted to achieve desired body weights. At week 13, ewe lambs were synchronized to the early follicular phase of the estrous cycle, all animals were euthanized following blood collection, and brain tissue containing the hypothalamus was collected. We selected tissue containing the arcuate nucleus from male and female sheep (n = 3/group/sex) and conducted triple-label immunofluorescence with primary antisera for kisspeptin, NKB, and cFos. In males, we observed fewer arcuate kisspeptin and NKB cells in FR animals (kisspeptin, 7.0 $\pm$ 2.7 cells/hemisection; NKB, 11.9 $\pm$ 4.3 cells/hemisection) compared to FM animals (kisspeptin, 29.0 $\pm$ 2.5 cells/hemisection; NKB, 31.3 $\pm$ 2.4 cells/hemisection). In addition, we observed fewer ARC kisspeptin cells expressing cFos in FR males (0.8 $\pm$ 0.7 cells/hemisection) compared to FM controls (11.4 $\pm$ 2.1 cells/hemisection). In females, we did not detect a difference in the number of arcuate kisspeptin or NKB cells between FR (kisspeptin, 63.4 $\pm$ 9.9 cells/hemisection; NKB, 63.1 $\pm$ 10.1 cells/hemisection) and FM animals (kisspeptin, 54.6 $\pm$ 8.2 cells/hemisection; NKB, 54.5 $\pm$ 8.1 cells/hemisection), but did observe fewer kisspeptin cells coexpressing cFos in FR females (3.4 $\pm$ 0.7) compared to FM females (15.0 $\pm$ 3.0 cells/hemisection). While future examination of ovariectomized females given subcutaneous estradiol is warranted, our data support the idea that in the presence of gonadal sex steroids arcuate kisspeptin and NKB are differentially regulated in male and female sheep during undernutrition.

Disclosures: S. Shuping: None. **J. Prado Balcazar:** None. **C. Atwater:** None. **J. Sommer:** None. **R. McCosh:** None. **C. Nestor:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.04/J23

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Measuring Estrogen Levels in POMC-Deficient Mice

Authors: *B. MCHOES, Z. THOMPSON;
Utah Valley Univ., Orem, UT

Abstract: The expression of the pro-opiomelanocortin gene (POMC) in the hypothalamus and pituitary leads to the production of several hormones, including α-melanocyte stimulating hormone (α-MSH) and β-endorphin (β-EP). α-MSH is a metabolic hormone that helps to regulate food intake in response to energy needs. Mutations in the POMC gene lead to excessive hunger and eventually obesity. The mice observed in this study have a specific recessive mutation in the POMC gene that results in little to no expression of the POMC gene in the hypothalamus. This leads to obesity and potentially infertility. Estrogen levels are a direct indicator of fertility in a mammal. The goal of this project is to measure and compare the levels of estrogen in female POMC-deficient mice with that of wildtype mice and mice heterozygous for the POMC mutation. Preliminary data show that POMC-deficient female mice have lower

uterus weights than wild-type mice; uterus weights can be used as a proxy for estrogen levels. Preliminary analyses also indicate that POMC-deficient female mice have abnormal estrus cycles. We plan to track the estrus cycles of the mice for several days before euthanasia and then collect a plasma sample for analysis via liquid chromatography/mass spectrometry to determine estrogen levels. This data will help us better understand how the POMC gene influences overall reproductive function.

Disclosures: B. McHoes: None. **Z. Thompson:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.05/J24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC CRSNG
FRQS

Title: The Effects of Ovarian Hormones on Membrane Progesterone Receptors in the Brain of Female Rats

Authors: *L. M. BUYNACK[1], T. ALLAW[1], L. ERNEDAL[1], S. PATEL[2], E. GOMEZ-PERALES[1], J. LACASSE[3], W. G. BRAKE[4];
[1]Concordia Univ., Montreal, QC, Canada; [2]Concordia Univ., Dollard-des-Ormeaux, QC, Canada; [3]CAMH, Brock Univ., Fonthill, ON, Canada; [4]Ctr. for Studies in Behav Neuro, Concordia Univ., Montreal, QC, Canada

Abstract: Ovarian hormones have been shown to influence a variety of memory systems. For example, place and response memory bias fluctuates across the rat estrous cycle. Female rats display a place memory bias when 17ß-estradiol (E2) is infused into the hippocampus. This corresponds to the link between the hippocampus and place memory bias. However, when E2 is infused into the dorsal striatum, the brain region correlated to response memory bias, no bias between strategies occurs. The medial prefrontal cortex (mPFC) is thought to play a role in the switching of the two strategies. Infusions of E2 into the mPFC result in female rats exhibiting a place memory bias. Progesterone, subcutaneously administered with E2 - one and four hours before the probe trial - leads female rats to predominantly use response memory. While much is known about the role and mechanisms of E2, less research has focused on progesterone. Fluorescence immunohistochemistry was used to observe the presence of mPRδ, the only membrane progesterone receptor exclusively found in the brain, in the mPFC and dorsal striatum. Brain tissue was collected from ovariectomized female Long Evans rats under three hormonal conditions: low E2 (5% E2 in cholesterol Silastic capsule), high E2 (10 µg/kg; 5% E2 capsule), and high E2 (10 µg/kg; 5% E2 capsule) plus progesterone (500 µg/kg). All subcutaneous injections occurred two hours before tissue collection. Preliminary results show

that mPRδ is present in the mPFC and dorsal striatum. The presence of mPRδ in these brain regions provides a putative non-genomic mechanism by which progesterone may exert its effects on place and response memory.

Disclosures: L.M. Buynack: None. **T. Allaw:** None. **L. Ernedal:** None. **S. Patel:** None. **E. Gomez-Perales:** None. **J. Lacasse:** None. **W.G. Brake:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.06/J25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant MH127850-01

Title: Modulating CRHR1 activity as a strategy to mitigate early-onset puberty and anxiety in rodent models of early life adversity

Authors: *A. BALAKRISHNAN, H. C. BRENHOUSE;
Psychology, Northeastern Univ., Boston, MA

Abstract: Modulating CRHR1 activity: a strategy to mitigate early-onset puberty and anxiety in rodent models of early life adversity*Angarika Balakrishnan [1], Heather C. Brenhouse [1]*

ABSTRACT:
Early life adversity (ELA) detrimentally affects human physiology and behavior, often mediated through dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis. The maternal separation (MS) paradigm serves as a validated method for inducing ELA in rodents, enabling the investigation of its impacts on neurobiological and behavioral changes into adolescence and adulthood. Previous research has demonstrated that MS from postnatal day 2 (P2) to postnatal day 20 (P20) in female rats prompts early onset of puberty and heightened acoustic startle response (ASR) later in life, which is consistent with observations in ELA-exposed humans. Pubertal timing is regulated by corticotropin-releasing hormone (CRH) signaling, with its receptor CRHR1 acting as a pubertal suppressor during healthy early development. Studies have also shown that chronic overstimulation of the CRHR1 receptor leads to its downregulation. We therefore postulate that premature puberty after MS is attributable to sustained activation of CRHR1 due to heightened CRH activity. Since MS has been shown to induce a CRH surge as early as P9, we hypothesize that chronic overactivation by CRH leads to CRHR1 downregulation. This downregulation results in early pubertal onset with increased anxiety-induced startle responses in adulthood. To investigate this hypothesis, we administered antalarmin (15 mg/kg), a CRHR1 antagonist, intraperitoneally from P8 to P19 during MS. We evaluated whether antalarmin administration delayed the age of pubertal onset, as well as adolescent anxiety-like behavior via the open field test at P38, and ASR at P40. Our findings

indicate that early targeting of the CRHR1 activity can prevent premature puberty onset in MS animals, accompanied by altered ASR compared to control reared animals. Additionally, immunohistochemical analysis of c-Fos expression indicates a possible connection between pubertal timing and the development of anxiety-related circuitry in the bed nucleus of the stria terminalis (BNST) and basolateral amygdala (BLA). These results offer insights into potential intervention targets for individuals exposed to ELA.
Keywords: Early life adversity, HPA axis, CRHR1 receptor, acoustic startle response, BNST

Disclosures: A. Balakrishnan: None. **H.C. Brenhouse:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.07/J26

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant 2239635

Title: Single-nuclei RNA sequencing reveals housing and photoperiod-dependent changes in hippocampal gene expression in seasonally social voles

Authors: *K. POWER, M. DAVIS, A. BEERY;
UC Berkeley, Berkeley, CA

Abstract: Meadow voles (Microtus pennsylvanicus) provide a unique opportunity to study neural mechanisms underlying social behavior, as they transition between social and non-social phenotypes depending on the season or photoperiod. Meadow voles housed in winter-like short days (SD), prefer social odors, huddle more, and spend more time in larger groups than voles housed in summer-like long day lengths (LD). These distinct behavioral phenotypes are accompanied by changes in hormone regulation, neurogenesis, and receptor densities of multiple neuropeptides linked to social behavior, especially in the hippocampus. To identify novel targets that may be driving social behavior, we used single nuclei RNA-sequencing (snRNA-Seq) in the hippocampus of female meadow voles. Meadow voles were housed in either LD (non-social phenotype) or SD (social phenotype), and either alone or with a same-sex, age-matched conspecific. This 2x2 design allowed us to dissociate effects on transcription due to photoperiod, housing, and their interaction. Photoperiod had the biggest effect on genes related to myelination in oligodendrocytes, consistent with seasonal changes in neuronal development. We previously found that social life history interacts with photoperiod to shape social behavior in meadow voles; in the present study, housing and the interaction of housing with day length had the most prominent impacts on gene expression. This study provides the first clustering of cell types in the hippocampus of a vole species, identifies pathways involved in myelination, inflammation, and plasticity as particularly susceptible to the environmental manipulations employed, and highlights the importance of social life history on gene expression in the hippocampus.

Disclosures: K. Power: None. **M. Davis:** None. **A. Beery:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.08/J27

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01 HD104418

Title: Insulin-like growth factor (IGF)-1 acts through Kiss1 neurons to influence metabolism and reproduction

Authors: M. WANG[1,2], ***M. NAVEED**[2,3], Y. XU[1], J. W. HILL[3,2];
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Abstract: Objective: Kisspeptin, encoded by the *Kiss1* gene, ties puberty and fertility to energy status; however, the metabolic factors that control *Kiss1* neurons need to be clarified. **Methods:** To evaluate the impact of insulin-like growth factor (IGF)-1 on the metabolic and reproductive functions of kisspeptin circuits, we created mice lacking IGF-1 receptors in *Kiss1* neurons ($IGF1R^{Kiss1}$ mice). Previous studies have shown that IGF-1 and insulin can bind to each other's receptors, permitting IGF-1 signaling in the absence of IGF-1R. Therefore, we also generated mice with simultaneous deletion of the IGF1R and insulin receptor (IR) in *Kiss1* neurons ($IGF1R/IR^{Kiss1}$ mice). **Results:** Female $IGF1R^{Kiss1}$ mice had a "metabolically healthy" profile with lower body weight and food intake, plus higher energy expenditure and physical activity. This phenotype was linked to higher pro-opiomelanocortin (POMC) expression and heightened brown adipose tissue (BAT) thermogenesis. Notably, $IGF1R^{Kiss1}$ mice also experienced delayed puberty and adult reproductive deficits. Male $IGF1R^{Kiss1}$ mice had lower gonadotropin and testosterone levels and impaired spermatogenesis. Thus, IGF-1 signaling in *Kiss1* neurons impacts metabolism and reproduction in a sex-specific manner. $IGF1R/IR^{Kiss1}$ mice had higher fat mass and glucose intolerance, suggesting IGF1R and IR in *Kiss1* neurons together regulate body composition and glucose homeostasis. **Conclusions:** Overall, our study shows that *Kiss1* IGF1R and IR have cooperative roles in body weight, energy balance, glucose homeostasis, puberty, and fertility.

Disclosures: M. Wang: None. **M. Naveed:** None. **Y. Xu:** None. **J.W. Hill:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.09/J28

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Ministerio de Ciencia e Innovación PID2022-142544OB-I00
Ministerio de Ciencia e Innovación RYC2019-028501-I
AGAUR Investigo 200076ID9

Title: Kiss1 neurons in the preoptic hypothalamus regulate testosterone synthesis in adult male mice

Authors: *C. CANAL CAPDEVILA[1,2], M. GIRONA DEL POZO[1,2], L. BENITO SANDOVAL[2], R. D. PALMITER[3], G. S. MCKNIGHT[4], A. QUINTANA[1,2], E. SANZ[1,2]; [1]Departament de Biologia Cel·lular, Fisiologia i Immunologia, Univ. Autònoma de Barcelona, [2]Inst. de Neurociències, Univ. Autònoma de Barcelona, Bellaterra (Barcelona), Spain; [3]Dept. of Biochemistry, Howard Hughes Med. Institute, Univ. of Washington, Seattle, WA; [4]Dept. of Pharmacology, Univ. of Washington, Seattle, WA

Abstract: Kisspeptin (*Kiss1*)-expressing neurons in the rostral periventricular region of the third ventricle (RP3V) are known to be crucial for female reproduction, orchestrating the preovulatory surge of gonadotropin hormone-releasing hormone (GnRH) and luteinizing hormone (LH) in response to estrogen and triggering ovulation and steroid synthesis. While these neurons have been extensively studied in females, adult male mice were reported to possess significantly fewer Kisspeptin-expressing neurons in the RP3V. Moreover, adult male mice do not respond either to estrogen or testosterone with an LH surge, which has been attributed to the loss of Kiss1 neurons. These observations led to the hypothesis that $Kiss1^{RP3V}$ neurons are responsible for the female-specific LH surge. In our study, we have used mouse genetics and cell-type specific tracing of neuronal projections to demonstrate that $Kiss1^{RP3V}$ neurons are an abundant neuronal population in male mice, presenting similar projection sites in both sexes. Moreover, cell-type specific gene expression analysis showed a highly similar expression profile of neuropeptides, receptors, and transcription factors. Additionally, specific activation of male $Kiss1^{RP3V}$ neurons expressing viral-encoded hM3Dq induced a surge in circulating testosterone levels. These findings demonstrate that $Kiss1^{RP3V}$ neurons are present and remain fully functional in adult male mice, capable of regulating the hypothalamic/pituitary/gonadal axis (HPG). We propose that these neurons may also play a significant role in reproductive behavior in adult male mice, potentially being critical for fertility and driving testosterone-mediated behaviors. Hence, understanding the physiology of this neuronal population will provide relevant information to tackle male reproductive diseases.

Disclosures: C. Canal Capdevila: None. **M. Girona del Pozo:** None. **L. Benito Sandoval:** None. **R.D. Palmiter:** None. **G.S. McKnight:** None. **A. Quintana:** None. **E. Sanz:** None.

Poster

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Program #/Poster #: PSTR232.10/J29

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R15 HD110963-01

Title: Expression of Avpr1a mRNA in neurons and glia in the developing mouse brain

Authors: *K. REESE[1], H. K. CALDWELL[2];
[1]Biol. Sci., Kent State Univ., Kent, OH; [2]Dept. of Biol. Sci., Kent State Univ. - Biol. Sci., Kent, OH

Abstract: Vasopressin (Avp) has been shown to play an important role in the sex specific neural regulation of social behavior. Additionally, there is growing evidence that Avp modulates aspects of neural development and that alterations in signaling through its Avp 1a receptor (Avpr1a) in a developing embryo can impact social behaviors in adulthood. Previous work from our lab found that transient disruption of Avpr1a signaling on embryonic day (E) 16.5 results in sex-specific behavioral effects in adult mice; though, the mechanism is still poorly understood. While data detailing Avpr1a binding in the developing mouse brain is scant, our lab has demonstrated that the Avpr1a mRNA is detected as early as E12.5 with no expression differences between males and females and that Avpr1a binding is observed in both males and females starting at E16.5, with changes in expression patterns over the course of development. What remains unknown is whether or not the expression of the Avpr1a is restricted to neurons, glia, or can be found in both. Though, we do have preliminary evidence to suggest that there are sex-differences in its cell-type expression. To address this knowledge gap, we will use Hairpin Chain Reaction (HCR) combined with immunohistochemistry (ICC) to examine the colocalization of Avpr1a mRNA and either NeuN (a marker for neurons) or S100B (a marker for glia). This approach is being taken due to the lack of antibodies against the Avpr1a. This work is important as understanding the cell types the Avpr1a is expressed in is critical to our understanding of how Avpr1a signaling during these developmental timepoints may be impacting neural development and behavior.

Disclosures: K. Reese: None. **H.K. Caldwell:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

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Program #/Poster #: PSTR232.11/J30

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: ANII FCE-1-2014- 1-103797
National Research Foundation Grant 87769

Title: Description and comparison of brain distribution of vasopressin receptor V1A in Rhabdomys pumillio and Rhabdomys dilectus

Authors: *D. E. OLAZABAL[1], N. PILLAY[2], A. P. FRONTÁN[3];
[1]Facultad De Medicina, Udelar, Montevideo, Uruguay; [2]Wits Univ., Johannesburg, South Africa; [3]Facultad De Medicina, Univ. De La República, Montevideo, Uruguay

Abstract: Vasopressin receptor 1A (V1AR) distribution in the brain has been studied extensively because of vasopressin (AVP) role in the regulation of aggressiveness, stress responses, reproductive and social behavior, among other functions. *Rhabdomys pumilio* (*R. pumilio*) and *Rhabdomys dilectus* (*R. dilectus*) are two sister rodent species that differ in their aggressiveness, anxiety, stress, and size of their social groups. In this study, we describe and compare the distribution of V1AR in these two species. V1AR binding in the brain of *R. pumilio* (8 females and 5 males) and *R. dilectus* (8 females and 5 males) adults was determined using autoradiography. Our results revealed significant species differences in the caudate putamen, nucleus accumbens, medial septum, diagonal band and ventral pallidum (higher in *R. dilectus*), and the dorsal subiculum (higher in *R. pumilio*). V1AR density in other brain regions, such as cortical areas, lateral septum, medial preoptic area, amygdala, and other regions of hippocampus, did not differ between the two species. Sex differences were found in *R. pumilio* in the anterior cingulate cortex, infralimbic cortex, diagonal band, ventral pallidum, posterior hypothalamus, and dental gyrus (V1AR density higher in males), and the dorsal subiculum (V1AR density higher in females). In contrast, there was only one sex difference in *R. dilectus*, higher V1AR density in the anterior BNST in females. The almost absence of sex differences in *R. dilectus* resemble other species, such as *S. teguina*, and suggest the existence of species-specific hormonal regulation of V1AR expression. Our study shows species-specific brain distribution of V1AR in *R. pumilio* and *R. dilectus* that are unique, but with some similarities with other species. However, no predictive pattern of V1AR distribution and density on social behavior was found. However, several areas related to anxiety, stress and aggressiveness showed higher density of V1AR in *R. dilectus*.

Disclosures: D.E. Olazabal: None. **N. Pillay:** None. **A.P. Frontán:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.12/J31

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: European Research Council Advanced Grant (TOGETHER)
Swedish Research Council Distinguished Professor Program (2021-00671)
Internal funds from Stockholm University

Title: An organotypic culture model of the mouse tuberoinfundibular dopamine (TIDA) network

Authors: *L. MORAN, P. WILLIAMS, C. C. BROBERGER;
Biochem. and Biophysics, Stockholm Univ., Stockholm, Sweden

Abstract: Tuberoinfundibular dopamine (TIDA) neurons in the hypothalamic arcuate nucleus play a key role in reproduction by acting as a brake on the release of the pituitary hormone, prolactin. Electrophysiological studies using acute brain slices preparations have revealed a number of cellular and network properties of TIDA neurons, including a rhythmic membrane potential oscillation, how hormones and neuromodulators can shift TIDA activity configurations, homeostatic feedback regulation, and stimulus-release DA relationship. However, acute *ex vivo* models are limited by tissue lifespan. An experimental platform that preserves gross tissue structure integrity and connectivity while enabling long-term manipulations is the organotypic slice culture (OTC). Here, we have developed and validated a mouse TIDA system organotypic model from transgenic DAT-Cre mice, comparing electrophysiology and morphology with the well-established acute slice preparation. The OTC's were prepared from 8-10-day old pups of both sexes and used 13-20 days later.
The electrophysiological properties were, with few exceptions, similar between TIDA neurons in OTC's and acute slices. Action potential parameters were consistent across preparations and firing types. The characteristic TIDA oscillation could be observed in a large proportion of neurons. However, differences were observed in the distribution of discharge patterns, with the OTC's showing a larger proportion of silent cells, a behaviour that is rare in acute preparations. Modest differences were seen in synaptic activity, in the form of higher excitatory frequency and higher inhibitory amplitude. - Structurally, the OTC, after a period of incubation, showed a similar anatomical organization to the TIDA system *in situ.* Cells were located in the dorsomedial aspect of the arcuate nucleus, and projections to the median eminence were visible albeit less distinct. *Post-hoc* reconstructed recorded TIDA neurons in the OTC exhibited greater neuronal process length but features like soma size, projection volume and branching remained similar. Finally, successful viral transduction of optogenetic constructs underscores the model's potential for genetic manipulation and further exploration of TIDA neuron structure and function.
In summary, the OTC offers an experimentally tractable system to study the TIDA neuron network, which recapitulates core features observed in the *ex vivo* slice. This new model of a key neuroendocrine population could shed light on e.g. the role of circulating factors and neuromodulators in the formation and plastic remodelling of the TIDA system.

Disclosures: L. Moran: None. **P. Williams:** None. **C.C. Broberger:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

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Program #/Poster #: PSTR232.13/J32

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01MH123523

Title: Single-cell multiomic characterization of the impact of sex and estrous cycle on cellular phenotype in the ventral hippocampus

Authors: M. C. TICKERHOOF[1], K. NDUKWE[1], A. SISTA KAMESHWAR[2], M. SUZUKI[2], ***M. KUNDAKOVIC**[1];
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Abstract: The ovarian hormone shifts are an important contributing factor to the increased prevalence of depression and anxiety disorders in women compared to men, as evidenced by the risk of such disorders peaking at menarche, during menopausal transition, and postpartum. In rodent females, anxiety- and depression-related behaviors also vary across the estrous cycle. Our lab previously linked these estrous cycle-dependent behavioral changes to changes in gene expression and chromatin organization in neurons of the ventral hippocampus (vHIP), a region critical for emotion regulation. Building on these earlier findings, in this study we aimed to characterize gene expression and chromatin accessibility within the mouse vHIP at single-nucleus resolution, within both neuronal and glial cell populations, across the estrous cycle and sex. Within the estrous cycle, we focused on diestrus (low estradiol, high progesterone) and proestrus (high estradiol, low progesterone). After the estrous cycle tracking for 3 consecutive cycles, nuclei from 11-week old diestrus (N=6) and proestrus (N=6) females were isolated and processed, together with those from aged-matched male C57BL/6J mice (N=6), using the 10X Chromium Single Cell Multiome (ATAC + Gene Expression) kit. We targeted 10,000 nuclei per replicate (3 biological replicates/group) and, following quality control, included over 55,000 nuclei across the three conditions. Using Seurat, we first characterized close to 30 different cellular clusters in the vHIP, including subclusters of excitatory and inhibitory neurons, astrocytes, microglia, oligodendrocytes, pericytes, ependymal, and endothelial cells. Then we analyzed differential gene expression and chromatin accessibility across broad cell types (e.g. excitatory neurons), sub-types (e.g. subclusters of astrocytes), and cells expressing genes of relevance to estrous cycle responsivity (e.g. *Esr1*). Subcluster and gene-of-interest analysis revealed differential gene expression and chromatin accessibility patterns between the sexes and across estrous cycle stages within specific cell types of genes relevant for neuronal function, behavioral stress response, and emotion regulation. These results demonstrate heterogeneity within broad cell types, including sex- and estrous cycle-dependent, cell subtype-specific differences in chromatin accessibility and expression of genes implicated in anxiety- and depression-related behaviors. This work will provide essential knowledge into the neurobiological mechanisms underlying sex bias in depression and anxiety disorders, paving the way for treatments that take hormonal state into account.

Disclosures: M.C. Tickerhoof: None. **K. Ndukwe:** None. **A. Sista Kameshwar:** None. **M. Suzuki:** None. **M. Kundakovic:** None.

Poster

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Program #/Poster #: PSTR232.14/J33

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant MH096050
NIH Grant DK095908

Title: Developmental thyrotoxicosis influences reward-motivated behavior and the ghrelin system

Authors: *L. T. DOUGLAS[1], A. HERNANDEZ[2];
[1]Hernandez Lab., Maine Hlth. Inst. for Research/University of Maine Grad. Sch. for Biomed. Sci. and E, Biddeford, ME; [2]Mol. Med., Maine Med. Ctr. Res. Inst., Scarborough, ME

Abstract: Thyroid disorders are highly prevalent in women and during pregnancy they may impact the development of the fetal brain, with neurological consequences later in life. We investigated the long-term effects of fetal thyroid excess on reward-motivated behaviors and the ghrelin system. We used a mouse model (*Dio3* KO mouse) with a non-functioning type 3 deiodinase (DIO3), the enzyme that breaks down thyroid hormones (TH). Loss of DIO3 activity results in developmental thyrotoxicosis, as the *Dio3* KO fetus is unable to clear maternal TH, with potential consequences for brain development. Previous observations have revealed a perinatal window of transient, high *Dio3* expression in reward-related brain areas where GHSR, the ghrelin receptor, is expressed, including the bed nucleus of stria terminalis, the nucleus accumbens and the amygdala. We hypothesized that perinatal TH excess disrupts the normal development of these brain regions and the ghrelin system, causing aberrant reward-motivated behaviors in adult life. We studied male and female wild-type (WT) and *Dio3* KO mouse littermates generated by reciprocal crosses of C57BL/6J and 129/SVJ mice heterozygous for the *Dio3* mutation. We analyzed reward-motivated behavior using a palatable food reward-conditioned self-administration runway (SAR) test and a conditioned place preference (CPP) test. Results indicated parent-of-origin specific increased motivation for palatable food reward, with *Dio3* KO mice showing resistance to extinction in the SAR assay, and *Dio3* KO mice with a C57BL/6J paternal background demonstrating increased CPP for palatable food reward compared to both WT controls and *Dio3* KO mice with a 129/SVJ paternal background. Serum acyl/total ghrelin ratio was significantly lower in *Dio3* KO mice compared to WT controls, and this observation was dependent on paternal genetic background, but not sex. Data from *Dio3* KO mice showed lower hypothalamic and higher striatal expression of the *Ghsr* compared to WT controls, as well as a blunted hypothalamic *Ghsr* response to fasting. These experiments suggest that abnormal TH levels during development play a role in the programming of the reward system, potentially including the ghrelin system. As abnormal acyl/total ghrelin ratios have been associated with alcoholism, obesity, and eating disorders, our findings raise the possibility that altered TH states during development influence the susceptibility to obesity and addiction via aberrant programming of brain circuitries regulating reward-motivated behaviors.

Disclosures: L.T. Douglas: None. **A. Hernandez:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

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Program #/Poster #: PSTR232.15/J34

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01DK126715
NIH Grant HD105386

Title: Role of estrogen-receptor alpha in the organization of melanocortin 3 receptor expression

Authors: *D. T. PORTER, R. CONE;
Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: The melanocortin 3 receptor (MC3R) has been shown to modulate critical physiological and behavioral processes. Recent work has shown that there are strong sexual dimorphisms in MC3R expression, with MC3R labeling patterns overlapping with the distribution of androgen and estrogen receptors in multiple regions of the hypothalamus. Thus, MC3R signaling likely impacts physiological responses to melanocortins differently in males and females. To test this, we have crossed a ESR1 floxed mouse to an MC3RCre to eliminate expression of Esr1 is in these cells, which are rendered unaffected by the differentiating effects of neonatal sex steroid exposure. We have found that male mice lacking ESR1 in MC3R neurons (MC3RESR1$^{-/-}$) have decreased body weight (BW) and lean mass (LM) with no difference in fat mass (FM) compared to wildtype (WT) littermates. Females showed no differences in BW, LM, or FM between WT and (MC3RESR1$^{-/-}$). Interestingly, male MC3RESR1$^{-/-}$ did not show any difference in glucose tolerance, food intake or preference to high fat diet. Further work will need to be done to elucidate the mechanism(s) through which estrogen signaling is having an effect on male but not female body composition.

Disclosures: D.T. Porter: None. **R. Cone:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.16/J35

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Molecular mechanisms underlying the regulation of metabolic function by ovarian hormones

Authors: *L. OULDIBBAT[1], D. ROCKS[1], M. C. TICKERHOOF[1], M. SUZUKI[2], M. KUNDAKOVIC[1];
[1]Fordham Univ., Bronx, NY; [2]Dept. of Nutr., Texas A&M Univ., College Station, TX

Abstract: Women face heightened risks of metabolic dysfunction, particularly during menopause. Via its effects in the hypothalamus, estradiol regulates weight, feeding, activity, and body composition, however underlying gene regulatory mechanisms are understudied. To address this question, we first examined metabolic phenotypes of ovariectomized (OVX) and ovary-intact (cycling) C57BL/6 female mice across the estrous cycle from 9-21 weeks of age. We focused on proestrus (high estrogen, low progesterone) and diestrus (low estrogen, high progesterone) estrous cycle phases to assess hormone fluctuation effects. Weight, feeding, activity and body composition were analyzed. At 21 weeks, OVX mice exhibited a 40% weight increase due to increased feeding and reduced activity, while cycling animals showed decreased food intake during proestrus, consistent with estrogen's anorexigenic effects. Body composition analysis revealed significant increases in fat mass in OVX animals. To attempt to "rescue" these phenotypes, we administered estradiol benzoate (EB) in OVX mice. Weight, activity, and body composition were analyzed in OVX animals receiving vehicle, 0.2 µg EB, and 1 µg EB every 4 days for 6 weeks beginning at 9 weeks, alongside cycling females. The 1 µg EB dose was sufficient to alter activity and reverse excess weight gain and fat mass in OVX females. Molecular mechanisms were preliminary explored through analysis of metabolically relevant genes, estrogen receptor 1 (*Esr1*), agouti-related peptide (*AgRP*) and pro-opiomelanocortin receptor (*Pomc*) in the hypothalamus. Differences in gene expression were found across the cycle and between OVX and cycling groups. Additionally, gene expression differences between OVX and cycling groups for *Esr1*, leptin receptor (*Lepr*), and ghrelin receptor (*Ghsr*) were observed in white adipose tissue. Due to the heterogeneity of hypothalamic nuclei, we are currently performing single-nuclei RNA-seq analysis of the hypothalamus across the estrous cycle and in OVX animals to identify cellular populations that are sensitive to ovarian hormone levels. Preliminary pilot experiments utilizing proestrus animals at 12 weeks revealed 28 distinct cell clusters and significant expression of metabolic genes in key estrogen-sensitive sub-regions utilizing cell-type specific marker genes. Our studies will be critical in linking metabolic phenotypes with estrogen-mediated molecular mechanisms in the female hypothalamus.

Disclosures: L. Ouldibbat: None. **D. Rocks:** None. **M.C. Tickerhoof:** None. **M. Suzuki:** None. **M. Kundakovic:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant HD-050470

Title: Temporal coordination of female reproductive axis activity by vasoactive intestinal polypeptide (VIP) expressing cells in the suprachiasmatic nucleus in mice

Authors: *C. PHUMSATITPONG, S. BEVER, L. J. KRIEGSFELD;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Circadian rhythms orchestrated by a master clock in the suprachiasmatic nucleus (SCN) are essential for ovulation in spontaneously-ovulating rodents. SCN signaling integrates with estradiol positive feedback to generate the preovulatory luteinizing hormone (LH) surge that initiates ovulation. We have previously shown that the SCN communicates directly to kisspeptin (Kp) and RFamide-related peptide-3 (RFRP-3) neurons to temporally balance key stimulatory and inhibitory input to gonadotropin-releasing hormone (GnRH) neurons required for LH surge generation. Because VIP-expressing neurons in the SCN (SCN^{VIP}) have been implicated in the LH surge, we employed a cell-specific chemogenetic approach to determine the functional role of this cell phenotype in female reproductive axis regulation. Specifically, female VIP-Cre mice were stereotaxically injected with either AAV-hM3Dq or hM4Di to express stimulatory (3Dq) or inhibitory (4Di) DREADDs in SCN^{VIP} cells. Following recovery, mice were injected with clozapine n-oxide (CNO) either in the morning, prior to the LH surge (3Dq experiment), or the afternoon, around the time of the LH surge (4Di experiment). Brains were collected to examine neuronal activation in Kp, GnRH, and RFRP-3 neurons using FOS as a marker. Morning stimulation of SCN^{VIP} neurons decreased RFRP-3 neuronal activation, whereas Kp and GnRH neurons were unaffected, suggesting a temporal gating mechanism at GnRH and/or Kp cells. In contrast, afternoon inhibition of SCN^{VIP} neurons led to an increase in neuronal activation in RFRP-3 neurons concomitant with a decrease in GnRH and Kp neurons, suggesting that VIP stimulation removes RFRP-3 inhibition on the GnRH and Kp systems. We confirmed this possibility by localizing afternoon injections of CNO to RFRP-3 cells, which led to comparable decreases in GnRH and Kp cellular activity. These data indicate the importance of SCN^{VIP} neurons in the temporally coordinated disinhibition of RFRP-3 neuronal activity required for stimulation of GnRH and Kp neurons around the time of LH surge.

Disclosures: C. Phumsatitpong: None. **S. Bever:** None. **L.J. Kriegsfeld:** None.

Poster

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Topic: F.02. Neuroendrocrine Processes and Behavior

Support: MH134119
NIH T32GM099608
NIH T32GM144303

Title: Dissecting the GRK-pß$_2$AR Axis in Learning and Memory

Authors: *B. SELZ, Y. XIANG;
Pharmacol., Univ. of California, Davis, Davis, CA

Abstract: Alzheimer's Disease (AD) is the 6th leading cause of death and is hallmarked by loss of the locus coeruleus diminishing adrenergic signaling, which is vital for memory formation and learning within the brain. Specifically, β-adrenergic signaling has been shown to facilitate memory retrieval and modulate spatial memory. In AD models, antagonism of β-adrenergic receptor (βAR) is proinflammatory with agonism of βAR ameliorating memory loss. Stimulation of β2AR drives both PKA and GRK phosphorylation of the receptor, which produce two distinct subpopulations in a single cell. While PKA phosphorylated β2AR remains at the surface, the GRK phosphorylated β2AR undergo endocytosis. We have previously established the vital role of the GRK2-Arr3-PDE4D5 in β2AR-induced nuclear cAMP in learning and memory. Here, we further dissect the contribution of these two subpopulations in transducing nuclear cAMP. To test this, FRET-based subcellular cAMP biosensors were transfected into Hek293 QBI cells and subsequently probed with various pharmacological inhibitors and subcellular targeted molecular tools. Our data reveal the distinct roles of subpopulations in transducing β2AR-induced nuclear cAMP. Future experiments will validate this mechanism in neurons, providing potential therapeutic targets for AD.

Disclosures: B. Selz: None. **Y. Xiang:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

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Program #/Poster #: PSTR232.19/K1

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CONAHCYT SASM 1004334
CONAHCYT FC 322333

Title: Gper-mediated actions influence pelvic floor muscle activation at micturition in female rabbits

Authors: *S. SANCHEZ MENESES[1], O. SANCHEZ GARCIA[3], D. L. CORONA QUINTANILLA[2], L. N. TOLEDO[4], M. MARTINEZ-GOMEZ[5];
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Abstract: Micturition requires the integral participation of the structural and functional components of the pelvic floor. Pubococcygeus and bulbospongiosus muscles contribute to the storage and expulsion of urine respectively. Morphophysiological alterations in these muscles are related to pathologies such as urinary incontinence, which is highly prevalent among menopausal or elderly women, likely in association with serum estradiol levels. Indeed, at least three different types of estrogen receptors may be involved. Our present study aimed to analyze the

effect of (GPER) blocking selectively the G-Protein Coupled Estrogen Receptor on the reflex activaton of bulbospongiosus (Bsm) and pubococcygeus muscles (Pcm) during micturition. Chihchilla nulliparous female rabbits were used to dissect both pelvic floor muscles and administering sequentially the G15, a selective antagonist of GPER; EMG and CMG were further simultaneously recorded. Data indicated that reflex activation of the muscles is disorganized in the storage and voiding phase of micturition. Cytometrography revealed alterations in the threshold, voided, and residual volumes at the bladder level; threshold and maximum pressures also changed following G15 administration. Our results indicate rapid actions mediated by estrogen influence pelvic floor muscle activity during micturition.

Disclosures: S. Sanchez Meneses: None. **O. Sanchez Garcia:** None. **D.L. Corona Quintanilla:** None. **L.N. Toledo:** None. **M. Martinez-Gomez:** None.

Poster

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Location: MCP Hall A

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Program #/Poster #: PSTR232.20/K2

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Oxytocin receptor co-expression with VGLUT1 and GAT1 in the mouse medial amygdala.

Authors: *C. SAPP, H. K. CALDWELL;
Kent State Univ., Kent, OH

Abstract: The medial amygdala (MeA) is an essential region of the Social Behavior Neural Network (SBNN), acting as an integral bridge and filter between incoming socially relevant olfactory input and the rest of the network. The MeA is also a relatively heterogenous region, consisting of many different cell types across three major subregions. However, every region in the SBNN expresses oxytocin receptors, and oxytocin, acting through its receptor, is essential to the modulation of social behavior through actions in this network. While oxytocin receptor signaling is an integral component of MeA functioning, the specific cell types the oxytocin receptor is expressed on in this region remain unknown and because there are no reliable antibodies for the oxytocin receptor, evaluating the cell types on which it is expressed continues to be a challenge. As a work around, we used HCR-FISH, a proprietary in situ technique, to identify the co-localization of oxytocin receptor RNA with VGLUT1 RNA (a marker of glutamatergic neurons) or GAT1 RNA (a marker of GABAergic neurons). Ultimately, this approach will allow for robust analysis of oxytocin receptor co-expression with excitatory (glutamatergic) or inhibitory (GABAergic) neurons, which is important for understanding how oxytocin receptor signaling contributes to the function of the MeA.

Disclosures: C. Sapp: None. **H.K. Caldwell:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.21/K3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01HD109337

Title: Noradrenaline has direct and indirect effects on the activity of female preoptic kisspeptin neurons

Authors: *S. PINTI[1], R. PIET[2];
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Abstract: In the female rodent, hypothalamic kisspeptin neurons in the rostral periventricular area of the third ventricle ($RP3V^{Kiss}$) are responsible for driving a surge in the secretion of gonadotropin-releasing hormone (GnRH) and, subsequently, of luteinizing hormone (LH), triggering ovulation a few hours later. Noradrenaline (NA) release increases in the preoptic area of the hypothalamus, which includes the RP3V, prior to the LH surge in rodents. Previous studies have shown that in vivo administration of exogenous NA stimulates LH secretion, and that of α1 adrenergic receptor antagonist, prazosin, blunts or prevents the surge entirely. While NAergic signaling appears to stimulate the GnRH neuronal network for the LH surge, the mechanisms of this modulation remain unclear. It is known that NA suppresses GnRH neuronal activity, suggesting its involvement in the LH surge occurs at some upstream driver of the GnRH neuron. We hypothesize that NA influences the surge by increasing $RP3V^{Kiss}$ neuronal activity. To investigate this, calcium imaging was performed on acute slices containing $RP3V^{Kiss}$ cells. These slices were obtained from diestrus mice which express the genetically encoded fluorescent calcium indicator, GCaMP6f, in kisspeptin neurons. Changes in GCaMP6f fluorescence were used as a proxy for changes in kisspeptin neuron activity (5-17 neurons per slice). Bath application of 100μM NA results in decreased GCaMP6f fluorescence in the majority (two thirds) of all observed $RP3V^{Kiss}$ cells, while a lesser proportion are excited (just over 5%) or unaffected (one third) under these conditions (10 slices from 9 mice). In the presence of 100μM prazosin, which prevents the surge in vivo, a greater proportion of excitations (18.7 ±8.5%. 6 slices from 6 mice) are observed than with the application of NA alone (6% ±3.1%. 6 slices from 6 mice. p= .03, unpaired t-test), without a change in the proportion of inhibitions (47.3% ±11.2% vs. 50% ±12.0%, respectively. p=.87). We next tested whether the effect of NA on $RP3V^{Kiss}$ neurons is direct by blocking electrical activity and synaptic transmission in the slice using a drug cocktail including a voltage-gated sodium channel blocker and receptor antagonists for $GABA_A$, NMDA, and AMPA receptors. In the presence of this cocktail, the proportion of inhibited neurons decreased from 83.3% ± 3.4% (4 slices from 3 mice) to 40.2% ±8.3% (5 slices from 5 mice. p= .003). This suggests that direct and indirect, excitatory and inhibitory effects occur in $RP3V^{Kiss}$ neurons in response to NA. Taken together, this reveals that NA at this concentration is primarily inhibitory, in contrast to our hypothesis.

Disclosures: S. Pinti: None. **R. Piet:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.22/K4

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NICHD Grant R01HD109337

Title: Suprachiasmatic Nucleus Neuromedin S Neurons Provide GABAergic Monosynaptic input to Preoptic Area Kisspeptin Neurons in Mice

Authors: R. PIET[1], *W. ABDULMAJEED[2], B. B. JAMIESON[3], S. B. THOMAS[3], Y. RIM[3], A. G. NOVAK[1], R. E. CAMPBELL[3];
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Abstract: In female rodents, projections from suprachiasmatic nucleus (SCN) neurons relay timing cues to the gonadotropin-releasing hormone (GnRH) neuronal network for the preovulatory surge. These cues are known to be integrated with estradiol positive feedback by Kisspeptin (KISS1) neurons of the preoptic area (POA) to drive GnRH neuron activity and the surge. We recently reported that SCN neurons release vasopressin (AVP) and stimulate female POA^{KISS1} neuron action potential firing in an estrous cycle stage-dependent manner. In the SCN, AVP is expressed in a subset of neuromedin S (NMS) neurons, a neuronal population essential for circadian rhythms. Moreover, a previous report indicates that exogenous NMS stimulates LH secretion in female rodents. SCN^{NMS} neurons might, therefore, play a role in timing the preovulatory surge. However, whether SCN^{NMS} neurons project to and regulate the activity of POA^{KISS1} neurons is currently unknown. To address this question, we combined anterograde viral tract-tracing, patch-clamp recording and optogenetic stimulation. Female NMS-Cre mice received stereotaxic injections in the SCN of adeno-associated viral vectors (AAVs) carrying the Cre-dependent sequence for mCherry. Fluorescence immunohistochemistry revealed that mCherry-expressing fibers come in close apposition with the great majority (>80%) of KISS1-immunoreactive neurons in the POA, suggesting a SCN^{NMS}-to-POA^{KISS1} neuron circuit. To interrogate this circuit, we next injected AAVs carrying Cre-dependent channelrhodopsin in the SCN of female NMS-Cre mice that also express the green fluorescent protein in KISS1 cells. Optogenetic activation of SCN^{NMS} neuronal fibers in the POA evoked postsynaptic currents (PSCs) in most KISS1 neurons recorded in whole-cell voltage clamp (-60 mV) in brain slices. Evoked PSCs had short latencies and were blocked by bath application of tetrodotoxin (TTX), indicating that they were generated by action potential-dependent release. Addition of 4-Aminopyridine (4-AP, a potassium channel blocker) to TTX rescued these responses, indicating the existence of a monosynaptic SCN^{NMS}-to-POA^{KISS1} connection. In addition, evoked PSCs were suppressed by bath application of a $GABA_A$ receptor antagonist but not by AMPA- and NMDA-type glutamate receptor antagonists, revealing that SCN^{NMS} projections release GABA

onto POA^{KISS1} neurons. Together, our findings provide direct evidence that monosynaptic GABAergic connections exist between SCN^{NMS} neurons and POA^{KISS1} neurons. Further studies are needed to determine the impact of these projections on POA^{KISS1} neuron activity and the role they might play in the preovulatory surge.

Disclosures: R. Piet: None. **W. Abdulmajeed:** None. **B.B. Jamieson:** None. **S.B. Thomas:** None. **Y. Rim:** None. **A.G. Novak:** None. **R.E. Campbell:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.23/K5

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant 2204750
NIH Grant 5T32GM144919

Title: Variations in Olfactory Bulb Gene Expression among Behaviorally Distinct Prairie Voles (Microtus ochrogaster)

Authors: *J. A. LOPEZ[1], K. R. GOSSMAN[2], A. PEREZ[3], A. S. SMITH[4], C. Y. PUERTA[1], S. BALIVADA[1], B. S. CUSHING[1], S. ROY[5], M. ISLAM KHAN[1];
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Abstract: Functional magnetic resonance imaging results indicate notable variations in connectivity levels both within and between the prosocial core (PC) and the olfactory core (OC) among male prairie voles (*Microtus ochrogaster*) exhibiting behavioral expressions of social affiliation and aggression[1]. Specifically, Illinois (IL) males which express higher levels of prosocial behavior and lower levels of aggression shower greater connective and core nodes than hybrid KI[2] (Kansas dam x Illinois sire), which display the lowest level of prosocial behavior. Behavioral differences are associated with differential expressions of underlying neural mechanisms within nuclei in the PC including oxytocin and estrogen receptor alpha (ERα)[3]. We hypothesis that differential behavior is associated with differential processing of olfactory cues, which we predict will be reflected in differential gene regulation with the OC. We tested this by comparing relative gene expression, using RNAseq, within the olfactory bulb of four groups of prairie voles, Illinois, Kansas and hybrid offspring, IK (Illinois dam & Kansas sire) and KI. Novogene™ KEGG enrichment analyses were employed for differential gene expression analysis. Preliminary data supports our prediction with at least 20 KEGG enrichment pathways distinguished between KS and IL. This included *oxytocin receptor* upregulation and *ERα* downregulation in IL compared to KS males. These findings support our prediction that processing social cues plays a role in the degree of expression of critical social behaviors and

that in addition to other genes that the known underlying neural mechanism in the PC are also directly involved in the OC.

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Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.24/K6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5T34GM145529

Title: 3d light-sheet imaging: differential expression of oxytocinergic neurons between behavioral distinct prairie voles (microtus ochrogaster)

Authors: *A. B. PEREZ[1], J. A. LOPEZ[1], E. I. SALAZAR[1], C. PUERTA[1], N. DESSAUER[2], S. BALIVADA[2], B. S. CUSHING[2];
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Abstract: Oxytocinergic neurons in the paraventricular nucleus of the hypothalamus (PVN) and the supraoptic nucleus (SON) been shown to play a major role in regulating a variety of behavioral and physiological functions including parturition, lactation, and social bonding Several recent studies have also shown expression of groups of oxytocinergic neurons within mammillary nuclei[1], which in mole rats have been described as playing a role in the formation of social bonds[2]. The goal of our study was to use light-sheet microscopy combined with IMARIS software to generate 3-D brain visualization to compare the expression of oxytocinergic neurons in the PVN, SON, and mammillary nuclei between groups of prairie voles that expressive differential levels of pro-social behavior. The prairie vole has been one of the primary models for studying the role of oxytocin in pair bonding and provides a unique model system as while all vole populations are socially monogamous, the degree of expression of the affiliative behavior and aggression differs by population, with Illinois expressing higher levels of affiliation and lower aggression than Kansas. This difference is increased in male hybrids KI (KS dam x IL sire). While 2D-immunohistochemistry studies have shown differences in the PVN[3] here we analyze the SON and for the 1st time expression in the mammillary nuclei. We predict full 3D expression will support previous findings in the male PVN and that there will also be significant differences in the mammillary nuclei, with IL expressing more than Kansas and KI males and that differences will be sexually dimorphic.

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Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.25/K7

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSTC-111-2636-B-007-007
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NSTC 112-2636-B-007-007
Brain Research Center under the Higher Education Sprout Project, co-funded by the Ministry of Education, Taiwan (112QR001EK) and the National Science and Technology Council, Taiwan.

Title: Thirst-driven hygrosensory suppression promoteswater seeking in Drosophila

Authors: *L.-A. CHU[1], A.-S. CHIANG[2], C. TAI[3];
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Abstract: Survival in animals relies on navigating environments aligned with physiological needs. In *Drosophila melanogaster*, antennal ionotropic receptors (IRs) sensing humidity changes govern hygrotaxis behavior. This study sheds light on the crucial role of IR8a neurons in both high humidity avoidance and water-seeking behavior. These neurons demonstrate a heightened calcium response toward high-humidity stimuli in hydrated flies and a reduced response in dehydrated flies, modulated by fluctuating levels of the neuropeptide leucokinin, which monitors the internal water balance. Optogenetic activation of IR8a neurons in thirsty flies triggers an avoidance response reminiscent of moisture aversion in adequately hydrated flies. Furthermore, our study identifies IR40a as a neuron associated with dry avoidance, while IR68a emerges as a moist attraction neuron. The dynamic interplay among these neurons, each with opposing valences, establishes a preference for approximately 30% relative humidity (RH) in well-hydrated flies and triggers water-seeking behavior in thirsty individuals. This research unveils the intricate interplay between sensory perception, neuronal plasticity, and internal states, providing valuable insights into the adaptive mechanisms governing hygrotaxis in *Drosophila*.

Disclosures: L. Chu: None. **A. Chiang:** None. **C. Tai:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.26/K8

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NS072337

Title: A hypothalamic circuit for circadian regulation of corticosterone secretion

Authors: *R. DE LUCA[1], O. RAMÍREZ PLASCENCIA[2], N. L. MACHADO[3], D. H. EGHLIDI[4], S. S. BANDARU[5], F. RAFFIN[6], N. VUJOVIC[7], E. ARRIGONI[8], C. B. SAPER[9]; [1]Neurol., Beth Israel Deaconess Med. Ctr. - Harvard Med., Boston, MA; [2]Neurol., BIDMC/ Harvard Med. Sch., Boston, MA; [3]Neurol., Harvard Med. Sch., BIDMC, Boston, MA; [4]Div. of Sleep Med. and Dept. of Neurol., Harvard Med. Sch. and Beth Israel Deaconess M, Boston, MA; [5]Neurol., Beth Israel Deaconess Med. Ctr. - Harvard Med. Sch., Boston, MA; [6]Neurol., Beth Israel Deaconess Med. Ctr. and Harvard Med. Sch., Boston, MA; [7]Sveta and Allein Sabel, Boston, MA; [8]Neurol., BIDMC and Harvard Med. Sch., Boston, MA; [9]James Jackson Putnam Prof, Harvard Med. Sch. Dept. of Neurol., Boston, MA

Abstract: The secretion of cortisol in humans and corticosterone (Cort) in rodents follows a daily rhythm orchestrated by the suprachiasmatic nucleus (SCN), which activates neurons in the paraventricular nucleus of the hypothalamus that produce corticotropin-releasing hormone (PVH^{CRH}). Tracing and lesion studies have identified the dorsomedial nucleus of the hypothalamus (DMH) as a crucial intermediary node in the daily Cort rhythm. The DMH is both innervated by the SCN and projects to the PVH, and lesions of the DMH eliminate the daily peak in Cort secretion. Our study focuses on understanding the circuit basis by which the DMH regulates the daily rhythm of Cort levels. Specifically, we examined how the projections from DMH glutamatergic (DMH^{Vglut2}) and DMH GABAergic (DMH^{Vgat}) neurons to the PVH^{CRH} neurons regulate the circadian rhythm of Cort, both in brain slices and through whole-animal recordings. We found in brain slices that optogenetic activation of the DMH^{Vglut2} terminals directly activated PVH^{CRH} neurons by AMPA-mediated signaling ($DMH^{Vglut2} \rightarrow PVH^{CRH}$). Whole-animal experiments showed that the ablation of DMH^{Vglut2} neurons or the *Vglut2* gene within the DMH resulted in a 50% reduction in the daily peak of Cort, highlighting the necessary role of DMH glutamatergic signaling. When we looked at the DMH^{Vgat} projections in brain slices, we found that the DMH^{Vgat} neurons do not directly synapse onto the PVH^{CRH} neurons, but optogenetic stimulation of the DMH^{Vgat} input reduced the spontaneous GABAergic input to the PVH^{CRH} neurons, effectively disinhibiting the PVH^{CRH} neurons. We also found that while sparing the PVH^{CRH} neurons, the DMH^{Vgat} input directly inhibited GABAergic neurons in the ventrolateral region of the PVH (peri-PVH; pPVH). Furthermore, we found that the $pPVH^{Vgat}$ neurons themselves directly inhibited PVH^{CRH} neurons through $GABA_A$ signaling, representing the potential intermediate node for DMH^{Vgat}-mediated disinhibition of the PVH^{CRH} neurons. Ablating or disrupting GABA transmission of DMH^{Vgat} neurons diminished the circadian peak of Cort only under constant darkness conditions. Although with a lower magnitude compared to DMH^{Vglut2} neuron stimulation, chemogenetic stimulation of DMH^{Vgat} neurons increased Cort, suggesting a role in disinhibiting PVH^{CRH} neurons, whereas ablation of the $pPVH^{Vgat}$ neurons resulted in increased Cort release at the onset of the active phase. In summary, our results identify two parallel pathways that transmit temporal information to the PVH^{CRH} neurons that cause the daily surge of Cort: a monosynaptic glutamatergic input from the DMH and an indirect disinhibitory $DMH^{Vgat} \rightarrow pPVH^{Vgat} \rightarrow PVH^{CRH}$ pathway.

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Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR232.27/K9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Howard Hughes Medical Institute
Pivotal Life Sciences Chancellor's Chair fund

Title: Neuroendocrine Circuit for Sleep-Dependent Growth Hormone Release

Authors: *X. DING[1], F.-J. HWANG[4], D. SILVERMAN[5], P. ZHONG[7], B. LI[2], C. MA[3], L. LU[8], Z. ZHANG[1], J. B. DING[4], Y. DAN[6];
[1]UC Berkeley, Berkeley, CA; [2]UC Berkeley, Richmond, CA; [3]Dept. of Mol. and Cell Biol., UC Berkeley, Berkeley, CA; [4]Neurosurg., Stanford Univ., Stanford, CA; [5]Dept. of Neurosci., [6]Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA; [7]Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE; [8]MCB, UC Berkeley, Dept. of Mol. and Cell Biol., Berkeley, CA

Abstract: Growth hormone (GH), which stimulates tissue growth and regulates metabolism, is released predominantly during sleep, but the underlying circuit mechanism is unknown. We found that GH release, induced by activating hypothalamic arcuate neurons that express GH-releasing hormone (GHRH), is markedly enhanced by both rapid eye movement (REM) and non-REM (NREM) sleep. The activity of GHRH neurons in the arcuate nucleus and somatostatin (SST) neurons in the arcuate and periventricular nuclei all depend on sleep-wake states; sleep-dependent GH release is regulated by the balance between GHRH and SST neurons. Furthermore, we identified a novel feedback pathway, in which GH enhances the excitability of noradrenergic neurons in the locus coeruleus and suppresses sleep. These results elucidate a circuit mechanism underlying bidirectional interactions between sleep and hormone regulation.

Disclosures: X. Ding: None. **F. Hwang:** None. **D. Silverman:** None. **P. Zhong:** None. **B. Li:** None. **C. Ma:** None. **L. Lu:** None. **Z. Zhang:** None. **J.B. Ding:** None. **Y. Dan:** None.

Poster

PSTR232: Neuroendocrine Anatomy: Physiology and Behavior

Location: MCP Hall A

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Program #/Poster #: PSTR232.28/K10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R01HD089495
R37HD043341
P51OD011106

Title: The Inhibitory Role of Opioid Neurons in Prepubertal GnRH Release in Male Rhesus Monkeys

Authors: *R. J. ADAM[1], M. L. GLOO[1], E. M. GELMAN[1], E. TERASAWA[2,3];
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Abstract: It has been shown in primates that GnRH/LH release during the prepubertal period is centrally inhibited such that the absence of gonads does not lead to elevated release in GnRH/LH. For example, during this period GnRH/LH levels in human patients with gonadal dysgeneses and gonadectomized prepubertal monkeys are very low. Additionally, previous studies in this lab suggest that GABA neurons are, in part, responsible for this "Central Inhibition." To further address the neuronal substrate responsible for central inhibition, in the present study we examined the role of opioid neurons in regulation of the GnRH neurosecretory system, by infusing the opioid receptor antagonist naloxone in the stalk-median eminence (S-ME) in two rhesus monkeys at 19.7-22.3 months age (Late prepubertal stage) and two 33.8-36.4 months (Mid-pubertal stage) using the microdialysis method. Naloxone (primarily an opioid μ receptor antagonist) at 10^{-8}M and 10^{-7}M concentrations was directly infused into the S-ME for 20 min, while a series of microdialysates were collected at 20 min intervals from the S-ME for measurements of GnRH release. Preliminary results indicated that GnRH release was stimulated by naloxone at a low dose (10^{-8}M) in late prepubertal monkeys ($p \leq 0.06$), whereas GnRH release was consistently inhibited by a higher dose naloxone (10^{-7}M, $p<0.01$) in mid-pubertal monkeys. Moreover, naloxone-induced GnRH increases in prepubertal males were 22 folds greater than those in pubertal males. The results are interpreted to mean that 1) in prepubertal males GnRH release in the hypothalamus is in part inhibited by opioid receptor sensitive neurons, while 2) in pubertal males naloxone becomes an opioid receptor agonist on the GnRH neurosecretory system. We further speculate that significantly larger responses to naloxone in prepubertal monkeys than those in mid-pubertal monkeys are attributable to the removal of opioid inhibition in the hypothalamus. Collectively, hypothalamic opioid neurons, such as ß-endorphin neurons, are also responsible for central inhibitory mechanism during prepubertal period in primates. Interaction between opioid and GABA neurons in the prepubertal hypothalamus remains to be investigated. (Supported by NIH grants R37HD043341**,** R01HD089495, P51OD011106).

Disclosures: R.J. Adam: None. **M.L. Gloo:** None. **E.M. Gelman:** None. **E. Terasawa:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.01/K11

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIH DP1-AT011979-01

Title: Deciphering the role of the celiac-mesenteric ganglia in immune response

Authors: L. REMÉDIO[1], ***Z. R. LEWIS**[2], E. THOMAS[2], S. YAO[2], K. SMITH[2], D. BERTAGNOLLI[2], A. CHAKKA[2], R. CHAKRABARTY[2], R. FERRER[2], J. GOLDY[3], J. GUZMAN[2], K. JAMES[2], B. NGUY[2], T. PHAM[2], C. RIMORIN[2], D. ROCHA[2], M. TIEU[2], A. TORKELSON[2], D. S. PETERKA[4], B. TASIC[2], R. O. COSTA[1];
[1]Allen Inst. for Neural Dynamics, Seattle, WA; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Allen Inst. For Brain Sci., Seattle, WA; [4]Zuckerman Inst., Columbia Univ., New York, NY

Abstract: The sympathetic nervous system (“fight or flight”) plays an important yet underappreciated role in modulating immune responses to infection via the innervation of organs such as the spleen, bone marrow and lymph nodes. The spleen is innervated by the celiac-mesenteric ganglion (CMG) in mice, and we posit that some neurons within the CMG respond to stimuli to modulate immune responses within the spleen. To address this hypothesis, we characterize the neuronal diversity within the CMG by scRNA-seq using a transgenic reporter mouse line that labels tyrosine hydroxylase-positive cells. We identify subclasses of CMG neurons, defined by transcriptional differences. By administering *Escherichia coli* lipopolysaccharide (LPS) endotoxin and performing scRNA-seq on neurons within the CMG, we find differential expression of genes that may be involved in splenic cell function following bacterial infection. These genes include toll-like receptors, chemokines and axon guidance factors. Coupled with anatomical description of the post-ganglionic innervation of the spleen we hope to leverage the transcriptomic insights to understand how interactions between the immune and nervous systems are functionally mediated.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

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Program #/Poster #: PSTR233.02/K12

Topic: F.04. Neuroimmunology and Neurovirology

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Contributions from NIHR BRC at South London and Maudsley NHS Foundation Trust and King's College London; and the NIHR Cambridge BRC (Mental Health and Immunity/Infection/Transplantation Themes, NIHR203312).

Title: Investigating central-peripheral immune interactions in depression via extra-axial inflammatory signals: a ^{11}C-PK11195 PET study

Authors: *B. EIFF[1], E. T. BULLMORE[3], M. CLATWORTHY[4], T. D. FRYER[5], C. M. PARIANTE[2], V. MONDELLI[2], L. MACCIONI[6], N. HADJIKHANI[7], M. L. LOGGIA[7,8], M. A. MOSKOWITZ[9], E. BRUNER[10,11], M. VERONESE[1,6], F. E. TURKHEIMER[1], J. J. SCHUBERT[1];
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Abstract: Although central and peripheral inflammation are consistently observed in depression, it remains unclear how these two systems interact. This study explores the role of extra-axial spaces, such as the skull bone marrow and dural sinuses, in mediating central and peripheral immune interactions.
A reanalysis was conducted on a dataset of 60-minute dynamic ^{11}C-PK11195 positron emission tomography obtained from 51 depressed subjects enriched for heightened peripheral and central immunity, along with 25 age and gender matched healthy controls. Translocator protein (TSPO) served as a marker of inflammation in the skull bone marrow regions overlying the occipital and parietal lobes, along with the dorsolateral prefrontal cortex. TSPO signal was also extracted from the upper viscerocranium, representing the facial bone region, as well as from the confluence of sinuses. Average standardized uptake values (SUVs) were calculated from 5 to 60 minutes. The analysis treated subject group as an independent factor and explored associations between extra-axial SUVs, TSPO SUV in the anterior cingulate cortex as a marker of central immunity, and peripheral cytokine concentrations (specifically tumor necrosis factor-α; TNF-α) as an indicator of peripheral immunity.
The findings reveal a nuanced relationship between immune activity across extra-axial spaces and both peripheral and central immunity. These interactions were particularly robust within the confluence of sinuses, but also present within the facial bone and parietal skull regions and were not found to be influenced by depression group status. Group dependent elevations in TSPO expression within the occipital skull region were found to be significantly associated only with central immunity.
These results align closely with recent anatomical findings and emphasize the importance of extra-axial spaces in mediating brain-body immune interactions. The venous sinuses, facial bone, and parietal skull regions are highlighted as pivotal locations for this crosstalk. When paired with

previous findings, these results suggest that the occipital skull region may host a specialized immune cell niche primed for supplying immune privilege to the brain during disease states. Together, these observations underscore the potential of extra-axial regions as promising therapeutic targets in neuroinflammatory conditions.

Disclosures: B. Eiff: None. **E.T. Bullmore:** None. **M. Clatworthy:** None. **T.D. Fryer:** None. **C.M. Pariante:** None. **V. Mondelli:** None. **L. Maccioni:** None. **N. Hadjikhani:** None. **M.L. Loggia:** None. **M.A. Moskowitz:** None. **E. Bruner:** None. **M. Veronese:** None. **F.E. Turkheimer:** None. **J.J. Schubert:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.03/K13

Topic: F.04. Neuroimmunology and Neurovirology

Support: University of Pittsburgh Physician Foundation Research Grant
VA VISN4 Competitive Career Development Fund
NIH Grant NS033730
Foundation for Anesthesia Education and Research Mentored Research Grant
NIH Grant GM150680

Title: Loss of neuronal MD-1 leads to increased inflammation in an imiquimod model of psoriasis

Authors: M. N. KELLER[1], J. D. JONES[2,3], K. M. ALBERS[5,3], *M. RITTER JONES[4]; [1]Dept. of Biol. Sci., [2]Dept. of Neurobio., [3]Pittsburgh Ctr. for Pain Res., [4]Dept. of Anesthesiol. and Perioperative Med., Univ. of Pittsburgh, Pittsburgh, PA; [5]Dept. of Neurobio., Univ. of Pittsburgh Dept. of Neurobio., Pittsburgh, PA

Abstract: The response to cutaneous inflammatory challenges requires the interaction of many cell types, which include immune cells, keratinocytes and sensory neurons. Unlike cutaneous peptidergic neurons, nonpeptidergic sensory afferents have only recently been demonstrated to influence the immune response. Neurturin (Nrtn), a glial cell line-derived neurotrophic factor produced by the skin, supports the survival and growth of nonpeptidergic, MrgprD-expressing, GFRα2 receptor-positive sensory neurons. Nrtn has also been implicated as a modulator of the skin pathogenesis observed in the inflammatory imiquimod model of psoriasis. Moreover, in mice that overexpress Nrtn (NrtnOE) in the skin, transcriptomic analysis of sensory ganglia shows upregulation of immune-response genes, suggesting Nrtn-responsive neurons have a role in modulating inflammatory challenges.
Towards understanding the role of nonpeptidergic neurons in immune signaling, we are investigating the functional relevance of immune genes upregulated in sensory ganglia by skin-

derived Nrtn. A gene of particular interest is lymphoid antigen 86 (Ly86), which encodes myeloid differentiation 1 (MD-1), an innate immune response protein predicted to have anti-inflammatory actions. Studies of MD-1 have demonstrated that decreased MD-1 mRNA expression is associated with proinflammatory disorders such as lupus and obesity, suggesting an anti-inflammatory function. To examine the role of MD-1 expressed by neurons, we used the imiquimod model of psoriasis to determine if loss of neuronal MD-1 impacts the inflammatory response. Imiquimod was applied daily to the ear of MrgprD+/MD-1 conditional knockout mice as well as littermate controls for 6 days. We then measured ear thickness to assess inflammatory edema and relative level of mRNAs encoding immune genes in skin and sensory ganglia. Immune cell profiles were also evaluated using flow cytometry. After 6 days of imiquimod treatment, the ear thickness of the neuronal MD-1 knockout mice was thicker relative to littermate controls, indicating continued inflammation. Transcriptional analysis of ear skin showed increased Ly6G, a molecule expressed on neutrophils. *In vitro* studies of neurons treated with imiquimod showed increased release of inflammatory cytokines from MD-1 knockout neurons, supporting an immune suppressor role for MD-1 in nonpeptidergic neurons. Further studies will delineate the detailed mechanism of by which neuronal MD-1 influences the immune response. These data support a role for nonpeptidergic neurons in the inflammatory response of skin and may lead to adjunctive approaches to facilitate resolution of inflammation.

Disclosures: M.N. Keller: None. **J.D. Jones:** None. **K.M. Albers:** None. **M. Ritter Jones:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.04/K14

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIH R01 GM143362

Title: Activation of cholinergic vagal efferents using Red-activatable channelrhodopsin (ReaChR) reduces cytokine levels

Authors: *K. PARK[1], A. CHEN[1,2], T. S. HUERTA[1], S. CHAUDHRY[1], M. NAGPAL[1], T. HEPLER[1], S. S. CHAVAN[1,2,3], E. H. CHANG[1,2,3];
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Abstract: Vagus nerve stimulation (VNS) is being investigated as a neuromodulation strategy to manage chronic inflammatory disorders by activating the inflammatory reflex, a neural circuit that decreases proinflammatory cytokine release via efferent cholinergic signaling pathways. Traditional VNS methods are predominantly invasive and rely on electrical stimulation but non-

invasive alternatives could increase the specificity of vagal fiber targeting and remove surgical risks. One such alternative, red-light optogenetics, employs red-light activatable channelrhodopsins (ReaChR) to stimulate genetically-targeted neural populations. We demonstrate that activating ReaChR in cholinergic vagal fibers diminishes cytokine production in an acute inflammation model.In this study, we specifically targeted vagus nerve cholinergic fibers by using ReaChR expressed in choline acetyltransferase-positive (ChAT) fibers in transgenic ChAT-ReaChR mice. In another group, transient receptor potential vanilloid subfamily member 1 (TRPV1)-ReaChR mice were used to activate sensory afferent vagal fibers. To induce acute inflammation, we administered lipopolysaccharide (LPS, 0.3 mg/kg) intraperitoneally under anesthesia, measuring the immune response via elevated tumor necrosis factor-alpha (TNFα) cytokine levels. A 635nm fiber optic was directed at the cervical vagus nerve for optogenetic VNS following LPS injection.Selective ReaChR activation of cholinergic fibers in the cervical vagus nerve stimulated the inflammatory reflex to inhibit splenic TNFa production to bacterial LPS (ChAT-ReaChR: 1.27 ± 1.44 pg/ug; control: 4.26 ± 0.97 pg/ug, $p < 0.001$), but no significant differences were observed in serum (ChAT-ReaChR: 538 ± 216 pg/ml; littermate: 447 ± 206 pg/ml, $p = 0.81$). In contrast to efferent activation, ReaChR stimulation of afferent subsets (TRPV1+) failed to show cytokine regulation in the spleen or in serum. An approximately 20% baseline heart rate drop was seen during both ChAT+ and TRPV1+ fiber excitation, but only ChAT+ activation elicited sustained bradycardia throughout the stimulation period. In summary, we show that ReaChR-based optogenetic activation of cholinergic vagal fibers reduces splenic cytokine levels during acute endotoxemia, suggesting the potential for its use as a non-invasive red-light-based VNS method.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.05/K15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Dr. Hunsberger's startup package

Title: The impact of pregnancy on mood, cognition, and microglia function in Alzhiemer’s disease mice

Authors: *P. ARIAS[1], H. C. HUNSBERGER[2];
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Abstract: Alzheimer's disease (AD) impacts six-million Americans, 2/3rds of whom are women. Women are more susceptible to developing AD due to a variety of social stress factors, differences in immune responses, and a decline in estrogen. Previous research has shown that pregnancy offers protective barriers to women, reducing the likelihood of developing dementia-like disorders. Notably, pregnancy has the ability to modify a woman's inflammatory response. This interaction between pregnancy and inflammation is critical given that there is microglial upregulation to clear amyloid plaque burden in the brain. In some instances, the activation of microglia can promote the phagocytosis and clearance of those toxic proteins, but if microglia become overactivated, a surplus of inflammatory mediators are released. Here we aim to understand how pregnancy impacts cognition and microglia activation in Alzheimer's disease mice. To answer these questions, we used behavior testing and immunohistochemistry to analyze cellular activity in the hippocampal region of male and female naive and postpartum mice. We found that 1) litter-bearing mice exhibit slight memory impairment with age and 2) litter size was indicative of memory impairment in AD mice. Interestingly, the sex of the pups also impacted affective behaviors. Furthermore, immunohistochemistry using microglia markers can give insights into the complexity of microglia function in naive and litter-bearing control or AD mice. Overall, this research provides a deeper understanding of how pregnancy influences brain pathology and AD symptoms.

Disclosures: P. Arias: None. **H.C. Hunsberger:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.06/K16

Topic: F.04. Neuroimmunology and Neurovirology

Support: Wellcome Grant

Title: Clozapine abates autoimmunity in a lupus mouse model

Authors: *T. B. NGUYEN, L. HE, K. SCHMACK;
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Abstract: Autoimmunity may play a role in the development of psychosis. Antipsychotic drugs, in turn, can downregulate the adaptive immune system. This raises the intriguing possibility that antipsychotic drugs might exert their therapeutic effects through modulating an autoimmune process. Here, we use a mouse model of systemic lupus erythematosus to show proof-of-concept that the antipsychotic drug clozapine can abate autoimmunity. First, we developed a novel protocol of chronically administering high-dose clozapine (120 mg/kg/day) to mice and verified that mice reached clinically relevant plasma concentration levels (median=218.7ng/mL). We then administered clozapine over the course of 9 weeks to "kika" mice, a mouse model of systemic lupus erythematosus. Kika mice are known to develop antinuclear autoantibodies,

increased spleen size and end organ damage akin to lupus seen in humans. We compared serum antinuclear antibodies by ELISA and spleen size by weighing between clozapine-treated kika mice (n=11) and an untreated kika control group (n=10). We found that serum antinuclear antibodies were significantly lower in clozapine-treated kika mice compared to untreated kika mice (median untreated anti-ssRNA optical density at 405nm=0.506 [IQR 0.41-0.63] vs treated=0.260 [IQR 0.18-0.40], $p<0.01$; median untreated anti-dsDNA=0.437 [IQR 0.36-0.55] vs treated=0.218 [IQR 0.15-0.39], $p<0.01$), indicating an effect of clozapine on autoimmune disease markers. In line with this, spleen mass was also significantly reduced in clozapine-treated kika mice compared to untreated kika mice (median untreated spleen mass= 0.405g [IQR 0.20-0.57g] vs treated=0.202g [IQR 0.19-0.24g], $p<0.01$), further corroborating a reduction in autoimmune activation by clozapine. Taken together, our results indicate that chronic clozapine exposure ameliorates disease markers in a mouse model of lupus, providing evidence that clozapine can abate autoimmunity. Further research is underway to uncover how clozapine interacts with the immune system that leads to this effect and to probe whether clozapine abates autoimmune psychosis.

Disclosures: T.B. Nguyen: None. **L. he:** None. **K. Schmack:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.07/K17

Topic: F.04. Neuroimmunology and Neurovirology

Support: SU BAP 22122027

Title: A Comparison: Three-Dimensional Characterization of Neuronal Networks in the Thymus of Young and Elderly Humans.

Authors: U. ONAL[1], I. HARMANKAYA[2], M. S. AYDIN[3], M. I. ALP[4], ***E. ERDOGAN**[5]; [1]Histology Dept., Hlth. Sci. Univ., Van Regional Training and Res. Hosp., VAN, Turkey; [2]Pathology, Selcuk Univ., Fac. of Med., Konya, Turkey; [3]Regenerative and Restorative Med. Res. Ctr. (REMER), Istanbul Medipol Univ., Istanbul, Turkey; [4]Neurosci. Res. Ctr., Istanbul Medipol Univ., Res. Inst. for Hlth. Sci. and Technologies (SABITA), Istanbul, Turkey; [5]Histology and Embryology, Selcuk Un. Med. Fac. (Tip Fak), Selcuklu, Konya, Turkey

Abstract: The thymus plays a crucial role in the body's immune response, serving as the site where T lymphocytes, an essential type of immune defense cells, mature. It provides various specialized microenvironments that support and guide the differentiation and selection of T cells.Aging induces structural changes affecting all physiological systems, including the immune system, leading to increased susceptibility to infectious diseases and contributing to various structural changes associated with cardiovascular, metabolic, autoimmune, and neurodegenerative diseases.Changes related to the 'thymic involution phenomenon,' which is

developmentally programmed and particularly limits the outputs of T lymphocytes from the thymus, have been highlighted in connection with the microenvironment, drawing attention to thymic adipocytes.For this purpose, our study obtained sections of human thymus tissue without any pathological diagnosis from the tissue archive of the Department of Pathology, Selçuk University Hospital, with ethical committee approval, comprising 26 samples from each group of young (average age 1 year) and elderly (average age 70 years) individuals. After applying optimal antigen retrieval protocols, sections of paraffin blocks were subjected to immunohistochemical labeling with specific markers for the peripheral nervous network. Data were statistically analyzed using fluorescence microscopy, and fluorescence intensities were measured. Additionally, tissue clearing was performed, allowing high-resolution imaging using lightsheet microscopic imaging, which offers a robust alternative to traditional imaging techniques with excellent penetration depth, enabling 3D visualization.
Our results show that in the well-defined involution process of human thymus tissue, where thymocytes are replaced by thymic adipocytes (beige adipocytes), immunolabeling of the neuronal network was detected at the same fluorescence intensity in both age groups, indicating that its metabolism is still active.
With these data, elements of the microenvironment representing the first step towards the production of artificial thymic glands that could be used to replace or augment damaged organs have been identified, highlighting the potential candidacy of thymic adipocytes.

Disclosures: U. Onal: None. **I. Harmankaya:** None. **M.S. Aydin:** None. **M.I. Alp:** None. **E. Erdogan:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.08/K18

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIH Grant HL152101
REZNIK23Y3
UF MBI Gator NeuroScholar Fellowship

Title: Effects Of Local Sympathetic Nerve Depletion On Airway Function In An Experimental Model Of Allergic Asthma

Authors: *P. TREVIZAN-BAÚ, A. L. FAGAN, S. P. AMIN, L. R. REZNIKOV;
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Abstract: The respiratory airways are in constant contact with inhaled pathogens and/or chemical irritants from the external environment. Airway-innervating neurons monitor the airway environment for potentially noxious or harmful stimuli; however, little is known about the role that airway nerves play on lung function in airway allergy and inflammation. Because it

is well documented that the sympathetic nerves modulate/regulate systemic inflammatory responses, we hypothesized that airway-innervating sympathetic neurons also play critical roles in regulating airway responses to pulmonary inflammation. The present study tested this hypothesis in an experimental mouse model of allergic asthma. We performed chemical sympathectomy of local sympathetic nerves in the lung via intranasal administration of 6-hydroxydopamine for three consecutive days (day 1-3). Control mice received vehicle (ascorbic acid) only. On day 7, we started administering intranasal IL-13, an effector molecule in allergic asthma, or vehicle control (saline) for four consecutive days (day 7-10) to mimic allergic asthma. Then, 20-24 h after the last IL-13 (or saline) administration, we evaluated pulmonary mechanics (flexiVent protocol) and, following flexiVent procedures, we collected bronchoalveolar lavage and lung samples. We observed that local airway chemical sympathectomy reduced tissue damping and tissue elastance in the lung, which are properties related to alveolar structure and tissue density, in both vehicle control and IL-13-treated mice compared to IL-13-treated mice with intact sympathetic airway nerves. Local airway chemical sympathectomy also decreased the density of neutral mucin-secreting goblet cells and mRNA expression for the gel-forming mucin (*Muc5ac*) in IL-13-treated mice compared to IL-13-treated mice with intact sympathetic airway nerves. By combining functional and molecular strategies, this study shows that airway-innervating sympathetic neurons regulate allergic airway responses and may serve as an underexplored therapeutic target for allergic asthma.

Disclosures: P. Trevizan-Baú: None. **A.L. Fagan:** None. **S.P. Amin:** None. **L.R. Reznikov:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.09/K19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The study was sponsored by Mitrix Bio, Inc

Title: Mitlet Therapy: A Novel Strategy for Enhancing Longevity and Combating Aging with Implications for Neuroscience

Authors: B. C. ALBENSI[1], T. BENSON[2], ***A. ADLIMOGHADDAM**[3];
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Abstract: Mitochondria are vital components incellular energy production, playing a crucial role in orchestrating variouscellular functions, including modulating the immune system. Typically locatedwithin cells, mitochondria can also be released into the extracellular spacealongside extracellular vesicles. Platelets, actively monitoring blood vesselsto uphold vascular integrity and bolster immune functions, are the primarysource of circulating mitochondria in the bloodstream.

Upon activation,platelets release extracellular vesicles, including a subset containingmitochondria known as "mitlets" or platelet-derived mitochondrialvesicles. Given the correlation between platelets and immune cells and theinvolvement of mitochondria in immunity, we aimed to elucidate the functions ofmitlets and evaluate their potential impact on aging, longevity, andinflammation, which are risk factors for neurodegenerative diseases such asAlzheimer's disease (AD).In this study, mitlets were extractedfrom the blood of young (2 months old) C57BL/6 mice and subsequentlytransplanted three times over seven days to groups of both young and aged (13months old) C57BL/6 mice. The effects on inflammation, longevity, andaging-related diseases such as Alzheimer's were investigated. Additionally,parallel transplants were conducted using mitochondria isolated from the liverof C57BL/6 mice.Mitlet transfusion significantly reducedearly markers of inflammation in aged mice. Although mitlet transplantationfrom Day 2 to Day 5 delayed subsequent signs of inflammation, it did notprevent it entirely. Mitlet therapy resulted in a considerable reduction inpro-inflammatory cytokine levels, particularly IL-6, which is known to beelevated in chronic inflammation associated with aging and neurodegenerativediseases like Alzheimer's. Injections of mitochondria isolated from the liverfailed to provide any protective effect; mice receiving these injections showedsimilar levels of inflammation as negative controls.Overall, our study indicates that mitlettherapy holds promise in alleviating inflammation, which is a critical factorin aging and age-related diseases such as Alzheimer's. Future advancements inthis area could potentially address immune senescence linked to decreasedmitochondrial energy production, offering a novel therapeutic strategy. Thisapproach shows potential for managing chronic inflammation and improvingoverall longevity, highlighting the importance of robust mitochondrial functionin maintaining health and combating neurodegenerative diseases.

Disclosures: B.C. Albensi: None. **T. Benson:** None. **A. Adlimoghaddam:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.10/K20

Topic: B.09. Glial Mechanisms

Title: Single-nucleus RNA sequencing of striatal microglia reveals distinct transcriptomic signatures of acute stress and chronic exercise

Authors: *M. G. CONNOLLY[1], Z. V. JOHNSON[2], L. CHU[3], N. JOHNSON[5], T. J. BUHR[6], E. MCNEILL[4], P. J. CLARK[4], J. S. RHODES[7];
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Abstract: Severe acute stress can produce long lasting decreases in voluntary physical activity that contribute to degraded mental and physical health. Stress also produces enduring molecular changes in the striatum, a brain region that regulates voluntary wheel-running and other motivated behaviors. Microglia, the primary immune cells of the central nervous system, have specialized functions in responding to stress, sensing changes in the striatum, and controlling neuronal activity. Thus, microglia are positioned at the interface between neural responses to stress and neural coordination of voluntary activity; however, the role of striatal microglia in stress-induced long-term suppression of voluntary activity remains unexplored. The present study employs single nucleus RNA-sequencing to investigate how stress and exercise impact the biology of microglia in the striatum. We find that stress-induced decreases in running behavior are associated with specific microglial activation profiles. Furthermore, we show that access to a running wheel is associated with an additional and distinct profile of microglia activation characterized by upregulation of complement components and phagocytosis pathways. Lastly, we find that distinct microglial gene sets are associated with general running (versus not running) and more subtle variation in genes with individual running levels. Taken together, our results contribute to a broader understanding of the diverse states that striatal microglia can assume in response to stress and exercise, and broadly suggest that microglia exhibit more nuanced functional responses to environmental perturbations than previously thought.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.11/K21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Cure Alzheimer's Fund
NIH

Title: Impact of peripheral inflammation on human microglia.

Authors: *S. SHERGILL[1], Y. LIN[2], M. THERRIEN[3], B. A. STEVENS[4];
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Abstract: Microglia are the brain’s immune cells and express multiple Alzheimer’s disease risk genes. Understanding the impact of these risk genes on microglia is essential to identify how disease is initiated and progresses. Recently, it has been shown that peripheral inflammation is a major component of AD pathogenesis, but we don’t know how microglia respond to this specific inflammation. We have recently demonstrated that using iPSC models can recreate microglia

states in vitro. However, in vivo models were needed to study interactions between brain and peripheral inflammation. In this study, we developed and validated an adaptation of cell villages for xenograft iPSC-derived microglia. We used cell lines from 40 donors, injected in P3 pups, and at six months old, mice were treated with saline or 1mg/kg lipopolysaccharide. Using single-cell RNA sequencing, we identified a global iMG responding to LPS shared across donors. These findings provide new models to study the role of genetic risk factors in human microglia.

Disclosures: S. Shergill: None. **Y. Lin:** None. **M. Therrien:** None. **B.A. Stevens:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.12/K22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: L. Calvier was supported by a postdoctoral fellowship grant from DFG (CA 1303/1-1). J. Herz was supported by grants from the NHLBI (R37 HL063762), NIA (RF1/RF3 AG053391), the NINDS and NIA (R01 NS093382 and R01 NS108115),
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and NIH/NIA SBIR grant to Reelin Therapeutics, Inc. (1R43AG084450) with L. Calvier and J. Herz (PIs Subcontract).

Title: Targeting the crosstalk between the brain and the periphery to dampen neuroinflammation in Alzheimer’s disease.

Authors: *L. CALVIER, J. HERZ;
UT Southwestern Med. Ctr., Dallas, TX

Abstract: Alzheimer's disease (AD) is characterized by two core pathologies: amyloid β plaques and neurofibrillary tangles. Despite therapeutic efforts focusing on these pathologies, success in the clinic has been limited. To address this challenge, alternative mechanisms are under investigation, and the presence of a low-grade, presymptomatic but sustained immune response has emerged as a third core pathology. This unresolved neuroinflammation relies on the activation of resident microglia and the recruitment of circulating leukocytes through a leaky blood-brain barrier (BBB). We hypothesize that restoring BBB integrity through targeted

intervention, focusing on the circulating endothelial factor Reelin, will reduce neuroinflammation and improve AD outcomes. Through examination of a cohort of AD patients and several AD mouse models, we observed that plasma Reelin increases with disease progression and is secreted by skull bone marrow niches. Direct injection of amyloid β 42 fibrils into the hippocampus also stimulated Reelin secretion from the skull bone marrow, suggesting a privileged communication path between the brain and this peripheral immune tissue. Moreover, depletion of plasma Reelin with an antibody in the AD mouse models 5xFAD and APP^{NL-F} decreased BBB permeability and leukocyte infiltration into the brain. This resolution of neuroinflammation was sufficient to restore normal cognitive functions, as evaluated by Y-maze and fear conditioning tests, in prevention and treatment studies. Our study challenges traditional views on brain-peripheral immune communication, revealing the activation of skull bone marrow niches during AD. It identifies new circulating factors from the skull bone marrow, such as Reelin, that propagate neuroinflammation and may serve as therapeutic targets to disrupt the chronic inflammatory loop in AD.

Disclosures: L. Calvier: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-funder of Reelin Therapeutics. **J. Herz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); co-funder of Reelin Therapeutics.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

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Program #/Poster #: PSTR233.13/K23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R21 AG087039

Title: Evaluation of the lh/cg receptor as a therapeutic target for sepsis-induced neuroinflammation and cognitive decline

Authors: *L. WAGNER[1], D. NACIONALES[2], G. TAKACS[1], J. K. HARRISON[3], P. EFRON[2], P. CHAKRABARTY[4], G. CASADESUS[5];
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Abstract: Sepsis has been increasingly associated with dementia and cognitive decline later in life. Loss of blood brain barrier (BBB) integrity and consequent peripheral immune cell infiltration has been implicated in sepsis-induced central nervous system (CNS) dysfunction and increased dementia risk. The LH/CG-hormone receptor complex is powerful immune regulator

critical in balancing the maternal immune milieu to support pregnancy. Human chorionic gonadotropin, one of two LHCGR ligands, has shown therapeutic benefits in sepsis treatment. Therefore, here we sought to address whether these properties extended to the CNS, by centrally administering hCG and evaluating its effectiveness in mitigating neuroinflammation, CNS dysfunction, and peripheral myeloid infiltration into the brain after cecum ligation and puncture - plus restrain stress, a more relevant model of the human sepsis syndrome. We performed these analyses in a double myeloid cell reporter mouse line ($CX3CR1^{GFP/+}$/$CCR2^{RFP/+}$) after 14 days of hCG or control treatment, and at 4-months post-CLP to address long-term benefits of treatment. Our preliminary findings support the long-term presence of peripheral cell infiltration/BBB breakdown in this sepsis model. These findings are paralleled by exacerbated microglial activation, and cognitive deficits that extend long-term post-sepsis. Our initial findings with a low hCG dose support the immune regulating and therapeutic properties of hCG in the CNS. Together, these data reveal a previously unknown role for the LH/CG receptor in the CNS and highlight a novel potential target for neurological conditions that involve peripheral myeloid infiltration as a pathogenic factor.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.14/K24

Topic: F.04. Neuroimmunology and Neurovirology

Support: NIH/NIMH R01MH122471
NIH/NINDS U01NS120836; R35NS111644
DoD W81XWH-21-1-0979
HDFCCC Laboratory for Cell Analysis Shared Resource Facility (NIH P30CA082103)
National Multiple Sclerosis Society
Valhalla Foundation
The Westridge Foundation

Title: A novel transcobalamin receptor autoantibody in autoimmune vitamin B12 central deficiency

Authors: J. V. PLUVINAGE[1,2], T. NGO[1,2], ***A. I. ASENCOR**[1,2,3], S. J. PLEASURE[1,2], M. R. WILSON[1,2];
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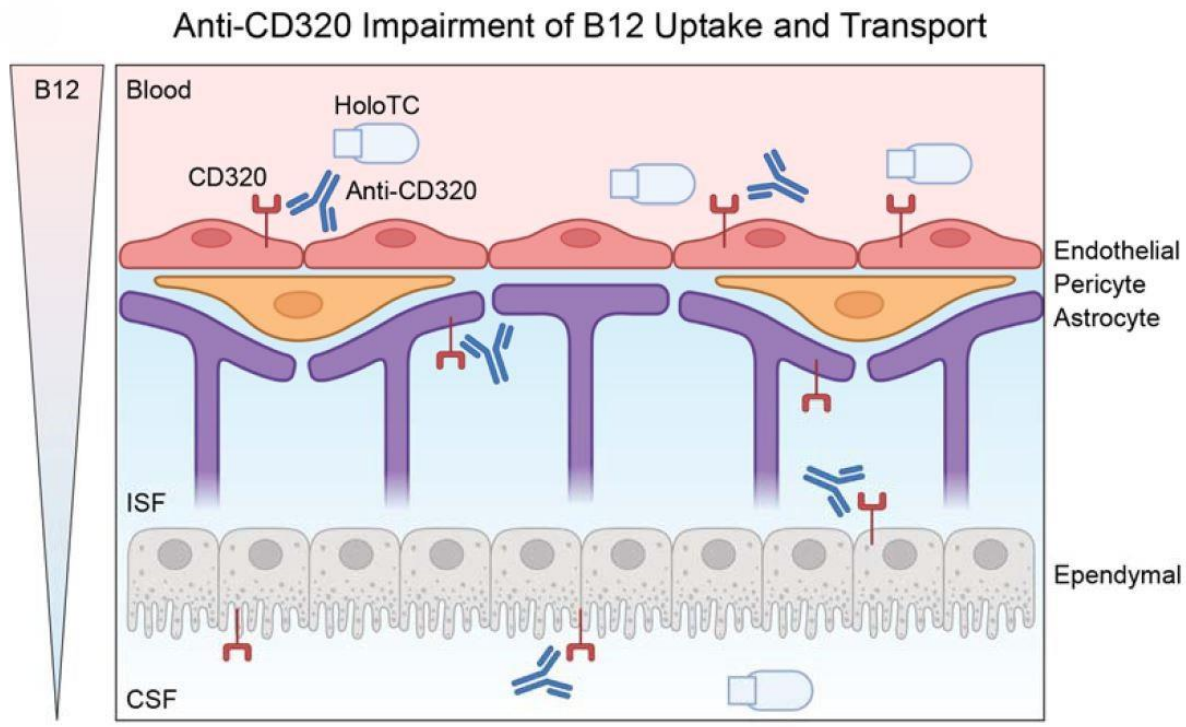
Abstract: Vitamin B12 is critical for hematopoiesis and myelination. Deficiency can cause neurologic deficits including loss of coordination, spasticity, and cognitive decline. However, diagnosis relies on vitamin B12 measurement in the blood which may not accurately reflect levels in the brain. Using programmable phage display, we identified an autoantibody targeting the transcobalamin receptor (CD320) in a patient with progressive tremor, ataxia, and scanning speech. Anti-CD320 impaired cellular uptake of cobalamin (B12) in vitro. Despite normal serum levels, B12 was nearly undetectable in her cerebrospinal fluid (CSF). Immunosuppressive treatment and high-dose systemic B12 supplementation were associated with increased CSF B12 levels and clinical improvement. Optofluidic screening enabled rapid isolation of a patient-derived anti-CD320 monoclonal antibody that impaired B12 transport across an in vitro model of the blood-brain barrier. Autoantibodies targeting the same epitope of CD320 were identified in 7 other patients with neurologic deficits of unknown etiology. Anti-CD320 was detected in 21.4% of a neuropsychiatric lupus cohort and predicted central B12 deficiency in multiple sclerosis. However, these individuals did not display any hematologic signs of B12 deficiency despite systemic CD320 impairment. Using a genome-wide CRISPR screen, we discovered that the low-density lipoprotein receptor (LDLR) serves as an alternative B12 uptake pathway in hematopoietic cells. These findings dissect the tissue specificity of B12 transport and elucidate an autoimmune neurologic condition that may be amenable to immunomodulatory treatment and nutritional supplementation.



Disclosures: J.V. Pluvinage: None. **T. Ngo:** None. **A.I. Asencor:** None. **S.J. Pleasure:** None. **M.R. Wilson:** 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.; Roche/Genentech, unrelated research grant funding, Novartis, unrelated research grant funding. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus);

Genentech, speaking honoraria, Takeda, speaking honoraria, WebMD, speaking honoraria, Novartis, speaking honoraria, CDI Laboratories, licensing fees. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Delve Bio, Inc., founder. F. Consulting Fees (e.g., advisory boards); Delve Bio, Inc., paid consultant.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.15/K25

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Analysis of Western and Mediterranean Diets on Brain Function and Cognition: A Systematic Review

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Abstract: Over the past 50 years, many natural nutrients have been replaced with artificial ones with the increase in processed foods. This study aims to analyze the role of a Western-style diet high in processed foods and a Mediterranean-style diet composed of produce and unsaturated fats on brain function and cognition.
A systematic review was conducted following PRISMA guidelines using various iterations of predetermined search terms such as “western diet”, “mediterranean diet”, and “brain cognition” in databases such as PubMed, Google Scholar, PsycInfo, and Cochrane. 696 articles were found in the initial search and narrowed to 208 after the removal of duplicates and articles not in the English language. Articles were then further narrowed based on relevance to the study (main findings) and presented results (showing a correlation between the type of diet and brain cognition, health, or function). 18 total sources were used in this systematic analysis.
Among the 18 sources, neuropsychological tests were administered along with observational and clinical studies to measure the effects of diet on brain cognition and function. Additionally, through qualitative studies, consumption of a Mediterranean diet was associated with increased cognitive function ($P = 0.003$). A Mediterranean-style diet was shown to have substantial changes in the gut microbiome and was correlated with a reduced risk for Alzheimer's and other neurodegenerative diseases ($P = 0.023$). In both human and animal models, the Mediterranean diet had increased effects on both global cognition ($P = 0.034$) and semantic memory ($P = 0.04$). A high fat and sugar intake associated with a Western Diet was shown to reduce hippocampal-dependent learning memory, impaired gluco-regulation, reduce levels of neurotrophins, and lead to neuroinflammation by disrupting the blood-brain barrier. A Western-style diet was associated with cognitive decline, risk for neurodegenerative diseases, and increased stress levels in both humans and animal models. Research conducted on Western and Mediterranean diets could provide further insight into the role diets play on neurodegenerative diseases and potentially lead to public health and clinical interventions for patients.

Disclosures: N. Bhimireddy: None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.16/K26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of Systemic Inflammation and Anticholinergics on Home-cage Behavior of Transgenic Alzheimer Mice

Authors: T. MEHTA[1], N. ANGULO[2], S. STATEN-LUSTY[2], R. PALKAR[2], V. IM[2], ***E. KIMCHI**[2];
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Abstract: Background: Understanding the interplay between Alzheimer's Disease (AD) pathology and systemic inflammation or cholinergic dysfunction is crucial for elucidating the mechanisms underlying acute disorders of cognition such as delirium. We therefore employed a multifaceted approach to investigate the effects of systemic inflammation and anticholinergics on multiple features of continuous home-cage behavior in a transgenic AD mouse model (5xFAD).
Methods: We examined how Locomotion, Rearing, Feeding, Drinking, and Nesting were affected by acute inflammatory or anticholinergic manipulations. We studied 5xFAD transgenic mice overexpressing amyloid and their wild-type (WT) siblings. We studied both female and male mice at two different ages: 4-7 months (n=36) and 8-10 months old (n=20). Systemic inflammation was induced via intraperitoneal injection of 0.5 mg/kg Lipopolysaccharide (LPS) from E.Coli, while on separate weeks anticholinergic effects were elicited using 2 mg/kg Scopolamine. We also performed control saline injections. Circadian influences were studied by injecting either during the active dark phase or resting light phase. Following injections, we recorded home-cage activity over 3 days using a novel intra-cage camera system that we recently developed (Camus).
Results: Preliminary analysis revealed that systemic inflammation suppressed spontaneous locomotion in both 5xFAD and WT mice, disrupting typical circadian rhythms and being more evident in dark periods. In contrast, scopolamine led to mild increases in spontaneous locomotion that were more evident in the light period. Analysis is ongoing to evaluate potential differential sensitivities between 5xFAD and WT mice to these manipulations.
Discussion: The effects of systemic inflammation and anticholinergic manipulations on spontaneous behavior in 5xFAD and WT mice may depend upon circadian influences. This reveals an opportunity to assess how these factors interact with the natural sleep-wake cycle, which is often disrupted in AD patients. This work may contribute to a more dynamic understanding of the behavioral manifestations of AD and associated changes in superimposed acute changes such as delirium.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.17/K27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R21AA029263-02

Title: Prenatal alcohol exposure impairs hippocampal spatial learning and impacts ciliary arterial resistance in middle aged transgenic F344-AD rats

Authors: *N. SAMIYA[1], S. BAKE[2], R. C. MIRANDA[3], F. SOHRABJI[4];
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Abstract: Prenatal alcohol exposure (PAE) can lead to cognitive deficits and increased depression related behaviors, which are early predictors of Alzheimer's Disease (AD). PAE may contribute to adverse cardiovascular and neurological health outcomes in adulthood, though we have limited research on the long-term effects on aging adults with FASD. Our previous studies related to PAE and stroke, a risk factor for dementia, have shown sex-dependent differences in behavioral assessment. We hypothesize that PAE in Fischer TgF344-AD rats will lead to sex-specific deficiencies and accelerate behavioral dysfunction, indicating premature aging.Wild-type (WT) Fischer F344 females were time mated with Fischer Transgenic (Tg) F344-AD males harboring the human Swedish mutation amyloid precursor protein (APP) and the presenilin-1 exon 9 deletion mutant (PS1). Pregnant dams were exposed to vapor ethanol or control air for 45 minutes daily (GD 11-16) to model consistent binge-like maternal exposure spanning the fetal neurogenic period. Offspring were tail sniped at 21 days and DNA extraction followed by PCR and gel electrophoresis for APP and PS1 confirmed transgenic and wildtype offspring. Offspring from both control and PAE groups underwent cognitive and depressive-like behavior testing. Barnes Maze was done at 10 months of age to assess cognition and spatial memory. At 11 months of age, social interaction test was done to assess depressive-like behavior. At 13 months of age, male and female offspring were assessed by high-resolution ultrasonography for blood flow parameters in the posterior ciliary arteries, which supply blood to most of the eye.Recall on Barnes Maze, assessed by time spent in the escape zone (during probe trial), was impaired in PAE Tg male and female rats. The Social Interaction test revealed significantly decreased time spent in the social chamber by PAE WT and Tg females compared to controls. PAE Tg offspring exhibited a significant increase in ciliary arterial acceleration (in mm/Sect. ^{2}, a measure of arterial resistance) compared to Tg controls, while there was a decrease in WT PAE ciliary

arterial acceleration compared to WT controls. PAE Tg positive offspring exhibited depressive-like behavior and impaired cognitive function, suggesting PAE may predispose offspring to develop AD associated pathology. PAE Tg positive offspring showed elevated arterial acceleration in the eye as adult offspring while PAE WT did not, suggesting changes in arterial resistance with AD. Understanding the effect of PAE on aging in a clinically relevant model for Alzheimer's Disease may allow for improved health management of people with FASD.

Disclosures: N. samiya: None. **S. Bake:** None. **R.C. Miranda:** None. **F. Sohrabji:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.18/K28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ministerio de Ciencia, Innovación y Universidades of Spain Grant RTI2018-095615-B-I00
Ministerio de Ciencia, Innovación y Universidades of Spain Grant PID2021-123865OB-I00
Comunidad de Madrid Grant S2017/BMD-3684

Title: Receptor Protein Tyrosine Phosphatase β/ζ regulates the effect of high fat diet-induced memory loss and the progression of amyloid plaques and neuroinflammation in a genetic model of Alzheimer's disease

Authors: T. FONTÁN-BASELGA[1], E. RIVERA-ILLADES[1], H. CAÑEQUE-RUFO[1,2], ***E. GRAMAGE**[1], J. ZAPICO RODRIGUEZ[2], B. DE PASCUAL-TERESA[2], M. VICENTE-RODRÍGUEZ[1], G. HERRADON[1];
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Abstract: Obesity is linked to neurodegenerative diseases through neuroinflammation. Pleiotrophin (PTN) is a cytokine that is upregulated in different neuroinflammatory disorders of diverse origin. PTN is a ligand and an endogenous inhibitor of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ. To investigate the role of RPTPβ/ζ in neuroinflammation and neurodegeneration, we used the APP/PS1 genetic model of Alzheimer disease (AD) and a high-fat diet (HFD)-induced obesity model. Three-month-old wild type (WT) mice were fed with standard chow or HFD (60 Kcal % fat) for three months. MY10, an exogenous RPTPβ/ζ inhibitor was administered intragastrically at different doses (60 mg/kg and 90 mg/kg). We observed that HFD produced long-term memory loss in the novel object recognition test and MY10 potentiated these effects on cognition, exacerbating short- and long-term memory loss. Moreover, MY10 treatment increased adult hippocampal neurogenesis, which was enhanced by

HFD, potentially contributing to HFD deleterious cognitive effects. These MY10 effects doesn't seem to be related to perineuronal nets intensity. In the APP/PS1 AD model, increased PTN levels in the brain were observed, suggesting that PTN overexpression takes part of a neurotrophic response in this genetic model of AD. To test this hypothesis, we studied the effect of MY10 in this model. Treatment with the higher dose of MY10 (90 mg/kg) significantly reduced the number of Aβ plaques in 8-10 month-old mice suggesting that RPTPβ/ζ plays an important role modulating Aβ plaque formation. In addition, we observed a significant decrease of astrocytes and microglia. Moreover, PTN expression was decreased with MY10 treatment. On the other hand, the analysis of mRNA proinflammatory markers such as *Il6, Il1b, Tnfa, Ptgs2, Cd68,* and *Hmgb1* revealed that the treatment with MY10 decreased their expression in a dose-dependent manner. Finally, we observed a differential regulation of genes implicated in eliminating protein aggregates such as *Mmp9, Bace1,* and *Ide* by MY10 administration.

Disclosures: T. Fontán-Baselga: None. **E. Rivera-Illades:** None. **H. Cañeque-Rufo:** None. **E. Gramage:** None. **J. Zapico Rodriguez:** None. **B. de Pascual-Teresa:** None. **M. Vicente-Rodríguez:** None. **G. Herradon:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.19/K29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U54 AG054345
NIH Grant R01 AG074566

Title: Impact of a High-Fat Diet on Neurodegenerative Disease Progression in the LOAD2.Plcg2M28L Mice: A Multi-Modal Analysis

Authors: C. RANGEL-BARAJAS[1], C. LLOYD[2], P. R. TERRITO[3], S. J. SUKOFF RIZZO[4], G. W. CARTER[5], M. SASNER[5], G. R. HOWELL[5], *B. LAMB[6], A. OBLAK[6];
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Abstract: Alzheimer's disease (AD) and related neurodegenerative conditions are characterized by a potent microglial-mediated immune response. Genetic investigations have identified microglial gene variants associated with AD risk. Phospholipase C gamma 2 (PLCγ2) emerged from genome-wide association studies (GWAS) as a novel AD risk gene. Subsequent in vitro, in vivo, and clinical studies have reinforced this link, positioning Plcg2 as a significant contributor to AD, housing common and rare risk variants with substantial effects on disease susceptibility.To assess the impact of Plcg2 gene expression on microglial function and disease

progression, we developed Plcg2M28L mice on an APOE4.Trem2R47H.hAβ (LOAD2) background (LOAD2.Plcg2M28L). Mice were fed a high-fat diet (HFD) from 2 months of age and monitored longitudinally until 18 months. Imaging and biomarker analyses were conducted at 4, 12, and 18 months, with additional cross-sectional analyses at 4, 12, 18, and 24 months. Post-mortem evaluations encompassed blood chemistry, immunohistochemistry, and molecular assessments, including transcriptomics and proteomics on brain tissue.Our comprehensive analysis revealed correlations between mouse and human LOAD phenotypes. At 18 months, male mice exhibited age-dependent microgliosis. Notably, HFD induced microgliosis in both male and female mice within the cortex, while in the subiculum, IBA1 expression increased solely in females.Our findings underscore the pivotal role of Plcg2 in LOAD, demonstrating that incorporating Plcg2M28L into a sensitized LOAD model (LOAD2) better recapitulates human AD outcomes in mice.

Disclosures: C. Rangel-Barajas: None. **C. Lloyd:** None. **P.R. Territo:** None. **S.J. Sukoff Rizzo:** None. **G.W. Carter:** None. **M. Sasner:** None. **G.R. Howell:** None. **B. Lamb:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Founder, Monument Biosciences. F. Consulting Fees (e.g., advisory boards); NervGen, Inc., SAB, Cleveland Clinic, SAB, Alzheimer's Association, MSAG. **A. Oblak:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.20/K30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Kavli Institute for Neuroscience at Yale University, Postdoctoral Fellowship to LaShae Nicholson

Title: Insulin resistance induced by high fat diet exacerbates Alzheimer’s phenotypes in knock-in mice with specific alterations in cortical inhibitory neurons and microglia

Authors: *L. K. NICHOLSON, T. KARRA, S. TANG, S. M. STRITTMATTER; Neurosci., Yale Univ., New Haven, CT

Abstract: Dysregulation in glucose metabolism has been implicated in Alzheimer’s Disease risk and progression of cognitive decline. The molecular and cellular mechanisms linking metabolic dysfunction and AD manifestations are poorly understood. Using WT (wild type) and AD (double homozygous knock-in AppNL−G−F /$Mapt^{hMAPT}$, dKI) mice, we studied the effect of brain hyperglycemia in two diabetic mouse models representing distinct mechanisms of glucose dysregulation. One group of mice was treated with and without 40 mg/Kg Streptozotocin (STZ) for 5 consecutive days at 8 months of age (n=25-30 mice per group). STZ treatment is known to result in glucose intolerance through the destruction of pancreatic beta cells, leading to reduced

insulin production. Alternatively, insulin resistance was induced in mice with a sustained high-fat diet (HFD). Starting at 3-4 months of age, a second cohort of mice received an ad libitum control diet (CtrD, ResearchDiets #D12450K) or high-fat diet (HFD, ResearchDiets #D12492) (n=15-20 mice per group). Both STZ and HFD mouse cohorts were aged to 10 months with confirmed hyperglycemia and then assessed for behavioral defects using Morris water maze. The AD-HFD, but not STZ-treated AD mice, exhibited exacerbated memory and learning impairments when compared to their respective WT and AD controls. Thus, brain metabolic state, rather than hyperglycemia alone, is associated with increased cognitive impairment in AD mice. Transcriptomic analysis of WT and AD mice, with and without HFD, revealed a prominent alteration of gene expression in a sub-population of Layer 2 (L2) inhibitory neurons. This population of L2 inhibitory neurons showed an HFD-induced increase in Meis2 expression and an AD plus HFD increase in Tshz1 expression. In addition, L2/3 excitatory neurons also displayed altered gene expression profiles between the same treatment groups. The transcriptomic analyses also revealed an AD-induced metabolic shift in microglia, with specific gene expression increases in AD-HFD mice that included Nrg3. The immunohistological distribution of Trem2 on activated microglia was altered in AD-HFD mice compared to AD mice, most possibly due to increased proteolytic processing. Nrg3 levels were also only increased in AD-HFD mice, coinciding with increased loss of Synapsin-PSD95^{+} puncta in AD-HFD as compared to AD mice in the cerebral cortex but not the hippocampus. These results show that brain metabolic states alter the pathological progression of AD with differential regional impacts on neuronal circuitry.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.21/K31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1F31AG076332-01A1
PF-SF-JFA-830658
1R01ES032440-01A1
ASAP-020527

Title: Alzheimer’S disease-associated gut microbes shape neuroinflammatory tone and disease outcomes

Authors: *L. BLACKMER-RAYNOLDS[1], M. M. SAMPSON[2], A. M. HAMILTON[3], A. YANG[1], A. KOZLOV[1], J. CHANG[4], S. KELLY[5], S. A. SLOAN[2], T. SAMPSON[6]; [1]Emory Univ., Atlanta, GA; [2]Human Genet., Emory Univ., Atlanta, GA; [3]Physiol., Emory Univ.,

Atlanta, GA; [4]Emory Univ. Sch. of Med., Atlanta, GA; [5]Emory Univ., Altanta, GA; [6]Cell Biol., Emory Univ., Atlanta, GA

Abstract: Changes in neuroinflammatory tone have been shown to modulate neuroimmune responses to Alzheimer's disease (AD) pathology and shape disease outcomes, however, extrinsic factors that modify neuroimmune activation remain poorly understood. The gut microbiome is one such factor, with the ability to shape peripheral and central immune activation, as well as AD pathologies. AD patients display unique changes in microbiome composition, however, the links between specific AD-associated gut bacteria, neuroinflammatory tone, and AD outcomes remain to be elucidated. To identify AD-associated bacteria that modify neuroinflammatory tone, wildtype germ-free mice were mono-colonized with type strains of bacteria species of interest (*Escherichia coli, Bacteroides thetaiotaomicron, Clostridium celatum, and Lactobacillus johnsonii*) for two weeks. AD-associated bacteria induced bacteria- and sex-specific changes in cytokine levels as well as myeloid cell gene expression within the brains of mono-colonized mice. In particular, *E. coli* was shown to induce a distinct, AD-associated neuroinflammatory phenotype that was characterized by increased MHC II antigen presentation as well as changes in boarder associated macrophage (BAM) and endothelial cell gene expression. Therefore, it was hypothesized that *E. coli* would exacerbate AD outcomes by modifying neuroinflammatory response to amyloid pathology. Indeed, exposure of conventional 5xFAD mice to *E. coli* via oral gavage for one month significantly accelerated the development of cognitive impairment. Further, *E. coli* exposed 5xFAD mice displayed altered neuroinflammation, with changes in both cytokine levels and myeloid cell gene expression. Together, these results highlight the unique neuroimmune modulatory potential of AD-associated gut bacteria. In particular, the present study demonstrates how increased intestinal exposure to non-pathogenic *E. coli* is sufficient to modify neuroinflammatory tone, cognition, and pathology in 5xFAD mice, highlighting the potential importance of this microbe for AD.

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Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.22/K32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CONAHCYT Grant 916176

Title: Identification of neurodegeneration markers of Alzheimer's disease present in the hippocampus of rats with Metabolic Syndrome

Authors: *E. FUENTES, A. DIAZ;
Univ. AUTONOMUS OF PUEBLA, Puebla, Mexico

Abstract: Currently, Alzheimer's disease (AD) is recognized by the World Health Organization as a public health problem due to the alarming prevalence worldwide with nearly 45 million cases and being the main cause of death in some european countries. Epidemiological studies predict that in the year 2050 the number of cases will triple and the risk of development at an early age will increase. On the other hand, reports indicate that in Mexico there are close to 1 million cases and the incidence is 27.3 (1000 adults/year). AD represents the most common form of dementia and is a neurodegenerative disorder associated with a progressive decline in cognitive functions and changes in personality that affect the quality of life of those who suffer from the disease. In recent years, a close relationship between ECDiD and the risk of AD has been proposed. Recent studies have shown the presence of neurofibrillary aggregations and neurodegenerative processes in patients with peripheral metabolic alterations (insulin resistance, dyslipidemia, among others) as occurs in type 2 diabetes. We worked with Male Wistar rats, they were randomly divided into four groups: Normocaloric diet (NCD), Hypercaloric-hyperglucid diet (HCD), Aβ 1-42 group and Hypercaloric-hyperglucid diet and Aβ 1-42. The diets were administered for 6 months ad libitum. The weight, size, abdominal diameter, body mass index and fat percentage were recorded weekly. Cellular deterioration was evaluated using the hematoxylin and eosin technique. The concentration of insulin and beta amyloid in the hippocampus was determined using the ELISA technique and the identification of protein deposits was carried out by impregnation with aminocupric silver. The results showed that the metabolic syndrome induced by the chronic intake of a hyperglycid diet does not modify the weight-height relationship, but it does modify the body composition, mainly favoring a loss of fat-free mass. Furthermore, it promotes hippocampal cellular damage similar to that caused by the presence of Aβ 1-42, characterized by alteration in cellular morphology and secondary lesions, and induces cerebral hyperinsulinemia, which represents a risk factor for the progression and severity of AD. Finally, it was identified that the chronic intake of hyperglycid diets intensifies the neurodegenerative response when AD is already present.

Disclosures: E. Fuentes: None. **A. Diaz:** A. Employment/Salary (full or part-time):; University Autonomus of Puebla, Research professor.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.23/K33

Topic: B.09. Glial Mechanisms

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NeuroNetwork for Emerging Therapies
NeuroNetwork for Emerging Therapies Tauber Family Student Internship

Title: Adipose-microglia crosstalk through adipose tissue extracellular vesicles: effect of obesity and prediabetes on CNS inflammation

Authors: *A. ALLOUCH[1,2], S. ELZINGA[1,2], M. NOURELDEIN[1,2], F. MENDELSON[1,2], J. HAYES[1,2], D. RIGAN[1,2], M. SAVELIEFF[2,3], J. HUR[2,3], K. GUO[4,2], Y. ZOU[5], E. L. FELDMAN[1,2];
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Abstract: Obesity, prediabetes, and diabetes induce adipose tissue inflammation, which is thought to be involved in the pathogenesis of many diabetic complications, including those of the CNS (central nervous system), such as cognitive impairment. Adipose tissue inflammation is also associated with increased production and release of adipose tissue-derived extracellular vesicles (ATEVs), which can induce insulin resistance and inflammation in neighboring and distant tissues. Furthermore, ATEVs can cross the blood-brain barrier and promote CNS inflammation possibly by activating microglia, the primary immune cells of the CNS. However, inflammatory mechanisms underlying this potential adipose-microglia crosstalk are unclear. As miRNAs are suggested mediators of peripheral and CNS inflammation, our goal was to assess effects of acute, intermediate, and chronic obesity and prediabetes on the miRNA content of ATEVs and inflammatory gene expression profiles of microglia. We induced obesity/prediabetes by feeding 5-week-old male C57BL/6 mice either standard diet (SD) or high fat diet (HFD) for 2 weeks, 1 month, or 3 months. At terminal, we isolated extracellular vesicles (n=6/group) from white epididymal adipose tissue. We confirmed the size and concentration of ATEVs via nanoparticle tracking analysis and analyzed ATEV miRNA content via NanoString nCounter. In parallel, single-cell RNA sequencing on hippocampal microglia (n=6/group) was used to assess changes in inflammatory gene expression. This allowed us to map associations between changes in ATEV miRNA expression to changes in microglia inflammatory gene expression. ATEVs from high-fat diet mice vs SD controls caused upregulation of NFκB in a human microglial cell line. We found that ATEV production was increased in white adipose tissue in HFD mice. Microglia of animals fed HFD for 1 month showed an early signature of endoplasmic reticulum and ribosome dysregulation, which shifted to an inflammatory signature after 3 months of feeding. These data indicate that HFD alters ATEV miRNAs, including increasing miRNA content that may signal to microglia and disrupt their inflammatory response in a time-dependent manner, further implicating adipose-microglia crosstalk as a potential mechanism by which peripheral inflammation spreads to the CNS.

Disclosures: A. Allouch: None. **S. Elzinga:** None. **M. Noureldein:** None. **F. Mendelson:** None. **J. Hayes:** None. **D. Rigan:** None. **M. Savelieff:** None. **J. Hur:** None. **K. Guo:** None. **Y. Zou:** None. **E.L. Feldman:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.24/K34

Topic: B.09. Glial Mechanisms

Support: Gatsby Charitable Foundation
NINDS R01NS092597
NIH DP1NS111132
HHMI Emerging Pathogens Initiative

Title: Early Life Respiratory Immune Challenges and Altered Brain Function

Authors: *K. MALACON[1], K. SHAMARDANI[2], J. KOCHALKA[3], M. MONJE[1];
[1]Neurol., Stanford Univ., Stanford, CA; [2]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [3]Stanford Univ., San Francisco, CA

Abstract: Nearly 6 million children in the United States are grappling with long COVID, and among them, between 2-44% experience symptoms of cognitive impairment, often described as “brain fog.” Such systemic inflammatory challenges, such as COVID or influenza A, can result in long-lasting dysregulation of neural cells and circuits. To investigate the neuroimmune and cognitive effects of respiratory immune challenges during early postnatal life, juvenile mice were exposed to respiratory immune challenges at P14: intratracheal gram-negative bacterial mimetic LPS or infected with influenza modeling mild infection or severe infection. Respiratory immune challenges, delivered at P14, resulted in impaired cognitive behavioral 4 weeks later at 6 weeks of age. Social behavior was unaffected. One week after respiratory immune challenge, intratracheal LPS resulted in white matter and cortical microglial reactivity and a loss of oligodendrocyte precursor cells (OPCs) in the white matter. Consistent with the elevated severity of symptoms and risk of mortality observed in human children with influenza, a well-tolerated “dose” of H1N1 influenza resulted in much more severe disease in young mice. In this severe influenza model, white matter microglial reactivity was markedly increased, and both OPCs and oligodendrocytes were decreased in subcortical white matter at day 7 following influenza infection. Juvenile mice with mild influenza exhibited white matter microglial reactivity and loss of oligodendrocytes at 7 days, prominent but less severe than in mice with severe influenza. Single nucleus RNA sequencing (snRNA-seq) was performed in this mild influenza model at this juvenile age, and initial results from 6 mice and ~60,000 single nuclei indicate inflammatory changes in microglia, astrocytes and oligodendrocytes. These results provide valuable insights into the neuroimmune response to respiratory immune challenges during critical developmental

stages, shedding light on potential mechanisms underlying cognitive impairment and suggesting avenues for targeted interventions in pediatric populations.

Disclosures: K. Malacon: None. **K. Shamardani:** None. **J. Kochalka:** None. **M. Monje:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Michelle Monje holds equity in Maplight Therapeutics..

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.25/L1

Topic: F.04. Neuroimmunology and Neurovirology

Title: Olfactory bulb neuroimmune responses to an intranasal virus-like challenge are primed by time of day

Authors: *G. PEARSON, B. FALCY, J. WANG, S. GOTTWALS, N. SANTOS, G. DENAROSO, S. AKLI, I. N. KARATSOREOS;
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Abstract: PURPOSE. Neuroimmune responses are critical for survival. This is particularly evident with neurotropic (brain-targeting) pathogens, in which impaired immune signaling following infection results in severe neuropathology and death. We previously found that the severity of neurotropic virus infection is impacted by time of day of infection. However, the mechanism by which time of day modulates this survival outcome remains unknown. We have previously demonstrated that the olfactory bulb (OB), a site of neurotropic virus entry into the brain, rhythmically expresses neuroinflammation-related transcripts. These rhythmically expressed transcripts are enriched in genes associated with functional aspects of microglia. We also found that antiviral-related transcripts are upregulated in the OB at active phase onset, a time of day associated with enhanced survival following neurotropic virus infection. Here, we tested the hypothesis that time of day primes the OB to differentially respond to an intranasal virus-like challenge. METHODS. For Experiment 1, we intranasally challenged male mice (age = 15 weeks) at resting phase onset (ZT0) or active phase onset (ZT12) with vehicle or poly(I:C) and collected tissues at 0-, 3-, 12-, and 24-hours post-inoculation (n = 4 mice/inoculation ZT/collection time, total n = 32 mice). OB transcriptional responses were measured using NanoString technology. For Experiment 2, we intranasally challenged male mice (age = 13 weeks) with vehicle or poly(I:C) at ZT0 or ZT12 (n = 4 mice/inoculation ZT/treatment, total n = 16 mice). We then isolated OB microglia at 24 hours post-inoculation and used imaging flow cytometry to analyze a population of cells characteristic of microglia. RESULTS. For Experiment 1, we found that intranasal poly(I:C) induced antiviral responses in the OB and that these responses unfolded more rapidly in mice challenged at ZT12 compared to ZT0. For Experiment 2, we found that time of day altered the number of OB microglia independent of

treatment, with more OB microglia at ZT12 than ZT0. Surprisingly, we also observed a high proportion of microglia that contained intrinsically fluorescent puncta. The number of intrinsically fluorescent microglia were reduced following intranasal poly(I:C) at both ZT0 and ZT12, but this reduction was enhanced at ZT12. CONCLUSIONS. Time of day primes the OB to mount differential antiviral and microglial responses to intranasal virus-like stimuli, which may provide an antiviral gating mechanism underlying differential susceptibility to neurotropic virus exposure via the nasal route.

Disclosures: G. Pearson: None. **B. Falcy:** None. **J. Wang:** None. **S. Gottwals:** None. **N. Santos:** None. **G. Denaroso:** None. **S. Akli:** None. **I.N. Karatsoreos:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.26/L2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AARF-21-850265
P20GM113123
U54GM128729

Title: Alzheimer's disease-associated pathological changes in the lungs of humans and the App^{NL-G-F} mouse model

Authors: *B. SAHU[1], S. NOOKALA[2], A. M. FLODEN[2], N. AMBHORE[3], S. VENKATACHALEM[3], C. K. COMBS[4];
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Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by brain accumulation of amyloid beta (Aβ) plaques, neurofibrillary tangles, neuron death, robust gliosis, and neuroinflammation, affecting around 55 million individuals worldwide. It is well known that brain immune changes are a component of the disease, but immune dysfunction in secondary organs has also been reported. To evaluate peripheral immune changes in AD, we examined the potential presence of lung dysfunction, as well as associated histological and immune changes, during the progression of the disease. Male and female C57BL/6 wild type and App^{NL-G-F} mice (8-9 months old) were sacrificed, and broncho-alveolar lavage fluid (BALF) and lungs were collected. The BALF cell pellet was used to quantify immune cell phenotype by flow cytometry, and the BALF supernatant was used to assess Aβ levels, cytokine concentrations, total protein content, LDH activity, and total IgE levels. Lungs were sectioned and stained with hematoxylin and eosin, Alcian blue, and Masson's trichrome. In addition, lung sections from wild-type and App^{NL-G-F} mice and human AD patients and healthy controls were immunostained

for APP, Aβ, and claudin 4. AD mice showed significantly increased pulmonary airway thickening and mucus and collagen production as indicated by hematoxylin and eosin, Alcian blue, and Masson's trichrome staining, respectively, compared to the wild-type controls. Female but not male *App*$^{NL-G-F}$ mice also demonstrated reduced B cell and neutrophil numbers and elevated IL-1β in their BALF compared to controls. Although there were no differences in BALF supernatant total protein content and LDH activity between wild-type and *App*$^{NL-G-F}$ mice, lungs from both sexes of *App*$^{NL-G-F}$ mice had decreased claudin-4 immunoreactivity, suggesting altered epithelial permeability. Additionally, male and female *App*$^{NL-G-F}$ mice lungs showed a significant increase in the total IgE level. BALF supernatant from male and female *App*$^{NL-G-F}$ mice also contained soluble Aβ1-40 and Aβ1-42 correlating with lung APP immunoreactivity. Human AD male and female lungs had reduced claudin-4 immunoreactivity and increased APP and Aβ staining compared to controls, verifying lung changes are also present in human disease. Similar to the female mice, human AD females but not males demonstrated numerous cytokine changes compared to control lungs. These data demonstrate disease-associated changes in the lungs of both human AD patients and the *App*$^{NL-G-F}$ mouse model. Further characterization of lung dysfunction and its contribution to brain changes may define a novel lung-to-brain contribution to disease.

Disclosures: B. Sahu: None. **S. Nookala:** None. **A.M. Floden:** None. **N. Ambhore:** None. **S. Venkatachalem:** None. **C.K. Combs:** None.

Poster

PSTR233: Brain-Body Interactions Regulating Inflammatory Signaling

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR233.27/L3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01DA055523

Title: Oral inoculation of Actinomycin meyeri induces neuropathologies associated with the early stage of Alzheimer’s Disease

Authors: *T. SALMAN[1], A. HAZZARD[2], D. JOHNSON[1], S. FITTING[3], W. JIANG[4]; [1]Microbiology and Immunol., [2]Med. Univ. of South Carolina, Charleston, SC; [3]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [4]Microbiology and Immunol., Med. Univ. of South Carolina (MU Neurosci. Inst. - Grad., Charleston, SC

Abstract: Several oral microbiomes (i.e., *Porphyromonas gingivalis*) can induce neuropathologies associated with Alzheimer’s disease (Dominy, Lynch et al. 2019). To date, there are very limited studies on cannabis-use altered oral microbiome as well as its impact on neuropathogenesis. Our laboratory reported that chronic cannabis smoking exhibited oral microbial dysbiosis and enriched *Actinomyces meyeri* compared to non-cannabis smokers (Luo, Z et al. 2021), the abundance of *A. meyeri* in saliva was inversely correlated with the first age of

cannabis use. Here, we investigated the impact of *A. meyeri* on neuropathology in B6 mice. The mice were orally inoculated with *A. meyeri* (Am), *A. odontolyticus* (Ao), and *N. elongata* (Ne) (5x10^7 CFU/time) twice/week for 6 months. At the end of the study, brain tissues were analyzed for Amyloid beta Precursor Protein (APP) and amyloid beta (Aβ)42 using Western, Meso Scale Discovery kit, and qPCR. Brain CD11b+ cells were isolated using MACS after removing myelin and analyzed CD206, iNOS, and Dectin-1 using flow cytometry. To investigate the mechanisms of oral *A. meyeri*-induced neuropathology, RNAScope in situ hybridization was applied with *A. meyeri* 16S RNAscope probe, Iba1, and TMEM119. The percentages of CD11b+ cells expressing CD206+ (median±SD, 2.73±2.02, 6.10±1.32, and 5.84±1.27), iNOS+ (0.12±0.02, 0.06±0.04, and 0.03±0.04), and Dectin-1+ (0.37±0.08, 0.14±0.04, and 0.15±0.04) for administration of Am, Ao, and Ne respectively. Am increased M1 and decreased M2-like myeloid cell activation vs. control groups ($P<0.05$, ANOVA). By using RNAScope in-situ hybridization, we determined the absence of *A. meyeri* translocation using a unique 16S RNAScope probe (n=4) and the non-region-specific activation of resident microglia (Iba1+) but not infiltrated macrophages (all Iba1+ cells were TMEM119+, a distinguishing marker of macrophage [negative] to microglia [positive]) in the brain (n=4). Notably, the percentage of increased expression of APP vs. PBS control was 28.37% ± 1.06 by qPCR and 32.3% ± 4.56 by western blot, and the percentage of increased Aβ42 protein production in the mice brain was 29.68% ± 6.94 (n=6) by the MSD kit. These findings reveal that chronic cannabis smoking-enriched *A. meyeri* in the oral cavity can play a significant role in the neurodegenerative process observed in AD. As the project progresses, we expect to find a novel mechanism of altered oral microbiome capable of leading to accelerated Aβ deposition through targeted genes and pathways in activated microglia using single-cell-RNASeq and specific bacterial products (i.e., metabolites) that could translocate to the brain and contribute to neuropathogenesis.

Disclosures: T. Salman: None. **A. Hazzard:** None. **D. Johnson:** None. **S. Fitting:** None. **W. Jiang:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.01/L4

Topic: D.04. Interoception

Title: Delineating In-Vivo T1-Weighted Intensity Profiles Within The Human Insula Cortex Using 7-Tesla MRI

Authors: *C. DALBY[1], A. DIBBLE[2], J. CARVALHEIRO[3], F. QUEIRAZZA[4], M. SEVEGNANI[5], M. HARVEY[6], M. SVANERA[6], A. FRACASSO[3];
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Glasgow, Glasgow, United Kingdom; [5]Sch. of Computing Sci., Univ. of Glasgow, Glasgow, United Kingdom; [6]The Ctr. for Cognitive Neuroimaging (CCNi), Glasgow, United Kingdom

Abstract: The integral role of the insula cortex in sensory and cognitive function has been well documented in humans, and fine anatomical details characterizing the insula have been extensively investigated ex-vivo in both human and non-human primates. However, in-vivo studies of insula anatomy in humans (in general), and within-insula parcellation (in particular) have been relatively limited. The current study leverages 7-tesla magnetic resonance imaging to delineate T1-weighted intensity profiles within the human cortex, serving as an indirect proxy of myelination. Our analysis revealed two separate clusters of relatively high and low signal intensity across the insula cortex located in three distinct cortical locations within the posterior, anterior, and middle insula. The posterior and anterior cortical locations are characterised by elevated T1-weighted signal intensities, contrasting with lower intensity observed in the middle insular cortical location. Importantly, the detection of the high T1-weighted anterior cluster is determined by the choice of brain atlas employed to define the insular ROI. Our results are robust at both individual and group levels, across two separate cohorts acquired in two separate sites (n1 = 21, Glasgow, UK; n2 = 101, Amsterdam, NL). These results reflect new insights into the insula anatomical structure, in-vivo, while highlighting the use of 7-tesla in neuroimaging. Specifically, the current study also paves the way to study within-insula parcellation at 7T and above, with further implications for individualized medicine approaches and their potential clinical applications.

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Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.02/L5

Topic: D.04. Interoception

Support: EIPOD4 fellowship

Title: The representation of PGE2-triggered sickness in the mouse insular cortex

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Abstract: During infections, mammals develop stereotypic symptoms such as fever, reduced appetite, warmth-seeking, and lethargy. These drastic changes in behavior and physiology are collectively known as sickness syndrome. The sickness syndrome is triggered by immune

mediators produced during systemic inflammation. Among the best-studied mediators of sickness is prostaglandin E2 (PGE2). Neurons can detect PGE2 through specific G protein-coupled receptors, and when injected into the brain, PGE2 can induce a sick-like state. While the role in sickness of diverse brain areas, including hypothalamic and brainstem nuclei, has been exquisitely dissected, the contribution of the cortex to this process remains less well defined. To expand our understanding of the cortical control of sickness, we combine whole-brain activity mapping, Neuropixel recordings in head-fix mice, and analysis of freely behaving mice undergoing PGE2-triggered sickness. So far, our data shows that PGE2 activates multiple brain areas previously associated with sickness, including brainstem and hypothalamic regions. Interestingly, the most significant activation at the cortical level is found within the insular cortex (IC), an area with a pivotal role in representing the body's internal state. Furthermore, preliminary in vivo neuronal recordings analysis from the IC points to a distinctive transition of ongoing neuronal activity during sickness. We envision that further analysis of our in vivo data will contribute to a better understanding of the involvement of the IC in sickness.

Disclosures: G.B. Kamm: None. **J.C. Boffi:** None. **M. Abd El Hay:** None. **D. Rajot:** None. **M.N. Havenith:** None. **M.L. Schölvinck:** None. **N. Renier:** None. **H. Asari:** None. **C.T. Gross:** None. **R. Prevedel:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.03/L6

Topic: D.04. Interoception

Title: Pseudotrial correction to control for confounding effects on heartbeat-evoked potentials during tasks

Authors: *T. STEINFATH[1,2], C. SANDER[3,4], V. V. NIKULIN[1], A. VILLRINGER[1,5,6];
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Abstract: Heartbeat-evoked potentials (HEP) refer to the brain's response to one's own heartbeat, reflecting its monitoring of cardiovascular function. Previous studies have shown that HEPs are sensitive to the focus of attention, with increased amplitudes associated with interoceptive focus, and reduced amplitudes associated with focus on external stimuli. In this context, investigating HEPs during tasks offers a valuable way to explore how processing of interoceptive stimuli is integrated with exteroceptive task-relevant activity.
However, this approach comes with a challenge, as task-related activity can overlap with HEPs, potentially leading to spurious results. To address this issue, we introduce a pseudotrial

correction procedure that subtracts an artifact template from the HEPs to control for heartbeat unrelated processes. We demonstrate its effectiveness in removing artifacts in a large EEG dataset of 1782 subjects who performed an auditory oddball task. Based on previous literature, we hypothesized that lower pre-stimulus HEP amplitudes before trials will be associated with greater stimulus evoked P300 amplitude, and faster reaction times (RT). We categorized target trials into high & low P300 amplitude as well as fast & slow RT conditions, and compared pre-stimulus HEPs between these conditions.
Before pseudotrial correction, we observed the hypothesized inverse relationship of pre-stimulus HEP amplitudes with P300 amplitudes ($p \leq 0.001$) as well as reaction times ($p \leq 0.001$). However, after employing the pseudotrial correction procedure, equivalence tests indicated the absence of meaningful differences between pre-stimulus HEPs sorted by P300 ($t1782 = -7.8$, $p < 0.01$) or RT ($t1782 = -7.5$, $p < 0.01$). This observation was substantiated by a surrogate heartbeat procedure we performed, which is based on shuffling R-peak onsets 1000 times and repeating the statistics with each randomization. Since original HEP effects were not greater than effects observed in the surrogate heartbeat data, we can conclude that no genuine HEP difference was present for P300 or RT sorted data (P300: $p = 0.39$, RT: $p = 0.09$).
Lastly, we show in simulated data that the application of a pseudotrial correction has the potential to uncover genuine HEP effects which might remain undetected in uncorrected data. In sum, our study provides a novel solution to overcome issues associated with the overlap between task-evoked activity and HEPs, and gives recommendations for future studies to avoid potential pitfalls when examining HEPs.

Disclosures: T. Steinfath: A. Employment/Salary (full or part-time):; Max Planck Institute for Human Cognitive and Brain Sciences. **C. Sander:** A. Employment/Salary (full or part-time):; Department of Psychiatry and Psychotherapy, University Hospital Leipzig, Leipzig, Saxony, Germany. **V.V. Nikulin:** A. Employment/Salary (full or part-time):; Max Planck Institute for Human Cognitive and Brain Sciences. **A. Villringer:** A. Employment/Salary (full or part-time):; Max Planck Institute for Human Cognitive and Brain Sciences.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.04/L7

Topic: D.04. Interoception

Support: NIH Grant AT011665

Title: Resting State Interoception Network in Rats

Authors: *F. ALKAABI[1,2], X. WANG[3], Z. LIU[3];
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Abstract: Even at rest, the body's internal environment stays active. The brain regulates organs through ongoing interoceptive processes via connected brain regions. It's unclear how these regions connect and interact for interoceptive functions. To this end, we used fMRI to probe the rat brain's interoception network. A 7T small animal MRI system (Bruker) scanned the brains of nine female Fischer rats initially anesthetized with 5% Iso/O2, maintained with 0.5% Iso/O2 and dex (0.015 mg/Kg/h). Body temperature was kept at 37°C. Head motion was minimized. Sessions with excessive motion were excluded. Each rat had 600 sec of data recorded. Processing utilized afni, fsl, and custom MATLAB scripts, with images registered to a standard template. Seed based correlation maps (SBC) were generated by averaging BOLD signals across regions of interest (ROI) and computing Pearson correlations. ROIs were chosen based on prior work linking them to interoception. Overlap scores were determined by voxel frequency across multiple SBCs.The following ROIs were used as seeds and were found to be part of the network: Insular (IC), Cingulate (Cg), Pre/Infra-limbic (PrL/IL), Primary Somatosensory (S1), Primary Motor (M1), Orbitofrontal (OFC) cortices, Thalamus (Thal), Amygdala (Amy), Striatum (Stria), Hippocampal Formation (Hipp), Cerebellum (Cere). The hypothalamus (Hypo) appeared to be functionally connected to Cg only. Other regions emerged as part of the network included both the Retrosplenial Dysgranular and Granular (RSD and RSG), the Primary and Secondary Visual (V1 and V2), the Primary and Secondary Auditory (Au1 and Au2), the Ectorhinal (EcT), both the Temporal and Lateral Parietal Association (TeA and LPtA), the Secondary Motor and Somatosensory (M2 and S2) Cortices, Corpus Callosum (CC), Fimbria, and the Midbrain (MB). Our analysis revealed the distributed nature of the interoception network and offered insights into the system's functional organization, prompting further exploration of its relationship with internal bodily signals.

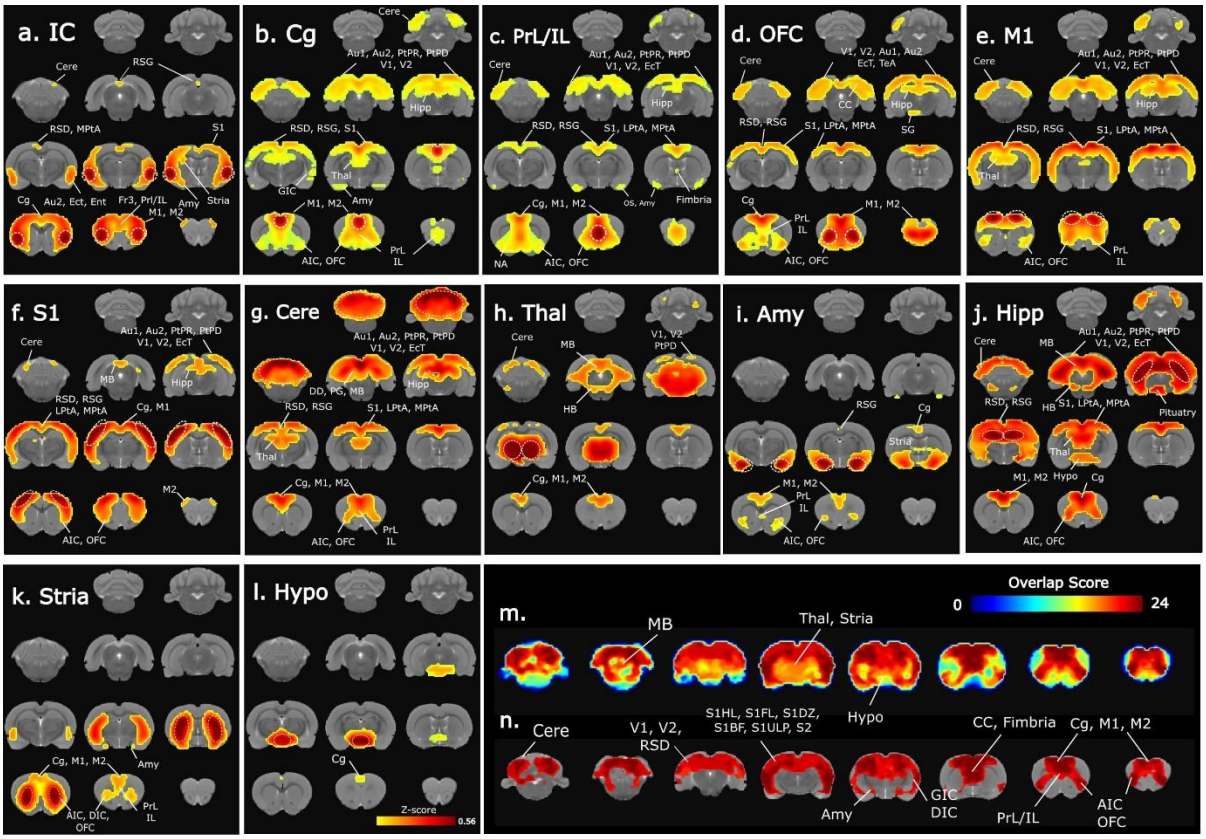

Figure 1: a-l Seed-based correlation maps depicting functional connectivity with the color bar representing z-scores. ROI in each map is delineated by circumscribing circles. ($p<0.01$, z threshold = 0.15). m and n: A heat map of the brain displays labeled regions along with their respective overlap scores. No threshold was applied to m, n was thresholded at 20. Regions with the highest scores were identified, with scores ranging from 0 (no connection to any ROI) to 24 (connected to all other ROIs).

Disclosures: F. Alkaabi: None. **X. Wang:** None. **Z. Liu:** None.

Poster

PSTR234: Central Pathways for Interoception

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Program #/Poster #: PSTR234.05/L8

Topic: D.04. Interoception

Support: NIH AG067153
UCI Susan Samueli Integrative Health Institute (SSIHI) Pilot award
Samueli Scholar award

Title: Single-cell transcriptomics reveals brainstem molecular maps of changes in response to electroacupuncture

Authors: *L.-Q. TONG[1], Z.-L. GUO[2], H. ZHANG[3], Z. TAN[1], X. XU[4];
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Abstract: Acupuncture has been employed as a therapeutic intervention for various diseases, including hypertension. However, the molecular mechanisms underlying the therapeutic benefits of acupuncture remain unclear. Here, we employed single-cell RNA sequencing to measure changes in gene expression, transcription regulatory network and intercellular communications induced by electroacupuncture (EA) in distinct cell types in the mouse brainstem. We conducted EA at the Neiguan (P6) acupoint, which serves as a model for studying how EA regulates cardiovascular function. Brainstem samples from two groups of mice, sham-EA control and EA (30 minutes treatment) animals, were collected for single-cell RNA-sequencing. Each group consisted of 6 male mice that were 3 months old. Exposure to EA led to differential expression of specific genes and alternations of biological signaling pathways in different cell types. Through evaluating the integrated signal of immediate early gene activation, we found that neural activity-associated expression of immediate early genes was decreased in GABAergic neurons but was increased in astrocytes. We performed single-cell regulatory network analysis and identified top regulons in major cell types. We further analyzed the intercellular communication by studying ligands-receptors interaction and found alterations in distinct signaling pathways across various cell types. Altogether, our findings provide a comprehensive view of the effects of acupuncture on transcriptomes of distinct cell types in the brainstem.

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Poster

PSTR234: Central Pathways for Interoception

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Topic: D.04. Interoception

Support: Fondecyt 1211359
Fondecyt 1210940

Title: Demyelination of prebötc neurons reduces the ventilatory response induced by hypercapnia

Authors: R. PULGAR[1], *J. EUGENIN[2], F. C. ORTIZ[3];
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Abstract: Central respiratory chemoreception is fundamental for the generation and modulation of the respiratory rhythm, allowing breathing to be adjusted to the physiological demands by activation of multiples interconnected nuclei in the brainstem. One of these nuclei is the pre-Bötzinger complex (preBötC), a small nucleus that is critical for the generation of inspiration. On

the other hand, myelin, a specialized membrane produced by oligodendrocytes that enwraps axons in the CNS, has been proposed as contributor to the regulation of neural excitability. However, myelin functional impact in the respiratory neural circuits has not been studied. Here, we addressed whether damage of myelin restricted to the preBötC impacts the respiratory function. So, we injected bilaterally lysolecithin (LPC, a myelin-destroying toxin) into the preBötC of 21-day postnatal CF1 mice (n=12), or PBS as a control (n=12) and the ventilatory effects were analyzed after 7 (n=6 controls, n=6 experimentals) and 30 (n=6 controls, n=6 experimentals) days post injection (dpi), using immunohistochemistry, whole animal plethysmography, and local field potential recordings. Immunohistochemistry revealed pronounced local demyelination lesions at 7 dpi in the preBötC associated with the increase of basal respiratory frequency in brainstem slices, suggestive of enhanced respiratory network excitability. In addition, plethysmography revealed that demyelination reduced the increases in respiratory frequency, minute volume, and tidal volume in response to hypercapnia compared to those observed in controls. After complete remyelination at 30 dpi the *in vivo* and *in vitro* respiratory responses returned to values observed in basal conditions. Finally, increased extracellular potassium concentration to enhance the respiratory neural circuit excitability also reduced the respiratory responses to hypercapnia. Our findings provide functional evidence that preBötC myelin can influence the respiratory response to hypercapnia by controlling the respiratory neural network excitability. We propose that demyelination of the preBötC increases respiratory network excitability, decreasing the ventilatory response induced by hypercapnia, as a result of a reduction in the dynamic range of the chemosensory response. Together, these findings highlight the relationship between neural excitability, chemoreception, and myelin in a physiological context.

Disclosures: R. Pulgar: None. **J. Eugenin:** None. **F.C. Ortiz:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.07/L10

Topic: D.04. Interoception

Title: A Drosophila Connectomics Approach to Understand Proprioception Processing in A Drosophila Connectomics Approach to Understand Proprioception Processing in the Central Nervous Systemthe Central Nervous System

Authors: *H. MALIK[1], M. GREANEY[1], E. HECKSCHER[2];
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Abstract: Proprioception is a critical sensory modality shared among all motile organisms and is required for efficient and coordinated motor behaviors. Although proprioception is well studied in the periphery and in local spinal reflex circuits, we do not know the full breadth of how proprioceptive information is dispersed and regulated in the central nervous system (CNS).

Which CNS neurons is proprioceptive information distributed to? What other sensory information does proprioceptive information combine with? How is this information regulated? What brain regions does this information go to? These questions are difficult to address in vertebrates because of the complexity of their CNS organization and methodological limitations. Therefore, to address these questions, I will utilize the Drosophila larva because it has a simplified proprioceptive and motor network. Additionally, there is a Drosophila electron microscopy (EM) dataset which can visualize each neuron in the larval CNS. Using this EM dataset, I will reconstruct all post-synaptic partners of the larval proprioceptors (second order proprioceptors) in one body segment. For each second order proprioceptor, I will identify the complexity of all sensory inputs it receives, characterize other inputs originating from the CNS to identify circuit motifs and sources of regulation, and identify the brain regions targeted by these neurons. Through this work, I will build a first of its kind network map of the early proprioceptive system. To date, this has not been accomplished in any other organism. I will identify fundamental organizational principles underlying the transmission, regulation, and utilization of proprioceptive information in the CNS. These principles will provide a foundation to understand how proprioceptive processing occurs in more complex organisms.

Disclosures: H. Malik: None. **M. Greaney:** None. **E. Heckscher:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.08/L11

Topic: D.04. Interoception

Support: Fonds Wetenschappelijk Onderzoek-Vlaanderen (FWO-Flanders) (G0E0520N)
National Natural Science Foundation of China, Mianshang (Project 32271049)
Shanghai Municipal Science and Technology Major Project (2019SHZDZX02)

Title: Conserved localization of the von Economo neuron across primate suborders

Authors: *S. STEINER[1], K. ZILLES[2], K. M. AMUNTS[2], C. SHERWOOD[3], P. R. HOF[4], A. Y. FALCHIER[5], G. S. LINN[6], H. C. EVRARD[7];
[1]Max-Planck-Institute for Biol. Cybernetics, Tübingen, Germany; [2]Res. Ctr. Jülich, Jülich, Germany; [3]George Washington Univ., Washington, DC; [4]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [5]Nathan Kline Inst. For Psychiatric Res., Orangeburg, NY; [6]Nathan Kline Inst., Orangeburg, NY; [7]Intl. Ctr. for Primate Brain Res., Shanghai, China

Abstract: The von Economo neuron (VEN) is an atypical spindle-shaped neuron occurring primarily in the anterior insular (AIC) and anterior cingulate (ACC) cortices in humans, great

apes, macaques, and a few large-brained non-primates (e.g., whale, elephant, cow). In humans, the VEN has been associated with affective and cognitive functions, as well as with self-conscious feeling deficits in specific mental disorders. The occurrence of the VEN in large-brained non-primate species suggests that the VEN could in fact occur across a wide range of primate species as well. It remains however unclear whether the VEN occurs in all primate species regardless of the size of the brain, and whether it occurs in comparable or in different sub-regions of AIC and ACC. Here, we conducted an extensive examination of 132 histological specimens across 3 primate suborders and 21 primate genera, including small-brained specimens. Employing classical histology, microscopy, and high-density stereological sampling, we identified VENs in 16 previously unobserved genera across all three primate suborders. VENs were identified in all 14 Catarrhini genera (33/35 specimens in total), and in 5 of the 6 Platyrrhini genera (8/18 specimens in total); Aotus (1 specimen) being the only genera in which VENs were presently not found. In the Strepsirrhini suborder, the only representative specimen (a galago) contained a sparse concentration of VENs, underscoring the critical role of high-density sampling and tissue quality in detecting rare cells. VENs were present in specimen with relatively small brain masses, such as the marmoset and galago, challenging an earlier notion that VENs occur exclusively in larger brains. VENs were distributed in layer 5 - often together with fork neurons (FN) - in a well delimited cluster located inside a region homolog or analog to the macaque ventral AIC, sharing the same cytoarchitectural features at least in old world monkeys and lesser apes. A significant VEN-containing cluster was identified in the ACC in one specimen of saimiris, whereas VENs tended to be sparse in the ACC in all other specimens. Stereological estimates showed VEN to PN count ratios varying from 3.3% in humans to 0.8% in spider monkeys. The ratio of VEN to PN perikaryal volumes (VEN index), ranged from 1.97 in humans to 1.25 in gibbons. These results underscore the evolutionary conservation and likely high functional relevancy of the VEN and FN, while contradicting previous theories linking VEN exclusively to human-specific cognitive functions. These results set the stage for investigations across primates of distinct ecological and socio-behavioral niches, including AIC, ACC as well as other cortical areas.

Disclosures: S. Steiner: None. **K. Zilles:** None. **K.M. Amunts:** None. **C. Sherwood:** None. **P.R. Hof:** None. **A.Y. Falchier:** None. **G.S. Linn:** None. **H.C. Evrard:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.09/L12

Topic: D.04. Interoception

Support: Max Planck Society
Fonds Wetenschappelijk Onderzoek-Vlaanderen (FWO-Flanders) (G0E0520N)
Shanghai Municipal Science and Technology Major Project

(2019SHZDZX02)
NSFC Mianshang

Title: Static and dynamic effects of electrical microstimulation of the anterior insula on brainwide connectivity in the macaque

Authors: *J. WOLLER[1], J. SMUDA[2,4], C. KLEIN[1], Y. MURAYAMA[3,4], N. K. LOGOTHETIS[4,2,5], H. C. EVRARD[4,2,6];
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Abstract: The ventral anterior insular cortex (vAIC) integrates homeostatic affective state predictions with interoceptive afferences, altering brainwide network states based on salience detection. The Forebrain Emotional Asymmetry model suggests that these effects could be lateralized, based on a parasympathetic "energy enrichment" left vAIC, and a sympathetic "energy expenditure" right vAIC (Craig, Trends in Cognitive Science 2005). As natural stimuli producing exclusive unilateral activation of vAIC are not known, we used local electrical microstimulation (EMS; 300-700 μA, 100Hz) in anesthetized macaques (n = 3; Remifentanil) to selectively activate the left or right vAIC during 7T fMRI. Following our prior individual general linear model (GLM) and network analyses (Smuda et al., SfN 2018), our new voxel-based group GLM analysis showed that left compared to right EMS triggered broader and more bilateral activations, specifically in cingulate, temporal, and occipital areas. For both stimulation sides, single-trial GLM and clustering analyses revealed two distinct activation patterns alternating across time: one 'closed' pattern limited to subcortical limbic and insular activations, and one 'open' pattern with broad cortical involvement, including auditory, vestibular and visual areas, both replicable across individuals. A group independent component analysis (ICA) detected the classical default mode (DMN), salience (SN), and central executive (CEN) networks, with high correlation with bilateral vAIC, in the resting state. This brainwide functional network did not differ between runs with left or right EMS, and rest. However, under both stimulation conditions, but not at rest, a component comprising bilateral anterior insula, thalamus, cerebellum, and contralateral amygdala emerged. These results highlight the causal role of the vAIC in dynamic regulation of brainwide networks, afforded by its rich club embedding and extensive self-referenced multisensory integration. The larger activation with left EMS could relate to affiliative behavior and exploration, with broader information integration during optimal energy states. The more restricted activation correlation during right EMS could indicate increased internal focus on homeostatic states supporting energetically costly activities. The fluctuation between activation closed and open patterns suggests that the influence of vAIC signaling may depend on dynamic state fluctuations to be identified. Further investigations are currently exploring the effect of unilateral stimulations that appear to recruit non-motor cerebellar regions often denoted in emotional paradigms.

Disclosures: J. Woller: None. **J. Smuda:** None. **C. Klein:** None. **Y. Murayama:** None. **N.K. Logothetis:** None. **H.C. Evrard:** None.

Poster

PSTR234: Central Pathways for Interoception

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR234.10/Web Only

Topic: D.01. Somatosensation – Pain and Itch

Support: PAPIIT-UNAM Mexico (grant no. IN202222 to M.C-L)
Eng. Elsa Nydia Hernández Rios, Unidad de Microspcopia Confocal, INB UNAM

Title: Hypothalamic CSF-contacting neurons projections towards the rostral agranular insular cortex: an immunofluorescence and ultrastructural analysis in rats

Authors: *M. ELTRABILY[1], G. MARTINEZ-LORENZANA[2], L. PALMA TIRADO[3], A. GONZÁLEZ-HERNÁNDEZ[4], M. CONDES-LARA[5];
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Abstract: Cerebrospinal fluid (CSF) contacting neurons are related to detection of CSF changes and other physiological processes within the central nervous system including nociception. Here, we describe the peptidergic nature of some CSF-contacting neurons within the hypothalamic paraventricular nucleus (PVN) and their projections towards the rostral agranular insular cortex (RAIC) in rats. In male Wistar rats, neuronal tracers (Fluoro-Gold™ in RAIC, Cholera toxin subunit B into the lateral ventricle, and BDA in the PVN) were used. Additionally, immunofluorescence and electron microscopy studies against Fluoro-Gold (FG), oxytocin (OT) and vasopressin (AVP) in the hypothalamus, besides, GABA and oxytocin receptors (OTR) in RAIC were performed. Our results show the presence of oxytocinergic or/and vasopressinergic CSF-contacting neurons along the PVN, electron microscopy images signal that these neurons could secrete those peptides into CSF through dendritic projections. Besides, some oxytocinergic CSF-contacting neurons were also labelled by anti-FG indicating that they send projections towards the RAIC. Inside the RAIC, ultrastructural analysis show that axons from PVN sustain synaptic connections with cortical GABAergic neurons that express OTR, also, OT molecules can be observed within the synaptic areas. Together, these findings indicates the possible role of CSF-contacting neurons in the neuronal modulation by releasing neuromodulators both at CSF and synaptic levels, in this case, at insular cortex level. Also, the results support our previous reports on oxytocin release inside RAIC that could trigger local GABAergic activity, however, its secretion towards CSF probably can modulate many areas close to the ventricles, that might be involved in nociception modulation among other physiological regulatory processes.

Disclosures: M. Eltrabily: None. **G. Martinez-Lorenzana:** None. **L. Palma Tirado:** None. **A. González-Hernández:** None. **M. Condes-lara:** None.

Poster

PSTR234: Central Pathways for Interoception

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Program #/Poster #: PSTR234.11/L14

Topic: D.04. Interoception

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NIH Grant R01HD096436
UC Davis Chancellor's Fellowship
NIH Grant P51OD011107

Title: Macaque interoceptive processing in healthy and pathological aging

Authors: *J. A. CHARBONNEAU[1], E. P. RAVEN[2], J. L. BENNETT[1], G. M. MOADAB[1], B. E. RUSS[3], J. MORRISON[4], M. G. BAXTER[5], E. BLISS-MOREAU[6];
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Abstract: Healthy aging is often characterized by changes to sensory processing, including a loss in sensitivity to sensory signals. Changes in sensation and perception of interoceptive signals—those conveying the internal state of the body—are poorly characterized in old age relative to exteroceptive signals (e.g., vision, audition, somatosensation). Human work suggests that interoceptive experiences shift in old age, which may have significant implications both for quality of life and longevity. Pathological aging—including neurodegenerative diseases like Alzheimer's disease (AD)—may be characterized by more dramatic shifts in interoceptive sensory experiences which can exacerbate other symptoms (e.g., cognitive changes). We characterized changes in two different domains of interoceptive processing in healthy monkeys across the lifespan and in a monkey model of early-stage AD—a heartbeat sensing task and a neuroimaging affective touch task. Monkeys participated in an eye tracking paradigm adapted from the human infant interoception literature to assess sensitivity to cardiac interoceptive signals. We measured monkeys' looking time patterns when visual stimuli were presented asynchronously vs. synchronously with their cardiac rhythms. Healthy older monkeys look for longer at asynchronous as compared to synchronous stimuli, like healthy younger monkeys do, indicating that they are sensing their heart beats. Compared to healthy older monkeys, older monkeys with induced early-stage AD (accomplished by the introduction of human amyloid beta oligomers into the lateral ventricle), no longer distinguished between asynchronous and synchronous trials. To assess changes to processing of affective touch signals, monkeys were exposed to fast (i.e., discriminative) and slow (i.e., affective) touch while lightly anesthetized and undergoing functional magnetic resonance imaging (fMRI). Brain areas responsible for processing interoceptive signals (insula, anterior cingulate, amygdala) were significantly more active during affective compared to discriminative touch in healthy young monkeys. However,

aged healthy monkeys exhibited no significant difference in neural activation by different touch types. Monkeys with induced early-stage AD exhibited changes to affective touch processing above and beyond those expected at their age. Taken together, our data suggest that there are dramatic alterations to interoceptive processing in healthy and pathological aging in macaques, providing the critical opportunity to model changes in humans and attempt interventions to preserve function.

Disclosures: J.A. Charbonneau: None. **E.P. Raven:** None. **J.L. Bennett:** None. **G.M. Moadab:** None. **B.E. Russ:** None. **J. Morrison:** None. **M.G. Baxter:** None. **E. Bliss-Moreau:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.01/L15

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R21NS135762
NIH RF1 NS114128-01A1S1

Title: Circadian dysfunction in Drosophila models of C9orf72-linked ALS/FTD

Authors: *O. AKPOGHIRAN, B. JENNY, K. KOH;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: The GGGGCC (G4C2) nucleotide repeat expansion (NRE) mutation in C9orf72 (C9) gene is the most common genetic cause of Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two overlapping neurodegenerative diseases on a spectrum. The C9-NRE mutation leads to several cellular abnormalities, including the appearance of highly toxic arginine-rich dipeptide repeats (DPRs). Sleep and circadian disorders are frequent comorbidities with ALS/FTD. However, the molecular and cellular mechanisms linking ALS/FTD to sleep and circadian disruptions are poorly understood. Here, we utilized *Drosophila* as a model system to investigate the impact of C9 NREs and DPRs on circadian rhythms. Using the GAL4/UAS system, we found that expression of C9 NREs or DPRs in *Drosophila* clock neurons resulted in a wide range of phenotypes. Notably, expressing proline-arginine (PR) repeats in *Drosophila* clock neurons resulted in long circadian periods and reduced rhythm strength despite the presence of the neurons, suggesting that circadian dysfunction precedes neuronal cell loss. Ubiquitous expression of G4C2 repeats in adults also resulted in similar circadian phenotypes. Furthermore, we observed reduced neuropeptide expression and morphological defects in the clock neurons expressing PR or G4C2 repeats. Surprisingly, increasing neuronal activity of clock neurons partially rescued PR-induced circadian phenotypes while decreasing neuronal excitability exacerbated them. Although neuronal hyperexcitability is thought to exacerbate ALS, these findings suggest that its effect may depend on the cell type and

the stage of disease. We also found that some genetic C9 modifiers previously identified using eye degeneration phenotypes have similar effects on circadian dysfunction, while others have opposite effects. This work suggests that shared and distinct mechanisms underlie C9-linked circadian dysfunction and neurodegeneration and provides us with a platform to discover molecular pathways linking C9 pathologies and circadian dysfunction.

Disclosures: O. Akpoghiran: None. **B. Jenny:** None. **K. Koh:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.02/L16

Topic: F.07. Biological Rhythms and Sleep

Support: NIH-NINDS R00 NS105942

Title: Sexually dimorphic roles for Drosophila circadian clock neuropeptides in regulating rest-activity rhythms

Authors: *S. CRESPO-FLORES, M. FETCHKO, H. M. GARNER, A. BARBER;
Rutgers Univ., New Brunswick, NJ

Abstract: Circadian clocks in the brain act as master pacemakers that synchronize molecular oscillators across an organism to allow anticipation of daily environmental changes. Across species, the circadian system consists of a central oscillator that is entrained by environmental inputs and controls behavioral and physiological outputs. In *Drosophila*, the brain clock network consists of ~150 neurons that express the core molecular clock components and is entrainable by light and temperature. Despite our advancements in understanding the brain clock network, we don't understand the molecular mechanisms underlying the known sexual dimorphism in circadian behavior in *Drosophila*. In this study, we evaluated the roles of circadian clock neuropeptides in modulating circadian rest activity behavior in male and female flies. I conducted a CRISPR deletion screen of eight candidate neuropeptides in the whole clock circuit and characterized changes in circadian locomotor behavior. We identified several hits that increase or decrease rhythm strength when knocked out in the whole clock network. We observed sexually dimorphic effects of neuropeptide knockout in the clock network. CRISPR deletion of Allatostatin A (AstA) or CNMamide (CNMa) increases rhythm strength in female, but not male, flies, with no effects on period length or daily activity amounts. Neuropeptide F (NPF) deletion has a more severe phenotype with female-specific increases in rhythm strength and male-specific decreases in rhythm strength accompanied by reduced daily activity, however clock-specific NPF deletion also reduced survival. Further restriction of CNMa and NPF deletion to DN1 neurons continues to increase female rhythm strength, suggesting that expression in this population has a key role. We also observed that this increase in female rhythm strength is mating status dependent, since it is absent in non-mated females. Ongoing work will address the

sexually dimorphic changes in rhythm strength by investigating the role of sex peptide and sex peptide receptor. Our findings suggest novel sexually dimorphic roles for three neuropeptides in mediating circadian locomotor behavior.

Disclosures: S. Crespo-Flores: None. **M. Fetchko:** None. **H.M. Garner:** None. **A. Barber:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.03/L17

Topic: F.07. Biological Rhythms and Sleep

Support: CAREER Award 1942167

Title: Central circadian clock control of Drosophila feeding and activity rhythms

Authors: *R. J. MEIER[1], S. SAURABH[2], L. M. PIREVA[2], R. A. MIRZA[2], D. J. CAVANAUGH[2];
[1]Univ. of Wisconsin-Madison, Madison, WI; [2]Loyola Univ. Chicago, Chicago, IL

Abstract: The circadian system produces ~24-hour cycles in diverse biological processes to establish and maintain rhythms of behavior and physiology. Circadian rhythms are commonly studied by examining daily oscillating rhythms of rest and activity; however, much less is known about other circadian processes, such as eating and digestion, the rising and falling of body temperature, and the releasing and suppressing of hormones. An emerging question is how the brain can concurrently regulate multiple of these circadian outputs. In *Drosophila melanogaster*, circadian rhythms are rooted in a well-defined neural network composed of only ~150 neurons. This “clock network” is divided into functionally and anatomically discrete subsets of cells, but the differential roles of each of these subsets and how they each contribute to rhythms of behavioral outputs is unknown. We have characterized the involvement of each of these subsets of clock cells in two prominent circadian behavioral outputs: feeding and locomotor activity. We produced cell-specific manipulations to either eradicate clock cell functioning or block neuronal communication in different subpopulations of clock cells and monitored the effects on feeding behavior and locomotor activity. Our results show that clock cells regulate feeding and locomotor activity rhythms in parallel, suggesting that circadian control of these two distinct behavioral outputs diverges in downstream circadian output cells rather than in core clock network cells. We find that the severity and nature of the effect of these manipulations varies according to the cell population targeted, revealing the hierarchical roles of these clock network neuronal clusters and their differential functions. For both feeding and locomotor activity, lateral clock neurons contribute only to the strength of circadian rhythmicity while dorsal clock neurons contribute only to the temporal distribution of activity. These findings provide insights into the cellular components of the *Drosophila* clock network. They uncover how various elements of the

circadian system, namely rhythm strength and phase, are differentially controlled, and they offer the first characterization of how clock network organization contributes to circadian feeding behavior.

Disclosures: R.J. Meier: None. **S. Saurabh:** None. **L.M. Pireva:** None. **R.A. Mirza:** None. **D.J. Cavanaugh:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.04/L18

Topic: F.07. Biological Rhythms and Sleep

Support: DFG Grants STE531/20-1,2
DFG GRK 2749-1

Title: The neural plasma membrane as circadian posttranslational feedback loop oscillator

Authors: *A. C. SCHNEIDER, M. STENGL;
Dept. of Biology, Animal Physiol. / Neuroethology, Univ. of Kassel, Kassel, Germany

Abstract: Behavior is usually organized in circadian (~ 24 h) patterns: sleeping, feeding, or mating occur at specific zeitgeber times (ZTs) per day. Such regular, predictive rhythms enable anticipation and synchronization of behavior within and between species. They are driven by endogenous clocks that are entrained to daily zeitgebers like the light-dark cycle.
In insects, the central "master clock" circuit is a neural network in the brain, while additional peripheral clocks exist in sensory neurons and other tissues. The current hierarchical model in chronobiology frames the clock as an autonomous transcription/translation feedback loop (TTFL) that drives the circadian expression of clock proteins, controlling all circadian oscillations of proteins, neural activity, and behavior. Instead, we propose that circadian clocks are based on a coupled system of TTFL and posttranslational feedback loop (PTFL) oscillators. We use electrophysiological analysis of olfactory receptor neurons (ORN) in the antennae of male hawkmoths (*Manduca sexta*) in combination with pharmacology and molecular genetics. The ORNs are endogenous circadian clock neurons. Their spontaneous ultradian spiking shows circadian modulation with maximal spiking during the nocturnal hawkmoth's activity phase. With application of various antagonists and agonists, in combination with molecular genetics, we search for ion channels involved in ZT-dependent rhythm generation. Preliminary work suggests that, rather than TTFL-dependent control, PTFL-dependent oscillators associated with plasma membrane-dependent signaling cascades generate the observed multiscale oscillations. Apparently, membrane potential oscillations are based on cyclic nucleotide- and calcium-dependent ion channels, which control sensitivity and kinetics of ORNs. Our current experiments search for links between these rhythms at timescales spanning several log units.

Disclosures: A.C. Schneider: None. **M. Stengl:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.05/L19

Topic: F.07. Biological Rhythms and Sleep

Support: BGSU Building Strength Grant

Title: Possible roles for the counterbalance between CD47 and CALR proteins in the circadian activity of glioma and normal neural cells

Authors: S. AFRIN, E. NTARE, C. W. CHANDLER, S. IRSHAD, V. S. MOHITE, *M. E. GEUSZ;
Dept of Biol. Sci., Bowling Green State Univ., Bowling Green, OH

Abstract: Apoptotic and non-apoptotic cells are removed by microglia and macrophages through phagocytosis triggered in part by cell-surface proteins such as calreticulin (CALR), an "eat me" signal. In contrast, leukocyte surface antigen CD47 inhibits this process by binding to the signal regulatory protein alpha (SIRPA) on phagocytes, providing a "don't eat me" signal. In the brain, CD47 protects neurons from excessive developmental synaptic pruning, and CD47 is expressed by astrocytes in response to interleukin-1β. Inflammatory signals also cause CALR release from neurons and microglia, thereby recruiting and activating microglia. This balancing of phagocytotic intensity by CD47 and CALR is likely to be important in depression, where brain inflammatory signals are elevated, and in Alzheimer's disease, which is promoted by brain injury. Because of the known daily rhythm in immune activity, we examined proteome and transcriptome databases to test whether CD47 or CALR expression are under circadian control. Significant circadian rhythms in mouse CD47 and CALR gene expression were found in samples taken from brain, liver, and kidney. Most important, the phase of CD47 and CALR rhythms were about 9 to 12 hours apart, suggesting a daily rhythm in cell vulnerability to phagocytosis. Cell removal could be more effective at a phase when cell-surface CALR is abundant and CD47 levels are low. CALR is ubiquitously expressed in cells. In cancer cells CALR mutations and persistent endoplasmic reticulum stress cause increased cell-surface CALR protein. We then examined the astrocytic cancer cell line C6 to test whether this model for glioblastomas also shows differential CD47 and CALR expression across cells, which would agree with the proteins being expressed in anti-phase circadian rhythms. Surprisingly, in C6 cells that were not treated to synchronize their intrinsic circadian clocks we found that CD47 and CALR protein expression were positively correlated according to confocal immunofluorescence microscopy, suggesting their rhythms may be in-phase. These results indicate glioma cells may evade phagocytosis by simultaneously expressing CALR and CD47, and this may be mediated by alterations in circadian timing control relative to non-tumor cells. CALR was highest in the C6 cells of early tumorspheres that develop from glioma stem cells in vitro. CD47 expression was present in low and high-density cultures. We also observed the elevated CD47 expression known to occur in

extracellular vesicles, which should be examined for possible circadian regulation of intercellular signaling.

Disclosures: S. Afrin: None. **E. Ntare:** None. **C.W. Chandler:** None. **S. Irshad:** None. **V.S. Mohite:** None. **M.E. Geusz:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.06/L20

Topic: F.07. Biological Rhythms and Sleep

Title: Effect of photoperiod on astrocytes in the suprachiasmatic nucleus

Authors: *A. SHANKS[1], A. PORCU[2];
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Abstract: Astrocytes are widely expressed in the nervous system, where they regulate neuronal activity and neuroplasticity. Astrocytes control circadian timekeeping in the suprachiasmatic nucleus (SCN) via glutamatergic signaling. Changes in astrocyte number have been previously observed in the hippocampus after long photoperiod exposure, suggesting that an alteration in astrocytes might be an additional homeostatic response to seasonal changes in the light environment. However, whether photoperiod alters astrocyte numbers and functions in the SCN remains unknown. To start addressing this gap in the knowledge, we exposed adult mice to 2 weeks of long photoperiod (19hrs of light and 5hrs of darkness, 19L:5D) or short photoperiod (5L:19D). We first assessed the effect of long and short photoperiods on SCN astrocytes numbers by analyzing the expression of glial fibrillary acidic protein (GFAP), a structural protein of astrocytes. The anterior-posterior (A-P) axis quantification revealed that the number of SCN GFAP-immunoreactive cells was greater in long versus short photoperiods within the medial region of the SCN. Astrocytes regulate the clearance of extracellular glutamate through glutamate transporters (EAAT1 and EAAT2). Therefore, we tested whether photoperiod altered astrocyte function by quantifying the expression of EAAT1 and EAAT2 after photoperiod exposure. Using in situ hybridization assay, we found increased Eaat1 and Eaat2 expression in the SCN of adult mice after long photoperiod exposure. The effects of light on the mammalian circadian system are achieved mostly through retina-derived glutamate release in the SCN, and the ionotropic-NMDA receptor (NMDAr) expressed in the SCN neurons is considered a key player in the light entrainment process. Therefore, we also analyzed the expression of NMDAr in the SCN neurons in response to photoperiod and found increased NMDAr subunit Grin2b in the SCN of mice exposed to a long photoperiod. Altogether, these data suggest that astrocyte organization and their extracellular glutamate regulation may represent a previously unidentified adaptation of the SCN regulating seasonal plasticity.

Disclosures: A. Shanks: None. **A. Porcu:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR235.07/L21

Topic: F.07. Biological Rhythms and Sleep

Support: R01GM143545

Title: Photic processing in the central circadian clock at the network level in real-time

Authors: S. A. MCGRAW[1], K. C. BENTON[1], I. A. SAVTCHOUK[1], *J. A. EVANS[2];
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Abstract: Daily rhythms are an important form of adaptive homeostasis that allows one to anticipate predictable, salient changes in the environment. Circadian rhythms are entrained to local conditions by daily light exposure through neural pathways that are well defined. However, modern patterns of light exposure disrupt daily rhythms and drive disease through mechanisms that remain unclear. For photoentrainment, glutamatergic inputs from the retina are transmitted to the central clock in the hypothalamus, the suprachiasmatic nucleus (SCN). Consistent with these findings, NMDA signaling in the SCN is sufficient to mimic the resetting effects of light on cellular and behavioral rhythms. To further examine SCN photic processing, here we conducted 2-photon calcium imaging of SCN neuronal responses to NMDA application at two different times of day. Computational mapping approaches highlight that large heterogeneity in cellular responses produce larger network-scale activation patterns that are gated by time of day. These data provide insight into SCN photic processing at the cellular and network levels, which may help to advance understanding of how light influences health and disease.

Disclosures: S.A. McGraw: None. **K.C. Benton:** None. **I.A. Savtchouk:** None. **J.A. Evans:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH NINDS R21NS120003
NIH NCI R01NS134885
Washington University Siteman Cancer Center

Title: Disruption of circadian signaling to GBM tumors desynchronizes intrinsic Per2 rhythms and slows disease progression

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Abstract: Glioblastoma (GBM) is the most common primary brain tumor in adults. Despite extensive research and clinical trials, median survival post-treatment remains at 15 months. Thus, all opportunities to optimize treatment and improve patient outcomes should be considered. Previous work has shown that murine and human GBM models have cell-intrinsic circadian rhythms in expression of the core clock genes Bmal1 and Per2. In a retrospective clinical study, our lab found that taking morning TMZ increased patient overall survival by 6 months compared to evening. This suggests that GBM has circadian rhythms that synchronize to the hosts' central clock. Understanding the mechanisms underlying circadian entrainment in GBM is essential to determine whether regulation of tumor biology and susceptibility to therapies varies with time of day. Here we tested whether host signaling synchronizes daily rhythms in GBM and regulates tumor growth. We transduced a murine GBM cell model (i.e., GL261) with a luciferase reporter of Per2 transcription (GBM-P2L) and confirmed that these cells had circadian rhythms in clock gene expression. We next implanted GBM-P2L cells into the basal ganglia of mice and imaged bioluminescence in vivo. We found that Per2 expression reliably peaked in the dark phase (CT16) in GBM xenografts implanted into C57 WT mice. Strikingly, GBM tumors grew slower and Per2 peaked at random times of day in mice that lacked daily rhythms in locomotion and Corticosterone secretion (i.e., VIP KO). As an independent measure of tumor size, we stained brain sections from tumor-bearing mice with the proliferation marker Ki67. We found that GBM tumors implanted into rhythmic C57 mice had increased Ki67 expression and tumor size, compared to tumors implanted into arrhythmic VIP KO mice. Our results suggest that GBM tumors integrate into circadian circuits of the brain and depend on clock-controlled cues, like vasoactive intestinal peptide (VIP), to grow at specific times of day. This work may inform personalized circadian medicine to improve individual GBM patient outcomes.

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Poster

PSTR235: Molecular Biology and Physiology of Clocks

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Topic: F.07. Biological Rhythms and Sleep

Support: Science Foundation Ireland SFI/12/RC/2273_P2
Saks Kavanaugh Foundation

Title: Gut Microbiota Regulates Stress Responsivity via the Circadian System

Authors: *G. TOFANI[1,2], S.-J. LEIGH[3,2], C. GHEORGHE[3,2], T. BASTIAANSSEN[1,2], P. SEN[1,2], G. CLARKE[3,2], J. CRYAN[1,2];
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Abstract: Many neuropsychiatric disorders are intricately linked to stress, and patients with these conditions often display alterations in their gut microbiota profile and in circadian regulation of hormonal and behavioral timekeeping processes. In this work we explore how the gut microbiota modulate diurnal oscillations in neuroendocrinal systems resulting in different stress responsiveness across the day, to shed light into how these gut microbes could be playing a role in neuropsychiatric disorders. Glucocorticoid hormones are key to regulating the organism's response to the environment and display a robust rhythm with a distinct peak around the sleep-wake transition, which is one of the synchronizing cues from the brain to peripheral tissues. Stress-induced glucocorticoid secretion will act on the same tissues leading to changes in state to cope with any stressors. Although the relationship between gut microbiota and stress is well established, it is still largely unknown how the circadian component of glucocorticoid secretion plays a role in such modulation. To this end, we investigate the circadian-stress interplay in the absence of gut microbiota in order to understand how gut microbes can shape the interactions between the stress and circadian systems. First, we establish that the gut microbiota regulates the diurnal rhythms in glucocorticoids, what is linked with alterations in circadian rhythmicity in the suprachiasmatic nucleus transcriptome. Next, by investigating the alterations in rhythmicity induced by the gut microbiota in the transcriptome and metabolome of the hippocampus and amygdala, we show a disruption in pathways key to maintaining appropriate stress responsiveness. Furthermore, we explore how these changes in rhythmic glucocorticoids are driven, demonstrating a hyper-activation of the HPA-axis following microbial depletion, what in turn drives time-of-day specific effects on the stress response and social behavior. Lastly, we investigate the compositional changes in the microbiota underlying the alterations in glucocorticoids to show that diurnal oscillations in gut microbes drive such changes. Together, our data offers compelling evidence that the microbiota regulates stress responsiveness in a circadian manner and is necessary to respond adaptively to psychological stressors throughout the day.

Disclosures: G. Tofani: None. **S. Leigh:** None. **C. Gheorghe:** None. **T. Bastiaanssen:** None. **P. Sen:** None. **G. Clarke:** None. **J. Cryan:** None.

Poster

PSTR235: Molecular Biology and Physiology of Clocks

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R37NS048471
Howard Hughes Medical Institute

Title: Intrinsic circadian rhythms of hypothalamic tanycytes contribute to JAK-STAT signaling, adult neurogenesis, and diet-induced weight gain

Authors: *D. IASCONE[1], M. LOPEZ VALENCIA[2], H. LIN[3], J. L. BEDONT[4], A. SEHGAL[1]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, Redwood City, CA, ; [3]Smith Col., Northampton, MA, ; [4]Kent State Univ., Kent, OH

Abstract: Circadian clocks are ubiquitous in tissues, regulate many biological functions, and their misalignment or disruption contributes to deleterious health consequences. Although molecular underpinning of the circadian clock is well-studied, the role of the clock in mediating metabolic signals from the periphery to the central nervous system is poorly understood. We hypothesized that circadian rhythms in tanycytes, specialized hypothalamic glial cells that gate the entrance of nutrients and hormones to the brain, might contribute to whole-body energy homeostasis through their regulation of leptin signaling and adult neurogenesis. In wildtype mice we observed significant rhythmic expression not only of core clock genes in tanycytes, but also in their protein levels of the leptin signaling effector Stat3, suggesting that tanycytes may undergo cell-autonomous circadian regulation of their metabolic functions. To assess the potential physiological role of intrinsic circadian rhythms in tanycytes, we used tanycyte-specific RaxCreER^{T2} mice to induce acute knockout of the core clock gene *Bmal1* through tamoxifen administration while labeling cells with tdTomato for lineage tracing experiments. We show that acute tanycyte-specific loss of *Bmal1* both abolishes rhythms of Stat3 expression in these cells and protects against high-fat diet-induced weight gain in both male and female mice. We also observed that tanycyte neurogenesis was inhibited compared to littermate controls only in females after acute *Bmal1* knockout while astrocyte production by tanycytes was increased in both sexes. These lineage tracing experiments further revealed that wildtype female mice have significantly more tanycyte-born neurons than males. Together, these results suggest that cell-autonomous transcriptional rhythms in a population of specialized hypothalamic glia contribute to whole-body energy homeostasis.

Disclosures: D. Iascone: None. **J.L. Bedont:** None. **A. Sehgal:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

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Topic: F.07. Biological Rhythms and Sleep

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NSTC 112-2811-H-320-001

Title: Reversing memory deficits in rapid eye movement sleep-deprived mice by TCU411 through the $GABA_B$ receptors

Authors: *P. VARINTHRA[1,3], S.-C. SHIH[2,4], L.-J. CHEN[5,6], L. YANG[2,4], M. NAGARAJAN[2,4], I. LIU[1,3];
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Abstract: Sleep is essential for maintaining both physical and mental health. Sufficient rapid eye movement (REM) sleep is crucial for promoting learning and memory, ensuring good sleep quality. Nevertheless, sleep deprivation (SD) in middle-aged and older individuals leads to a reduction in REM sleep, which worsens memory problems and increases the chance of developing dementia and causing accidents. The inhibitory GABAergic system is the key neurotransmitter that influences sleep quality and contributes to memory formation. Many medications for sleep work by targeting $GABA_A$ receptors; however, these exhibit a wide range of adverse side effects. TCU411 is a Taiwanese indigenous herbal medicine; its essential oil contains antioxidant properties and demonstrates the potential to promote sleep quality in wild-type mice. However, the impact of TCU411 in ameliorating memory impairments in REM sleep-deprived mice has yet to be explored. The treatment of 300 mg/kg TCU411 did not affect the locomotor activity of the mice. The T-maze test demonstrated that SD resulted in a decline in working memory, which was restored by TCU411 treatment. Additionally, a western blot study revealed an upregulation of$GABA_BR1$ expression in the hippocampus of SD mice, which can be attenuated by TCU411 administration. In addition, the administration of baclofen, an agonist of the $GABA_B$ receptor, to mice noticed a decline in their working memory function but reversed by treatment with TCU411. Our results demonstrate that SD indeed impairs working memory performance, and TCU411 can significantly reduce its impact by mediating the $GABA_BR1$ expression level.

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Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

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Program #/Poster #: PSTR236.02/L26

Topic: F.07. Biological Rhythms and Sleep

Support: VA BX000798
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1R21AG083236

Title: Most dynorphin neurons in the zona incerta-perifornical area are active in waking relative to NREM and REM sleep

Authors: *P. SHIROMANI[1], A. VIDAL-ORTIZ[2];
[1]Ralph H Johnson VA Healthcare Syst., Charleston, SC; [2]Res., Ralph H Johnson VA Med. Ctr., Charleston, SC

Abstract: Introduction: Dynorphin is an endogenous opiate localized in many brain regions and spinal cord, but the activity of dynorphin neurons during sleep is unknown. Dynorphin is an inhibitory neuropeptide that is coreleased with orexin, an excitatory neuropeptide. We use microendoscopy to test the hypothesis that, like orexin, the dynorphin neurons are wake-active. **Method:** Dynorphin-cre mice (n=3) were administered rAAV8-Ef1a-Con/Foff 2.0-GCaMP6M into the zona incerta-perifornical area, implanted with a GRIN lens (Gradient Reflective Index), and electrodes to the skull recorded sleep. One month later, a miniscope imaged calcium fluorescence in dynorphin neurons during multiple bouts of wake, NREM, and REM sleep. **Results:** Unbiased data analysis identified changes in calcium fluorescence in sixty-four dynorphin neurons. Most of the dynorphin neurons (72%) had the highest fluorescence during bouts of active and quiet waking compared to NREM or REM sleep; a subset (20%) were REM-max. **Conclusion:** Our results are consistent with the emerging evidence that the activity of orexin neurons can be classified as wake-max or REM-max. Since the two neuropeptides are coexpressed and coreleased, we suggest that dynorphin-cre-driven calcium sensors could increase understanding of the role of this endogenous opiate in pain and sleep.

Disclosures: P. Shiromani: A. Employment/Salary (full or part-time):; Veterans Administration Research Service, Medical University of South Carolina. 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, VA Merit Grants. **A. Vidal-Ortiz:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH IAA AOD22011-001-00000; MOA-AI-21002-01
ORISE Research Participation Project DOE Contract DOE contract number DE-SC0014664

Title: Understanding the relationship between moderate-dose nerve agent exposure and sleep-wakefulness

Authors: *A. N. SANTORO, J. H. MCDONOUGH, Jr.;
USAMRICD, APG-EA, MD

Abstract: Exposure to organophosphorus nerve agents results in cholinergic inhibition causing immediate and long-term detrimental health outcomes to humans and animals. One prominent long-term effect that has been documented is disruption to the sleep-wakefulness cycle, specifically increased rapid eye movement (REM) sleep. However, a robust paradigm for characterizing and treating these impacts in a rodent model has not yet been established. In this study, we conducted two experiments: first, we established a model for quantifying the impact of moderate-dose nerve agent intoxication on the sleep-wakefulness cycle in rats; second, we investigated the efficacy of the anticholinergic drug scopolamine as a pharmacologic intervention to counteract the impact nerve agents have on REM sleep outcomes.In Experiment 1, Sprague Dawley rats were implanted with subcutaneous telemetry devices to measure EEG and EMG activity. These signals were used to quantify wakefulness, slow wave sleep, and REM sleep before and after moderate-dose nerve agent exposure. Percent time spent in each state and number of times entering each state during the rats' light (sleep) and dark (wakefulness) cycles were quantified. We found that both male and female rats spend significantly less percent time in the REM state across the 12-hr light cycle post-exposure (M = 5.48) compared to baseline (M = 6.72), $p < .001$. However, only male rats entered the REM sleep state significantly more times across the 12-hr light cycle post-exposure (M = 13.15) compared to baseline (M = 11.15), $p < .001$, while female rats did not display this increase.In Experiment 2, rats received the above exposure paradigm. After exposure, rats were assigned to one of four groups in which scopolamine treatment was self-administered via sucrose drinking water over three days (control, 0.0065 mg/ml, 0.011813 mg/ml, 0.027 mg/ml) and sleep-wakefulness outcomes were quantified. In this experiment, the findings of Experiment 1 were not replicated; no significant differences in percent time in or number of times entering the REM state post-exposure compared to baseline were identified. These inconsistent data highlight the need to further investigate the impact that moderate-dose nerve agent exposure can have on the sleep-wakefulness cycle. Establishing a reproducible model to understand this relationship can help us better understand how to improve the quality of life for survivors of nerve agent exposure.

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Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

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Topic: F.07. Biological Rhythms and Sleep

Support: Veteran Administration Merit Research Award; I01BX002661 and I01BX006240-0
National Institute of Health (through the RO1 grant; AA028175-01)

Title: Predator odor trauma model of PTSD downregulates the Period-1 gene expression in the basolateral amygdala and causes sleep disruption.

Authors: *M. S. PARIKH[1], A. CHISCHOLM[2], D. KEMPURAJ[3], R. ALHAKIM[3], R. SHARMA[4], P. SAHOTA[5], M. M. THAKKAR[6];
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Abstract: Background: Recently we have observed the antisense-induced downregulation of the Period1(Per1) gene in the basolateral amygdala (BLA) affects the circadian regulation of sleep (sfn2023). BLA regulates traumatic memory, however, its specific role in sleep disturbances, the hallmark of post-traumatic stress disorder (PTSD) remains unclear. Thus, we hypothesize that the downregulation of the Per1 gene in the BLA, but not in the suprachiasmatic nucleus (SCN), is responsible for sleep disruption in PTSD. To test our hypothesis, we performed two experiments. The first experiment evaluated sleep disturbances in mice exposed to predator odor trauma (POT). The second experiment examined the expression of Per-1 in the BLA of mice exposed to POT. **Methods:** *Experiment 1:* Mice were surgically implanted with wire electrodes targeted towards BLA (to record local field potentials) and implanted with cortical sleep electrodes to assess for sleep-wake patterns. We recorded a stable sleep-wake baseline before the onset of traumatic memory acquisition (TMA) and continued until the completion of traumatic memory recall (TMR). One hour after light onset, mice were divided into two groups: 1) POT; and 2) no odor control (NOC). TMA was performed on Day 1. Mice were housed in an aluminum-colored cage (conditional stimulus; CS) for 5 min. Two scoops of soiled cat litter (unconditional stimulus; US) were introduced, in the contextual cage for 15 min. On completion, mice were returned to their respective home cages and left undisturbed until TMR on Day 4. TMR was performed the same way except for this time, the mice were exposed to CS only, without US. The same protocol was carried out simultaneously with unsoiled cat litter NOC, compared to the POT group. *Experiement-2:* Mice were exposed to TMA (as described above) and were assessed for anxiety-related stress in the elevated plus maze (EPM) 2-hr after light on set on Days 0, 1, and 2 respectively. On day 2 mice were euthanized in the middle of the light/dark period respectively, to confirm the temporal dynamics of PER1 gene expression.
Results: Exp1: POT-exposed mice showed significant sleep disruption following TMA, compared to the NOC. Traumatic markers such as gamma and theta frequencies were found significantly higher in the POT compared to the NOC on both the TMA and TMR period.
Exp 2: POT-exposed mice displayed significant downregulation of Period1 gene expression on day 2 light period. They also showed a higher anxiety index in the EPM compared to the NOC group.
Conclusion: POT-induced mice showed significant sleep disruption and reduced Per1 gene expression, providing significant insight into therapeutic interventions for PTSD.

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Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

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Topic: F.07. Biological Rhythms and Sleep

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Wellcome Trust Strategic Award 098461/Z/12/Z

Title: Deficient synaptic neurotransmission results in a persistent sleep-like cortical activity across vigilance states in mice

Authors: *M. C. C. GUILLAUMIN[1], C. D. HARDING[2], L. B. KRONE[3], T. YAMAGATA[4], M. KAHN[5], C. BLANCO DUQUE[3], G. BANKS[6], P. M. NOLAN[7], S. N. PEIRSON[1], V. V. VYAZOVSKIY[8];
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Abstract: Growing evidence suggests that brain activity during sleep, as well as sleep regulation, are tightly linked with synaptic function and network excitability at the local and global levels. We previously reported that a mutation in synaptobrevin 2 (*Vamp2*) in restless (*rlss*) mice results in a marked increase of wakefulness and suppression of sleep, in particular REM sleep (REMS) as well as increased consolidation of sleep and wakefulness. In the current study, using finer-scale *in vivo* electrophysiology recordings in both male wild-type (n=5) and

rlss (n=5) mice, we report that spontaneous cortical activity in *rlss* mice during NREM sleep (NREMS) is characterised by an occurrence of abnormally prolonged periods of complete neuronal silence (OFF-periods), often lasting several seconds, similar to the burst suppression pattern typically seen under deep anaesthesia. Increased incidence of prolonged network OFF-periods was not specific to NREMS, but also present in REMS and wake in *rlss* mice. Slow-wave activity (SWA) was generally increased in *rlss* mice, while higher frequencies including theta-frequency activity were decreased, further resulting in diminished differences between vigilance states. The relative increase in SWA after sleep deprivation was attenuated in *rlss* mice, suggesting either that *rlss* mice experience persistently elevated sleep pressure, or, alternatively, that the intrusion of sleep-like patterns of activity into awake state diminishes the accumulation of sleep drive. We propose that deficit in global synaptic neurotransmitter release leads to 'state inertia', reflected in an abnormal propensity of brain networks to enter and remain in a persistent 'default state' resembling coma or deep anaesthesia.

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Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

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Program #/Poster #: PSTR236.06/L30

Topic: F.07. Biological Rhythms and Sleep

Support: Navy BUMED Program of Record for Toxicology

Title: New approach methodologies for rapid reassessment of potential endocrine disrupting chemicals for neurohormone melatonin receptor activation

Authors: *L. OLSEN[1], M. WAGNER[2], M. NAIDU[3], J. G. ROHAN[4];
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Abstract: Circadian rhythm (CR), a highly conserved biological process significantly disrupted in modern society, follows 24-hour cycles that influence sleep and physiological health. A previously unknown effect prior to regulatory assessment, the common carbamate pesticide carbaryl activates neurohormone melatonin receptors with CR disruption occurring at levels below daily exposure limits. An updated assessment of safety recommendations for tens of thousands of previously reviewed chemicals is urgently required for situations where CR disruption may produce occupational or health risks.Drug discovery *in silico* methods were leveraged to identify chemicals with CR disruption potential. Using carbaryl as the seed molecule, ChemMine structural similarity was performed (80% structural similarity, PubChem

Fingerprint database). Cheminformatics approach and exposome databases were utilized to categorize chemicals by functional groups, lipophilicity, and melatonin receptor docking potential.As we previously presented, the top 50 structurally similar compounds were mostly carbamate pesticides and their metabolites (54%). Half contained a methylcarbamate functional group and 14% included functional groups similar to methylcarbamate. Most methylcarbamate groups were attached to monocyclic benzene (46%) or bicyclic naphthalene (6%). Only a fraction exist in metabolite (8%) and pathway (12%) databases. Lipophilicity ranged from 1.5 - 5.8, with 42% predicted to have moderate solubility and permeability with low metabolism. Considering compound lipophilicity and that previous studies suggest compounds with methylcarbamate attached to naphthalene have higher molecular docking potential to putative melatonin binding pockets, 5 priority compounds of concern were identified.Potential CR disrupting chemicals were identified using new approach methodologies. Among the compounds identified, 5 were prioritized as compounds of concern based on physicochemical properties that anticipate organism exposure and melatonin receptor activation. The results from this study contribute to addressing the urgent need to investigate potential CR disrupting effects of environmental and occupational exposures. No DoD endorsement implied. Supported by Navy BUMED Program of Record for Toxicology.

Disclosures: L. Olsen: A. Employment/Salary (full or part-time):; Leidos Inc. **M. Wagner:** A. Employment/Salary (full or part-time):; Leidos Inc. **M. Naidu:** A. Employment/Salary (full or part-time):; Leidos Inc.. **J.G. Rohan:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

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Topic: F.07. Biological Rhythms and Sleep

Support: Department of Psychology

Title: Orexin-2 receptor antagonist mitigates fentanyl-induced sleep disruption in C57BL/6J mice

Authors: *N. W. COOPER, D. ZEBADÚA UNZAGA, C. E. OLSON, H. A. BAGHDOYAN; Psychology, Univ. of Tennessee, Knoxville, TN

Abstract: Orexin is an excitatory hypothalamic peptide that promotes wakefulness primarily by activating orexin-2 receptors (OX2Rs) and modulates motivated behaviors by acting at orexin-1 receptors (OX1Rs) (10.3389/fnmol.2018.00220). In humans, dual OXR antagonists are FDA approved for the treatment of insomnia (10.1159/000514963). In rats, the OX2R antagonist JNJ-10397049 enhances sleep (10.1124/jpet.109.152009). Mice are widely used for mechanistic studies of sleep, yet effects of this OX2R antagonist on sleep in mice have not been reported. Opioids are standard of care for pain management despite causing sleep disruption in humans

(10.1124/mol.119.119107). Sleep disruption exacerbates pain, leading to an increase in opioid requirement that further disrupts natural sleep while increasing the risk of addiction and overdose. Fentanyl significantly disrupts sleep in C57BL/6J (B6) mice by increasing wakefulness (10.1152/jn.00266.2021) and eliminates sleep at higher doses (10.1096/fasebj.2021.35.S1.01706). This ongoing study is testing the hypothesis that OX2R antagonism decreases fentanyl-induced sleep disruption. Adult, male B6 mice (n=6) were housed in a 12:12 light/dark cycle. Injections (0.3 mL) were made 1.5 h after lights on or 15 min before the lights off phase. Mice received vehicle (15% DMSO in DI water) or the OX2R antagonist JNJ-10397049 (10 mg/kg) 15 min before receiving a sleep-disrupting dose of fentanyl (0.3 mg/kg). Using a repeated measures, crossover design all mice received all treatments and all mice were tested during light and dark phases. Immediately after fentanyl administration 4-h recordings of EEG and EMG were obtained via subcutaneous telemeters (DSI HD-X02). Wake, NREM sleep, and REM sleep were analyzed in 10-s epochs. One of two sleep scorers was blinded to treatment condition. Data were analyzed using Wilcoxon signed-rank tests. Nine of the 10 significant (P=0.03) changes caused by the OX2R antagonist occurred during the dark phase. Blocking OX2Rs decreased the fentanyl-induced increase in percent wake, NREM latency, and wake duration. The fentanyl-induced increase in wake duration (https://www.abstractsonline.com/pp8/#!/10892/presentation/21199) was also decreased by the antagonist during the light phase. Pretreatment with the OX2R antagonist in the dark phase increased percent NREM, number of wake and NREM episodes, NREM duration, number of wake-to-NREM transitions, and number of NREM-to-wake transitions. These preliminary results are consistent with the wake-promoting role of orexin and suggest that the OX2R may be a viable target for reducing fentanyl-induced sleep disruption.

Disclosures: N.W. Cooper: None. **D. Zebadúa Unzaga:** None. **C.E. Olson:** None. **H.A. Baghdoyan:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.08/L32

Topic: F.07. Biological Rhythms and Sleep

Support: JST OPERA JPMJER2001

Title: Presynaptic proteins play a crucial role in regulating both the quality and quantity of mammalian sleep

Authors: *K. YAMAMOTO[1], Y. JIANG[2], D. TONE[2], R. G. YAMADA[2], H. R. UEDA[2]; [1]Osaka University/RIKEN, Suita/Osaka, Japan; [2]RIKEN (BDR), Suita, Japan

Abstract: Sleep is one of the familiar life phenomena. We become sleepy and fall asleep at night, and when we lack sleep time, we feel more sleep pressure. In the context of sleep study,

sleep pressure is generally defined with NREM delta power, EEG power spectrum density at low frequency (0.5–4.0 Hz) in NREM sleep. However, the molecular basis is still unsolved. Recently, some studies indicated that the phosphorylation in synaptic proteins are upregulated during the accumulation of sleep pressure. Presynaptic proteins are also targets of such phosphorylation, suggesting that synaptic vesicle release also changes dynamically in the sleep-wake cycle. Developmental epileptic encephalopathy (DEE) is a disease characterized by epileptic seizures because of excessive neuroexcitation and developmental delay or regression. In some patients with DEE, sleep disorders have been observed, which suggests a relation between neurotransmission and sleep. However, detailed research on this relation is limited.In this study, we focused on a presynaptic protein in which genetic mutations were found in DEE patients. We expressed the mutant genes in mice brains with adeno-associated virus (AAV) and evaluated sleep phenotype by the Snappy Sleep Stager (SSS) method, a non-invasive sleep analysis method using respiration patterns as an indicator, and electroencephalography/electromyography (EEG/EMG). As a result, we found some mutants that change the sleep duration significantly in SSS measurement. In addition, we found from EEG/EMG analysis on the mice that expresses the mutant gene showed increased NREM delta power. In addition, we examined the response against sleep deprivation to evaluate the effects on sleep homeostasis. Interestingly, the mice with extended sleep showed a significant increase in recovery NREM sleep, whereas the mice with prolonged wakefulness exhibited the shorten recovery NREM sleep. These results suggested that the function of presynaptic proteins is involved in the regulation of sleep duration and the accumulation of sleep pressure. Additionally, we examined sleep regulation by phosphorylation of the target gene and the relation with other sleep-related kinase or phosphatase. In the poster presentation, we also explain the above results.In summary, we found the gene for a presynaptic protein that enhances both sleep and wakefulness, and alters the quality of sleep. Our results suggest that the gene in focus plays a role in the physiological regulation of sleep.

Disclosures: K. Yamamoto: None. **Y. Jiang:** None. **D. Tone:** None. **R.G. Yamada:** None. **H.R. Ueda:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.09/L33

Topic: F.07. Biological Rhythms and Sleep

Support: STI 2030-Major Project 2021ZD0203400
the National Natural Science Foundation of China 82151308
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Optometry and Vision Science, Wenzhou Medical University J01-20190101

Title: 40hz light flicker promotes sleep and glymphatic activity via adenosine signaling

Authors: *J.-F. CHEN;
Wenzhou Med. Univ., Wenzhou, China

Abstract: Light flickering at 40 Hz is receiving increased attention for its ability to reverse pathological features of Alzheimer's disease, and traumatic brain injury in animals, but the neurochemical underpinning is still unclear, which has hampered its therapeutic development. We have recently demonstrate that light flickering frequency-dependently (with maximal effect at 40Hz) triggered an immediate and sustained increase (up to 3 hours after flickering) in extracellular adenosine levels in the primary visual cortex and other brain regions. We uncovered that cortical (glutamatergic and GABAergic) neurons, rather than astrocytes, as the cellular source, and the intracellular adenosine generation from AMPK-associated energy metabolism pathways and adenosine efflux mediated by equilibrative nucleoside transporter-2 (*ENT2*) as the molecular pathway responsible for extracellular adenosine generation. Importantly, brief 40 Hz (but not 20 Hz and 80 Hz) light flickering promote sleep in mice and also children with insomnia by decreasing sleep onset latency, increasing total sleep time. Furthermore, 40 Hz light flickering robustly enhanced glymphatic influx- and efflux- circulations as evident by fluorescence tracing and dynamic contrast-enhanced MRI analyses. This glymphatic effect of 40Hz flikcering is attributed to increased aquaporin-4 polarized expression in astrocyte endfeet and enhanced cerebral blood flow through adenosine signaling. Collectively, our findings establish the ENT2-mediated adenosine signaling as the neurochemical basis for 40 Hz flickering-induced sleep and glymphatic activity. This also reveals a novel non-invasive strategy for promoting sleep and enhancing glymphatic activity, with high translational potential to eliminate metabolic waste from the brain. The intrinsic link between the high cellular metabolism imposed by gamma oscillation and adenosine generation, together with the multiple physiological effects of adenosine as a dual neuromodulator, homeostasis regulator, uniquely position the adenosine signaling system to serve as the unique and pivotal neurochemical basis for the biological effects of 40 Hz light flickering.

Disclosures: J. Chen: None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

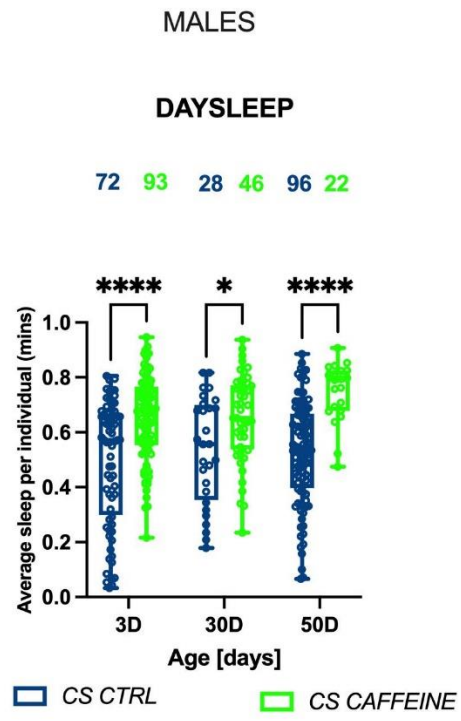
Program #/Poster #: PSTR236.10/Web Only

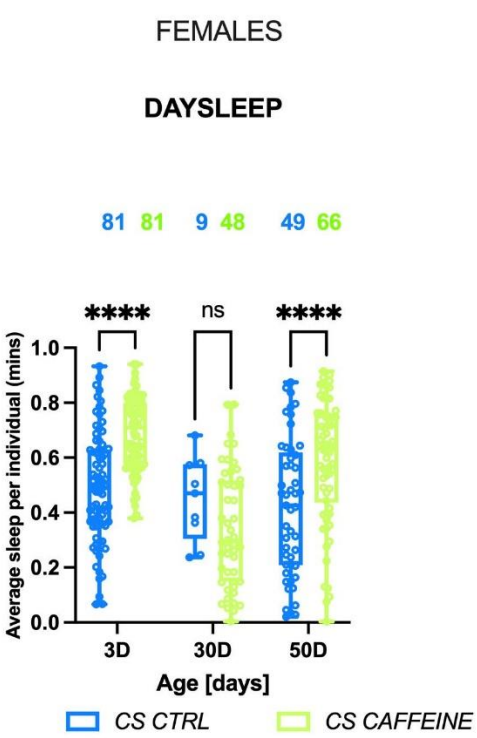
Topic: F.07. Biological Rhythms and Sleep

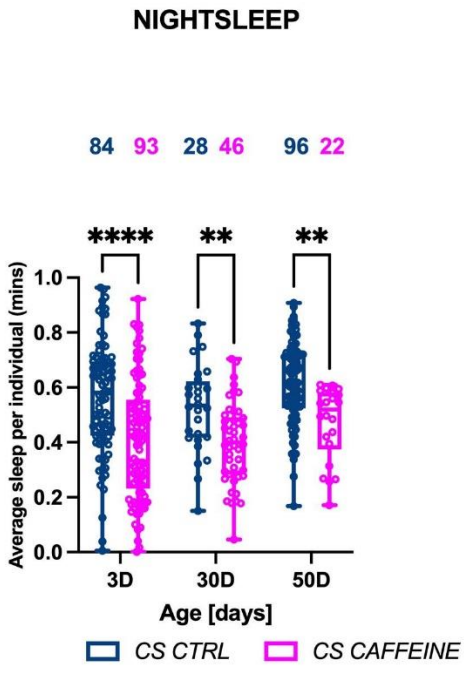
Title: Influence of caffeine on siesta and nighttime sleep in Drosophila melanogaster

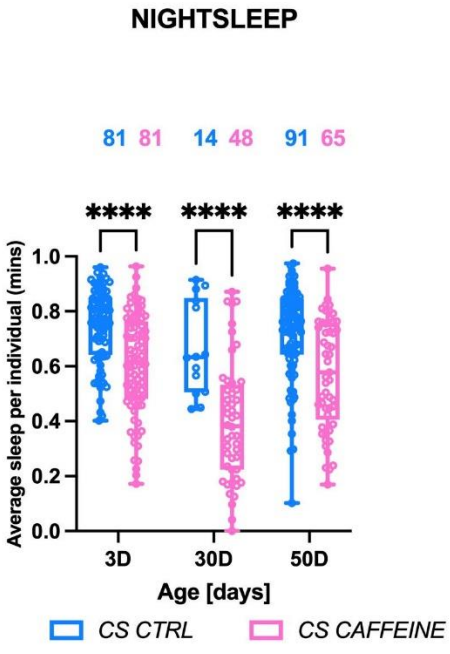
Authors: *D. M. BHATTACHARYA;
Neurosci., Sheffield Inst. for translational Neurosci., Sheffield, United Kingdom

Abstract: Caffeine, a plant-derived psychostimulant, has been shown to decrease sleep and enhance dopaminergic neuron activity in mammals by competitive antagonism to adenosine receptors (AdoR). Interestingly, it also reduces sleep in the fruit fly Drosophila melanogaster. However, whether it influences daytime sleep (siesta) as much as nighttime sleep and whether this is executed by the adenosine signalling pathway has not yet been determined. It is also unknown whether its influence varies with sex and age and what types of cells it influences the most, those that are involved in sleep timing and regulation (clock cells), or those that are crucial for voluntary movement (dopaminergic neurons). To answer these questions, we examined the role of caffeine in both siesta and nighttime sleep of males and females of Canton-S wild-type flies (WT) of different age (3d, 30d and 50d), and in transgenic flies with overexpressed or silenced dAdoR in all neurons (using the pan-neuronal driver elav-Gal4), circadian clock cells (tim-expressing neurons and pdf-expressing neurons), glial cells, and dopaminergic neurons (th-expressing cells). We found that females are more sensitive to caffeine than males and that older flies sleep longer during the day than young ones. However, caffeine treatment decreases the siesta of flies with overexpression of dAdoR in all neurons, clock cells, and th-expressing cells. In turn, silencing of dAdoR increases siesta. Therefore, the observed differences in the daytime sleep of D. melanogaster appear to depend on signalling through adenosine receptors. In our study, we found that the concentration of the caffeine, the sex, and age of flies, their genetic background, can modulate the effect of caffeine on daytime and nighttime sleep. All of this can help us to examine more deeply the caffeine signalling mechanism in Drosophila, which may be important for clinical trials of adenosine receptor-based therapy.









Disclosures: D.M. Bhattacharya: None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.11/L34

Topic: F.07. Biological Rhythms and Sleep

Title: Sleep evaluation in cynomolgus monkeys using polysomnography

Authors: *Y. HORIMOTO[1], K. HAYASHIDA[2], T. UCHINO[2], Y. NUMATA[2], Y. MITSUKURA[3];
[1]Shin Nippon Biomed. Labs., kagoshima, Japan; [2]Shin Nippon Biomed. Labs., Ltd., Kagoshima, Japan; [3]Keio Univ., Kanagawa, Japan

Abstract: Rodents are widely used in sleep research, despite their sleep pattern differing from humans due to their nocturnal nature. Conversely, non-human primates are diurnal and share many similarities with humans in not only sleep pattern, but also anatomy brain function. Thus, sleep evaluation in non-human primates may provide more translational value compared to other animal species. Polysomnography is the gold standard in assessing sleep stages using electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG).In this study, we investigated the effects of lemborexant (orexin receptor blocker) and ramelteon (melatonin receptor agonist) on sleep in cynomolgus monkeys. In addition, we preliminarily evaluated sleep stages using only electrocardiography (ECG) in several animals. During the experiments, six male cynomolgus monkeys (5-8 years old) were housed individually in a room with a 12 h light/dark cycle (lights on from 7:00-19:00). Vehicle, ramelteon (0.1 mg/kg), and lemborexant (3 mg/kg) were orally administered at approximately 17:00. EEG, EOG, and EMG were recorded from implanted electrodes, and the data during the dark cycle (19:00-7:00) were analyzed using NeuroScore software (Data Sciences International, MN, USA). Sleep stages were classified into wake; rapid eye movement (REM); and non-REM sleep N1, N2, and N3. Lemborexant significantly increased the duration of non-REM N3, total non-REM, and total sleep compared to the vehicle. Ramelteon had no significant effect on any sleep stage or total sleep when compared to the vehicle. Preliminary sleep evaluation using only ECG showed similar distributions of sleep stages in the vehicle-, lemborexant-, and ramelteon-treated animals to sleep evaluation from Polysomnography. Although further studies are required, the present results indicate that lemborexant, but not ramelteon, at the doses in the current study, increased non-REM sleep time in cynomolgus monkeys, and that sleep stage evaluation using only ECG may be a useful non-invasive method for sleep research in monkeys.

Disclosures: Y. Horimoto: None. **K. hayashida:** None. **T. Uchino:** None. **Y. Numata:** None. **Y. Mitsukura:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.12/L35

Topic: F.07. Biological Rhythms and Sleep

Support: NIGMIS T32 Grant GM132494
NCI Grant 5R21CA276027-02

Title: Orexin neuronal activity and disrupted sleep are driven by tumor-produced ghrelin in a murine model of breast cancer

Authors: *B. D. ELLIOTT[1], C. O. KISAMORE[1], W. H. WALKER[1,2], R. J. NELSON[1,2], A. C. DEVRIES[1,2,3];
[1]Neurosci., West Virginia Univ., Morgantown, WV; [2]Cancer Institute, West Virginia University, Morgantown, WV; [3]Medicine, West Virginia University, Morgantown, WV

Abstract: Sleep disturbances are common for individuals with breast cancer and are associated with reduced quality of life, increased symptom severity, and elevated mortality risk from all causes. In terms of reduced quality of life, nearly 80% of breast cancer survivors report insomnia and fatigue. Animal models of breast cancer are consistent with the clinical findings, as mice with mammary tumors exhibit fragmented NREM sleep and decreased bouts of wake during their active periods. We have previously demonstrated that tumors are associated with an increase in wake-promoting orexin/hypocretin (OH) neuronal activity. This upregulation is independent of peripheral and central pro-inflammatory cytokine levels. Additionally, ghrelin concentrations are increased in the serum of tumor-bearing mice relative to tumor-free mice. Further studies revealed that mammary tumors express ghrelin mRNA. As ghrelin binds to growth hormone secretagogue receptor 1 alpha located on OH neurons, we sought to determine the significance of tumor-produced ghrelin on sleep. We hypothesized that tumor-evoked ghrelin production is functionally relevant for the increase in OH activity and sleep disruption observed in mice with mammary tumors. To test this, mice were implanted with sleep recording telemetry and received bilateral 100 Âµl orthotopic injections of nonmetastatic 67NR cancer cells (1.0x105 cells/injection) or a vehicle (DMEM) into the fourth and ninth inguinal mammary glands. Fifteen days after inoculation, we provided a once daily injection of the ghrelin antagonist JMV2959. Once tumors reached a size of 1.5 cm, tissues were collected mid-dark phase. To assess OH activity, hypothalamic brain sections were double-stained to determine the percentage of OH neurons expressing cFos. We predicted that OH activity would be increased in tumor-bearing mice, and that administration of JMV2959 would normalize OH activity and restore sleep. To test our hypothesis that ghrelin produced by the tumor specifically is driving disrupted sleep, we next used CRISPR-Cas9 to knock out the genetic sequence for ghrelin in 67NR cells and generated two identical GHRL-/- cell lines (1G9 and 2G2). Mice underwent sleep transmitter implant as described and were then inoculated with either the 67NR, 1G9, or 2G2 cells. We predicted that mice bearing tumors generated from the GHRL-/- cell lines would demonstrate normalized OH activity and sleep. Together, these studies highlight a novel mechanism by which nonmetastatic breast cancer is capable of altering the brain and behavior. Future studies will

address the causal role of OH neuronal activation in disrupting sleep in individuals with mammary cancer.

Disclosures: B.D. Elliott: None. **C.O. Kisamore:** None. **W.H. Walker:** None. **R.J. Nelson:** None. **A.C. DeVries:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.13/L36

Topic: F.07. Biological Rhythms and Sleep

Support: R21 AG080335 (to AP)
2RF1 AG050518 (to JRF)

Title: Interplay between aging, sleep, and kynurenic acid: Focus on orexin activation in the lateral hypothalamus

Authors: *M. REESE, S. MILOSAVLJEVIC, J. R. FADEL, A. POCIVAVSEK;
Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Sleep is a vital process, as it plays an important role in daily function such as maintaining optimal metabolic balance. Throughout the lifespan, disturbances in sleep, which can be experimentally modeled by sleep deprivation (SD) in rodents, may contribute to age-related decline in brain function. The lateral hypothalamus (LH) is a brain region that regulates sleep-wake homeostasis, and it is particularly susceptible to age-associated neurogenic changes. With advanced age, the number of neurons expressing the neuropeptide orexin within the LH is significantly reduced. However, little is known about the relationship between age-related dysfunction of the LH and the kynurenine pathway (KP) of tryptophan metabolism. Aging is characterized by the accumulation of KP metabolites, including kynurenic acid (KYNA), a neuromodulator synthesized by kynurenine aminotransferase II (KAT II) enzyme. When sleep is dysregulated, brain KYNA levels are also higher.We hypothesize that SD-induced accumulation of KYNA contributes to exacerbated disruptions of orexinergic activation in the aged brain. To assess activation of orexinergic neurons following SD, we evaluated the colocalization of cFos, a marker for immediate-early neuronal activation, and orexin in the LH of male and female rats using dual label immunohistochemistry (IHC). We determined the percentage of activated orexinergic neurons in young (3-4 months; N = 3-7 per group) and old (22-24 months; N=3-7 per group) male and female Fischer 344/Brown Norway F1 hybrid rats. Experimentally, the rats received either ad libitum sleep or underwent SD from Zeitgeber time (ZT) 0, the onset of light phase, to ZT6 using automated sleep disruption chambers. To inhibit de novo KYNA synthesis in the brain, rats were treated with KAT II inhibitor (PF-04859989, 30 mg/kg, s.c) at ZT0, or vehicle. At ZT6, animals were euthanized, and brain tissue was collected for IHC analysis. Employing peroxide activated diaminobenzidine (orexin) with nickel (cFos) reaction, we

observed that there was an interaction between sleep and sex, notably in males ($P < 0.01$). Under ad lib sleep, old rats had higher orexin activation compared to young rats. With SD, orexin activation increased in young rats, but surprisingly decreased in old rats. Treatment with KAT II inhibitor prevented the basal elevation in orexin activation with age and mitigated the impacts of SD ($P < 0.05$). Taken together, we determined that SD influences activation of orexinergic neurons in an age-dependent manner, and place novel attention on KAT II inhibition, a strategy to reduce excessive KYNA, as a potential therapeutic target to reduce the effects of sleep loss as aging progresses.

Disclosures: M. Reese: None. **S. Milosavljevic:** None. **J.R. Fadel:** None. **A. Pocivavsek:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.14/L37

Topic: F.07. Biological Rhythms and Sleep

Support: R21 AG080335 (to AP)
R01 NS102209 (to AP)

Title: Brain-specific elevations in kynurenic acid reduces sleep duration in rats

Authors: *C. A. GRANT, K. M. RENTSCHLER, C. J. WRIGHT, A. POCIVAVSEK; Pharmacology, Physiology, and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Individuals who suffer from neurocognitive disorders, such as age-related dementias or schizophrenia, often suffer from sleep disturbances. Kynurenic acid (KYNA) is a tryptophan metabolite implicated in the pathophysiology of these disorders. Modest increases in KYNA, which acts as an antagonist at N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine (α7nACh) receptors, result in cognitive impairments and alterations in sleep-wake behavior (Pocivavsek et al. *Sleep* 2017). Specifically, KYNA negatively impacts rapid eye-movement (REM) sleep and increases wakefulness. The goals of the present project were to determine the impact of a dose response (0 µM, 1 µM, 3 µM, 10 µM; dissolved in PBS) elevation of KYNA locally in the brain. Wistar rats (N=10 female, 7 male) were cannulated with guide targeting the lateral ventricle and simultaneously implanted with telemetry devices to record electroencephalogram (EEG) and electromyogram (EMG) polysomnography. Upon a week of recovery from surgical procedures, a within animal design employed intracerebroventricular (ICV) infusion to deliver each dose of KYNA (1 µl/min for 10 min) at Zeitgeber time (ZT) 0, the start of the light phase, to rats across different 24-hour recording days. Vigilance states were classified into wake, REM sleep and non-REM (NREM) sleep by an artificial intelligence neural network in 10 sec epochs and reviewed by an expert hand-scorer . We presently evaluated vigilance state durations from ZT 0-4 and found a dose-dependent impact of KYNA on sleep

duration. Notably, 3 µM KYNA increased wake duration by 35.01% ($P<0.01$), whereas 10 µM KYNA resulted increased wake duration by 41.91% ($P<0.05$), reduced REM sleep duration by 37.97% ($P<0.01$), reduced NREM sleep duration by 32.47% ($P<0.05$) between ZT 0-2 when compared to vehicle infusion. Importantly, the impact of KYNA on sleep-wake behavior was transient, as vigilance state durations returned to basal levels (vehicle infusion) from ZT 2-4, which also corresponds to the timeframe wherein exogenous application of KYNA was estimated from the brain. Our findings provide novel support for the hypothesis that brain-specific elevations in KYNA cause a significant decrease in NREM and REM sleep and an increase in wakefulness. Taken together, the implications of our study place further attention on the role of the kynurenine pathway, a pharmacologically targetable metabolic pathway, and KYNA in regulating sleep behavior.

Disclosures: C.A. Grant: None. **K.M. Rentschler:** None. **C.J. Wright:** None. **A. Pocivavsek:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.15/M1

Topic: F.07. Biological Rhythms and Sleep

Support: R21 AG080335 (to AP)
2RF1 AG050518 (to JRF)

Title: Effects of kynurenine aminotransferase II inhibitor on age-associated changes in sleep

Authors: *S. MILOSAVLJEVIC, J. A. MCQUAIL, J. R. FADEL, A. POCIVAVSEK; Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Adverse effects of insufficient sleep progress with aging, wherein sleep disturbances may contribute to exacerbated cognitive decline and onset of neurodegenerative disorders including Alzheimer's disease and Parkinson's disease. We hypothesize that kynurenic acid (KYNA), an endogenous antagonist of cholinergic neurotransmission, plays a mechanistic role in age-related sleep dysfunction. The small molecule KYNA is an astrocyte-derived metabolite of the kynurenine pathway of tryptophan catabolism, synthesized primarily by kynurenine aminotransferase II (KAT II) in the brain. An excess of KYNA in the brain results in dysfunctional sleep architecture (Pocivavsek et al. 2017 *Sleep*). Male Fischer 344 x Brown Norway F1 hybrid rats (N=8, 6 months; N=8, 24 months) were implanted with telemetry transmitters for continuous electroencephalography (EEG) and electromyography (EMG) data acquisition. EEG/EMG waveforms were annotated in 10-sec epochs and classified into rapid eye movement (REM) sleep, non-REM (NREM) sleep and wakefulness by a trained artificial neural network, a supervised predictive machine learning technique with high accuracy, and reviewed by an expert hand-scorer. Aged rats exhibited increased NREM sleep duration and reduced wake

duration during the latter half of the dark phase (P<0.01). The higher number of REM bouts (P<0.001), NREM bouts (P<0.001) and wake bouts (P<0.001) in the dark phase in aged rats suggests more fragmented sleep. Next, we pharmacologically inhibited the KAT II enzyme to reduce brain KYNA levels and improve sleep behavior in aged rats. We previously established that brain penetrable irreversible KAT II inhibitor PF-04859989 had a long-lasting effect on sleep-wake homeostasis (Milosavljevic et al. 2023 *Transl Psychiatry*). As our present findings pointed to sleep disruptions primarily during the dark phase, rats were treated with vehicle or PF-04859989 (30mg/kg, s.c.) at Zeitgeber time (ZT) 6 and sleep quality was evaluated for 24 hours. KAT II inhibitor treatment restored sleep-wake behavior in aged rats such that NREM sleep and wake durations between ZT18-24 were at the level of young rats. Sleep architecture was also more consolidated in aged rats following KAT II inhibitor treatment with a reduced number of REM sleep, NREM sleep and wake bouts in the dark phase. Taken together, our data suggest that mitigating elevations in KYNA in aged rats effectively treats sleep-wake behavioral abnormalities experienced with aging. Reducing brain KYNA levels via inhibition of KAT II enzyme could be a potential therapeutic target for improving deteriorating sleep quality associated with aging.

Disclosures: S. Milosavljevic: None. **J.A. McQuail:** None. **J.R. Fadel:** None. **A. Pocivavsek:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.16/M2

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant 5R01MH092638

Title: The Effect of Zolpidem on Sleep Physiology and Declarative Memory over a Nap in Schizophrenia

Authors: *J. ZHANG[1], D. MYLONAS[1], M. SJOEGAARD[1], C. TALBOT[2], D. S. MANOACH[1];
[1]Massachusetts Gen. Hospital/ Harvard Med. Sch., CAMBRIDGE, MA; [2]Arizona State Univ., Tempe, AZ

Abstract: Background Schizophrenia (SZ) is characterized by abnormal sleep physiology that correlates with cognitive deficits and symptoms. Sleep spindles, slow oscillations (SOs), and their temporal coupling have been shown to promote sleep-dependent memory consolidation. We showed that although eszopiclone enhanced sleep spindles, it did not improve memory, potentially due to the disruption of spindle-SO coupling. Previous research has demonstrated that zolpidem, a $GABA_A$ agonist, can enhance declarative memory by boosting sleep spindles while preserving the integrity of spindle-SO coupling in healthy adults. In this study, we investigate

whether zolpidem has similar benefits on memory and sleep physiology in SZ. Methods Twelve SZ patients and 13 healthy controls (HC) were included in the study that comprised an adaptation and two counterbalanced experimental naps. On experimental days, all participants completed a Word Pair Task around noon, followed by either placebo or 10mg Zolpidem. They then took a nap with high-density EEG before being retested on the task. We investigated the drug effect on spindles, SOs and their coupling for both SZ and HC across all electrodes. We controlled for multiple comparisons using cluster-based permutations. We also calculated Pearson correlation coefficients between the change in memory performance over a nap and sleep oscillations. Results Zolpidem significantly increased spindle density (p=0.001) while preserving SO density, coupled density and the phase of the coupling in both groups. However, neither increases in spindle density in zolpidem vs. placebo, nor spindle density during either nap correlated with memory. Nor did coupled density during either treatment condition correlate with memory. Memory consolidation did not differ between groups or treatment conditions (ps>0.3). Conclusions Contrary to our expectations, although zolpidem increased spindle density, it did not significantly affect sleep-dependent memory consolidation. This may reflect that the study is under-powered, or the memory task lacks sensitivity. Alternatively, the absence of a memory effect could be attributed to zolpidem's failure to also enhance SO-spindle coupling. Zolpidem may have increased isolated spindles, as coupled density was not affected despite an overall increase in spindle density. These findings are in line with prior research showing that coupled spindles are stronger predictors of memory than overall spindle activity.

Disclosures: J. Zhang: None. **D. Mylonas:** None. **M. Sjoegaard:** None. **D.S. Manoach:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.17/M3

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R35 NS132223
NIH NHLBI T32 5T32HL007909-25

Title: Molecular and cellular mechanisms by which GABAergic neurons control Drosophila sleep

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Abstract: Inadequate sleep furthers the progression of diseases like Alzheimer’s and depression and contributes to non-neurological conditions like diabetes, obesity, and heart disease. A key hurdle in effectively treating these conditions is understanding the fundamental elements of our biology that necessitate sleep, and by extension the structures and molecular pathways that sense

sleep need and effect sleep drive. To do this we leveraged the genetic amenability and economies of scale of *Drosophila* to activate different subsets of neurons using a library of GAL4 drivers and UAS-TrpA1 to screen for neurons which regulate sleep when activated. We found that activation of GABAergic neurons using the promoter of the vesicular GABA transporter (*vGAT*) driving GAL4 both acutely induced sleep and reduced subsequent sleep drive. To investigate what genes are activated within the neurons captured by vGAT-GAL4 after spontaneous sleep at the end of the night, wake at the end of the day, and after extended (12h) night-time sleep deprivation, we sorted vGAT-GAL4 labeled neurons using FACS and performed RNA sequencing. We found that transcripts encoding genes involved in mitochondrial oxidative phosphorylation are among the most significantly upregulated following sleep deprivation and after a day of normal wakefulness, suggesting enhanced energetic demands after extended wake. To further narrow down which GABAergic neurons regulate sleep we used the intersectional split GAL4 approach with an activation domain (AD) driven by the glutamic acid decarboxylase 1 (*Gad1)* promoter and DNA binding domains (DBD) driven by enhancers for genes identified in GABAergic clusters of the fly brain single cell atlas. We found 11 split combinations which strongly induced sleep during activation with TrpA1. These results suggest there may be at least one discrete population of GABAergic cells in *Drosophila* which regulate sleep and/or sleep drive, and similar to what has been described in other sleep regulating neurons there may be a role for mitochondria in the sensing or transduction of sleep need within these GABAergic neurons.

Disclosures: A. Perera: None. **C. Rosensweig:** None. **R. Allada:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.18/M4

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AG062398

Title: Acute Sleep Deprivation Significantly Affects Hippocampal Gene Regulation in Aged Animals

Authors: *C. REMEDIES[1], N. A. STORCH[2], W. G. PLEDGER[2], Y. VANROBAEYS[4], T. ABEL[4], L. C. LYONS[3];
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Abstract: Acute sleep deprivation affects more than one third of the U.S. population, resulting in memory and cognitive performance deficits, and increased risk of metabolic and neurological disorders. In older adults (age 65+), sleep deprivation occurs at an even higher frequency (~50%)

and co-occurs with multiple age-related diseases including dementia and stroke, potentially exacerbating these pre-existing conditions. To date, the vast majority of sleep deprivation research has been performed in animal models in young populations leaving a gap in understanding the relationship between sleep deprivation and age-related diseases. Given the interactions of sleep deprivation with many neurological disorders and neurodegenerative diseases, we investigated gene expression changes in the hippocampus of 22-month-old male C57BL/6J mice following 5 hours of sleep deprivation. The hippocampus plays a critical role in spatial memory and is highly susceptible to the effects of acute sleep deprivation. Mice were gently handled for 5 hours (tapping and cage shakes as needed) to induce a sleep-deprived state. Sleep deprived and non-sleep deprived animals were dissected at the same time to avoid gene expression change confounds due to variations in circadian time. Unbiased RNA sequencing revealed 471 differentially expressed genes (DEG) in the hippocampus following acute sleep deprivation in old males. In comparison with our previously published data on young males (3-4 months) given the same treatment, 116 of the 471 old male DEGs were shared between young and old males, with 355 DEGs unique to old males. As seen by gene ontology pathway analysis of molecular function and biological processes, pathways significantly affected by acute sleep deprivation in young and old animals included protein kinase inhibitor activity, protein phosphatase activity, and regulation of RNA splicing. Gene changes unique to aged males were significantly enriched in pathways for negative regulation of synapse organization, multicellular organismal homeostasis, and potassium ion transmembrane transporter activity. Genes involved in negative regulation of synapse organization could exacerbate age-related changes in neural function and plasticity seen in aged populations. Thus, even in animals that may have age-related changes in sleep patterns and gene regulation, acute sleep deprivation still has a significant effect on gene regulation. The current study provides insights into age specific changes induced by sleep deprivation and will aid in our understanding of the role sleep deprivation plays in age-related disease susceptibility.

Disclosures: C. Remedies: None. **N.A. Storch:** None. **W.G. Pledger:** None. **Y. Vanrobaeys:** None. **T. Abel:** Other; Scientific Advisory Board of EmbarkNeuro and Scientific Advisor to Aditum Bio and Radius Health. **L.C. Lyons:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.19/M5

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AG062398

Title: Molecular resilience to acute sleep deprivation in females

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Abstract: Sleep deprivation represents a significant public health problem, with more than one third of U.S. adults experiencing sleep deprivation on a regular basis. The hippocampus is particularly susceptible to the effects of acute sleep deprivation with changes seen in gene expression, cellular signaling, synaptic plasticity and long-term memory. Evidence is increasing for sex-specific behavioral differences following acute sleep deprivation. However, almost all of the molecular studies on sleep deprivation have examined changes in males, particularly in rodent studies. To address this knowledge gap, we investigated changes in gene expression following acute sleep deprivation in female mice. Using an unbiased RNA sequencing approach, we found that females appeared more resilient to changes in gene expression in the hippocampus after acute sleep deprivation. Compared to the 636 significant changes in hippocampal gene expression seen in male mice, we found only 99 genes in the hippocampus exhibited significant differential expression from sleep deprived females compared to non-sleep deprived females. Moreover, in the proestrus stage during peak estrogen levels, female mice had no significant changes in hippocampal gene expression following sleep deprivation. We hypothesize that hormone signaling, particularly estrogen, may mediate the molecular resilience observed across estrous stages to sleep deprivation. Estrogen targets two distinct receptor families, ERα/ERβ and G protein-coupled estrogen receptor (GPER1), the latter involving activation of kinase signaling cascades including cAMP/PKA signaling. Because sleep deprivation is known to impair cAMP/PKA signaling, we investigated whether pharmacological inhibition of GPER1 signaling altered the molecular resilience to sleep deprivation in females. Following 5 hr of sleep deprivation using gentle handling, brain tissues were collected. Non-sleep deprived mice were dissected at the same time to avoid any confounds in gene expression due to variations in circadian time. Estrous stage of the mouse was determined post-sacrifice. Using qPCR, we assessed gene expression from G-15 treated sleep deprived and non-sleep deprived female animals and found that inhibition of GPER1 signaling pathways with G-15 diminished the molecular resilience seen in females to sleep deprivation. Although this study does not address the mechanisms through which sleep deprivation induces changes in gene expression, these results suggest that estrogen signaling through GPER1 contributes, in part, to the molecular resilience observed in females.

Disclosures: N.A. Storch: None. **P.J. Rote:** None. **C.E. Remedies:** None. **Y. Vanrobaeys:** None. **T. Abel:** Other; Scientific Advisory Board of EmbarkNeuro and Scientific Advisor to Aditum Bio and Radius Health. **L.C. Lyons:** None.

Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR236.20/M6

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Fellowship F31HL165896 to WPA
NINDS Grant R01NS131664 to ACJ and DCM

Title: Characterization of cell type-specific synaptic protein expression and function in hypothalamic hypocretin/orexin (H/OX) neurons

Authors: *W. P. ARMSTRONG IV[1], D. C. MARTINELLI[2], M. E. CARTER[3], A. C. JACKSON[1];
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Abstract: Hypocretin/orexin (H/OX) neurons are a lateral hypothalamic cell population defined by their expression of the H/OX neuropeptide and are critical regulators of sleep-wake states. Dysfunction of the H/OX system in multiple species leads to severe sleep-wake disruptions, such as the human sleep disorder narcolepsy. Despite a large body of work investigating the circuitry and behavioral role of H/OX neurons, comparatively little is known about the molecular mechanisms underlying the function of their excitatory, co-transmitting synapses. In probing our lateral hypothalamic single-cell RNA sequencing database, we identified a suite of transcripts encoding putative synaptic proteins that appear to be specifically enriched in H/OX neurons. One of these transcripts, *C1ql3,* encodes complement component 1q-like protein 3 (C1QL3), and is uniquely and robustly expressed in H/OX neurons within the lateral hypothalamus. In other brain regions, C1QL3 is known to be critical for the stability of excitatory synapses. We therefore hypothesized that C1QL3 may play an important role in the function and integrity of H/OX synapses and may regulate their wake-promoting effects throughout the brain. To accomplish this, we first developed a novel epitope-tagged knockin mouse to localize C1QL3 protein expression in both H/OX cell bodies and their axons in regions of the brain known to be densely targeted by H/OX axons and implicated in the regulation of sleep-wake states. We next conditionally knocked out *C1ql3* from H/OX neurons of both male and female mice, resulting in diminished axonal H/OX+ puncta in the locus coeruleus (LC), a key target region of H/OX axons, without affecting the total hypothalamic H/OX cell number in conditional knockout mice compared to uninjected controls. Additionally, we performed blinded EEG/EMG analysis of *C1ql3* conditional knockout mice to study the sleep-wake behavior of mice lacking *C1ql3* in H/OX neurons. We observed multiple disruptions in sleep-wake architecture, consistent with some key signatures of H/OX dysfunction in both mice and human narcolepsy. These results support our hypothesis that C1QL3 may be an important regulator of both H/OX synaptic function and sleep-wake behavior.

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Poster

PSTR236: Sleep Regulation: Molecular, Cellular, and Pharmacological

Location: MCP Hall A

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Program #/Poster #: PSTR236.21/M7

Topic: F.07. Biological Rhythms and Sleep

Support: NIMH Grant R01MH112739

Title: Parallel strategies for genetic targeting of transcriptionally-distinct melanin-concentrating hormone (MCH) neuron subpopulations in the lateral hypothalamus

Authors: *M. S. ANTONY[1,2], L. A. SCHWARZ[3], A. C. JACKSON[2];
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Abstract: The lateral hypothalamic area (LHA) coordinates crucial innate behaviors through heterogenous, yet poorly understood neuronal cell types and circuits. Melanin-concentrating hormone (MCH; encoded by *Pmch*)-expressing neurons, are uniquely expressed in the LHA and are key contributors to this complex physiological control. In rodents, excitation of MCH neurons leads to increases in REM sleep and increases in wake-related behaviors, such as feeding, exploration, and learning. Given its paradoxical role in promoting diverse wake- and sleep-related behavioral states, it is hypothesized that MCH neurons are functionally and molecularly heterogenous. Building upon foundational anatomical and developmental studies, our single cell transcriptomic analysis of the LHA described a large set of discriminatory markers that identify MCH neuron subpopulations. These subpopulations are defined by the presence (MCH^{Tacr3+}) or the absence (MCH^{Tacr3-}) of *Tacr3*, which encodes the neurokinin-3 receptor of the tachykinin family (NK3R). We hypothesize that MCH subpopulations operate through functionally distinct, parallel subcircuits that exhibit unique neuroanatomical projections and synaptic properties, and that *Tacr3* expression may be leveraged to dissociate these subcircuits. Our project has defined a multi-pronged approach to gain genetic access to MCH^{Tacr3+} and MCH^{Tacr3-} subpopulations and their neuroanatomical projection targets through use of novel mouse lines, intersectional viruses and cell type-specific cell ablation reagents. We first demonstrated the cell type specificity of the proposed mouse lines, viruses and reagents through fluorescence *in situ* hybridization and immunohistochemistry in both male and female mice. After validation of these novel genetic tools, we identified brain subregions with distinct enrichment of MCH^{Tacr3+} or MCH^{Tacr3-} axonal projections. Finally, we used slice electrophysiology and optogenetics to interrogate MCH subcircuits *in vitro* and to understand MCH synaptic properties in identified subregions. Through this multi-pronged approach, we aim to characterize the unique anatomical properties of MCH subcircuits to further our understanding of circuit-level synaptic and behavioral mechanisms mediated by transcriptionally-distinct MCH subpopulations.

Disclosures: M.S. Antony: None. **L.A. Schwarz:** None. **A.C. Jackson:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.01/M8

Topic: G.02. Reward and Appetitive Learning and Memory

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Title: Calibration of context-evoked feeding by a dorsal hippocampus-prodynorphinergic lateral septum-to-lateral hypothalamus circuit

Authors: *T. GOODE[1,2,3,4], B. FERRIS[5,6], J. B. ALIPIO[1,2,3,4], D. CHIZARI[5,6], N. SACHDEV[5,6], A. BESNARD[1,2,3,4], D. PATHAK[1,2,3,4], M. KRITZER[1,2,3,4], A. CHUNG[1,2,3,4], X. XU[7], X. DUAN[8], E. MACOSKO[5,6], A. SAHAY[1,2,3,4];
[1]Harvard Med. Sch., Boston, MA; [2]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA; [3]Harvard Stem Cell Institute, Cambridge, MA; [4]BROAD Institute of Harvard and MIT, Cambridge, MA; [5]BROAD Inst. of Harvard and MIT, Cambridge, MA; [6]Stanley Center for Psychiatric Research, Cambridge, MA; [7]Univ. of California, Irvine, CA; [8]Univ. of California, San Francisco, CA

Abstract: Adaptive initiation or termination of motivated food-seeking behavior is thought to depend on the successful recognition, linkage, and relay of internal states, external contextual cues, and food reward outcomes. While diverse sources of subcortical inputs to the lateral hypothalamus (LH) are thought to regulate consumption behaviors in response to internal states, we know less about how sensory and contextual information from cortical sites and the hippocampus contributes to feeding. Human brain imaging has identified dysregulation of a dorsal hippocampal-LH network in binge eating, but mechanistic instantiation of such a pathway is lacking. Here, we deploy single-nucleus RNA sequencing, anterograde and retrograde viral synaptic mapping, *ex vivo* synaptic physiology, conditional mouse genetics, and cell- and pathway-specific optogenetics and behavior to define such a pathway in mice. We identify an evolutionary conserved and discrete *Prodynorphin*-expressing subpopulation of *Somatostatin*-expressing inhibitory neurons (*Pdyn* INs) in the dorsolateral septum (DLS) that receives primarily dorsal, but not ventral, hippocampal inputs. *Pdyn* INs inhibit LH GABAergic inhibitory neurons and confer context- and internal state-dependent calibration of feeding. Viral deletion of *Prodynorphin* in the DLS mimicked effects seen with optogenetic silencing of DLS *Pdyn* INs, suggesting a potential role for PRODYNORPHIN-KAPPA OPIOID RECEPTOR signaling in the DHC-DLS-LH pathway in contextual regulation of food reward-seeking. Together, our results suggest that the hippocampus has evolved to recruit an ancient feeding circuit module through *Pdyn* DLS INs to link contextual information and consumption.

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Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.02/M9

Topic: G.02. Reward and Appetitive Learning and Memory

Support: This work was supported by the DICBR of the NIAAA [ZIA AA000455 to AJK].

Title: A role for medial septum glutamate neurons in acquisition of reward seeking behaviors

Authors: *M. MARKS[1], S. RAMOS-MACIEL[2], A. KESNER[3];
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Abstract: In order to survive, all animals must be driven to explore their environment in a goal directed manner. Behaviors that lead to goals are reinforced by the rewarding properties of those goals, and animals learn to repeat these behaviors. The behaviors involved in such processes are termed reward seeking behaviors. A hallmark of many psychiatric illnesses, including substance use disorders, is maladaptation in reward seeking. The medial septum (MS) is a brain region with canonical roles in learning, navigation, and theta rhythmicity- all cognitive processes with importance in exploration- but its role directly in reward seeking is unclear. Recent studies have shown that glutamate neurons in the MS (MS^{GLU}) are reinforcing when stimulated with optogenetics, can influence mesolimbic dopamine activity, and optogenetic excitation of MS^{GLU} neurons can enhance exploratory locomotion. Yet, how these properties of optogenetic excitation of MS^{GLU} neurons factor into reward seeking in a naturalistic manner remains unknown. We recently found that chemogenetic excitation of MS^{GLU} neurons enhances strategy switching in mice (a form of cognitive flexibility). In the current set of experiments, we continued using a chemogenetic approach to further our understanding of how MS^{GLU} neurons contribute to exploratory/reward seeking behaviors in a novel and familiar environment and hypothesized that perturbing these neurons would alter acquisition of operant responding for a natural reward. We first found that enhancing MS^{GLU} neuron activity enhanced exploratory behavior, such that these mice do not habituate to an open field arena over three days of exposure, despite not hyper-locomoting on day 1 of exposure. We also found that inhibiting MS^{GLU} neurons hinders acquisition of a simple operant reward-seeking task (FR1). Once the task was learned, however, subsequent inhibition no longer altered rewarding-seeking behavior, suggesting that MS^{GLU} neurons are important to the initial learning of a reward-related association, potentially via a mechanism involved with environmental exploration that leads to association of action-outcome learning. While there is still more to learn about MS^{GLU} neurons during reward seeking, our research suggests that these neurons, along with other neurons within the MS, may be a therapeutic target for substance abuse and other psychiatric illnesses related to maladaptation in reward seeking.

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Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.03/M10

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant F31MH133373

Title: A dorsoventral gradient of reward representations in the lateral septum

Authors: *J. ISAAC[1], S. KARKARE[2], H. BALASUBRAMANIAN[3], M. MURUGAN[4];
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[4]Princeton Neurosci. Inst., Emory Univ., Decatur, GA

Abstract: The lateral septum (LS) is a brain region that has been implicated in a broad range of behaviors ranging from kinship to aggression to feeding. Early self-stimulation studies also suggested a critical role for the LS in reward-related behaviors. Since then, more emphasis has been placed on its position as a relay between the hippocampus and hypothalamus, especially as it pertains to the regulation of aggressive behaviors. However, there is now emerging evidence that the LS may contain anatomically and functionally distinct compartments along its anatomical axes. In particular, various representations of locomotion have been identified along the dorsoventral (DV) axis of the LS and spatial transcriptomic studies have revealed spatially distinct clusters of various molecular markers within the LS. Thus, this study seeks to characterize the reward representations along the DV axis of the LS using cellular resolution calcium imaging. Specifically, LS neurons (n = 740 neurons, 12 mice) at varying depths along the DV axis were imaged in freely behaving mice during a sucrose reward operant assay. The proportion of reward-responsive neurons was quantified, and their responses were characterized as either excited (positively modulated) or inhibited (negatively modulated) by sucrose reward. Preliminary analyses suggest that there is a gradient of reward representations along the DV axis of the LS such that a greater proportion of excited reward-responsive neurons are located in the dorsal LS, while a greater proportion of inhibited reward-responsive neurons are located in the ventral LS. Additionally, viral tracing studies have identified distinct LS projection populations to several reward centers within different DV compartments of the LS. For example, LS neurons projecting to the nucleus accumbens (NAc) are found in the dorsal LS, while those projecting to the ventral tegmental area (VTA) are found in the intermediate LS. Future work aims to characterize the reward representations of these projection populations and map them back on to the previously described DV gradient. Finally, optogenetic manipulation experiments will be performed to determine the contribution of these LS projection populations to reward-related behaviors such as self-stimulation.

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Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.04/M11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DA 044980

Title: Phasic dopamine release in the rat prelimbic prefrontal cortex preferentially encodes salience rather than valence in Pavlovian reward and fear tasks

Authors: *M. J. MARTIN[1], J. B. TORRES[2], M. SADDORIS[3];
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Abstract: In associative learning, animals acquire the relationship between stimuli and salient outcomes, as well as the relevant actions to approach (appetitive) or avoid (aversive) these predicted outcomes. However, animals also must flexibly adapt behavioral strategies when relationships between these stimuli change. Decades of work have implicated phasic dopamine (DA) release as an important component for the formation of associative representations, both appetitive and aversive. However, the majority of this work has focused on the mesolimbic pathway between the ventral tegmental area (VTA) and the nucleus accumbens, where phasic DA supports the acquisition of appetitive associations and mediates conditioned approach. In contrast, less is known about the phasic release properties of the mesocortical pathway composed of VTA projections to areas of the prefrontal cortex, including the prelimbic (PL) cortex, a region vital for regulating behavioral flexibility during extinction learning. Although research has determined the functionality of the mesocortical pathway, fewer studies have characterized phasic DA release during adaptive learning situations. Therefore, this study sought to investigate the phasic release of DA in the PL during different Pavlovian tasks. To accomplish this, we expressed a fluorescent biosensor (AAV-hSyn-GRAB_DA) in the PL of rats, allowing for the rapid detection of *in vivo* DA. Phasic DA signals in the PL were then recorded in awake and behaving rats via fiber photometry during both Pavlovian reward conditioning and fear extinction tasks. During Pavlovian reward learning, we found phasic increases in DA for both rewards and reward-predictive cues, as well as briefer responses to non-predictive cues. For the aversive task, we used two extinction procedures - one in which an expected shock was omitted (standard extinction), and another in which a novel cue was presented at the time of expected shock (NFE). Here, phasic DA showed high levels of sustained release for fear-predictive cues, but also produced a large second release at the NFE cue signaling shock omission. Rats exhibited significantly greater DA release during cues that predicted an aversive rather than rewarding outcomes, supporting prior work that mesocortical DA release differs from valence-related release patterns (i.e., increases for appetitive stimuli and inhibitions for aversive) in the mesolimbic pathway. Collectively, these data suggest that DA release in the PL tracks the

salience of stimuli, which may be vital for deploying attentional resources towards acquiring and updating behavioral actions toward emotionally-relevant stimuli.

Disclosures: M.J. Martin: None. **J.B. Torres:** None. **M. Saddoris:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

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Program #/Poster #: PSTR237.05/M12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: T32 MH112507
BWF CASI 1019469

Title: Mechanisms in the lateral habenula that underlie rewards-based behavioral state changes

Authors: *J. CHANDRA[1,2], J. MUIR[2,3], E. LEWIS[1,2], N. MEJIA[2], C. K. KIM[2,3];
[1]Univ. Of California Davis Neurosci. Grad. Program, Davis, CA; [2]Ctr. for Neurosci., [3]Dept. of Neurol., Univ. of California, Davis, Davis, CA

Abstract: Major depressive disorder (MDD) is a prevalent and pressing issue whose root cause in the brain involves changes in numerous different circuitries. MDD is often associated with dysregulated reward-seeking behavior, including lowered approach tendencies and heightened avoidance responses. To study the regulation of reward-seeking behaviors in animal models, we can deliver unexpected aversive outcome during the acquisition of rewarding stimuli. Animals must then decide whether to avoid potential future aversive outcomes, or whether to continue to approach the rewarding outcome. A growing body of literature has implicated the lateral habenula (LHb) as an important and relevant brain region that may be involved in regulating approach-avoidance behaviors. The LHb can act like a hub that receives several inputs and sends outputs to other brain regions involved with reward regulation, such as the dopaminergic and serotonergic systems. Here, we seek to pinpoint a direct relationship between LHb neuronal activity and dynamic changes in reward-seeking behavior in the face of conflict. We hypothesize that neuronal activity in the LHb shifts under “approach” versus “avoidance” behaviors, and that modulation of LHb neuronal activity may influence an animal’s susceptibility to engaging in avoidance behavior. To address this question, I have characterized how mice adapt to unexpected aversive outcomes (foot shocks) delivered during reward-seeking, while simultaneously measuring LHb activity during this approach-avoidance task using fiber photometry recording of GCaMP activity. I observed that mice actively suppress their reward-seeking behavior for ~5 minutes after receiving a bout of unexpected foot shocks, before returning to baseline rates of reward-seeking. LHb activity in mice is specifically modulated during the “avoidance” state, suggesting that LHb activity dynamically tracks changes in behavioral state during approach-avoidance conflict. To assess whether neuronal activity in the LHb can manipulate the balance between approach and avoidance behavior, I utilized an inhibitory opsin, ArchT, to silence

neuronal activity in the LHb during the same behavioral task described. Compared to control animals, mice with inhibited LHb displayed longer time spent in the “avoidance” state, prior to returning to reward-seeking (2-way ANOVA interaction $F_{2,16}$=6.4, P=0.0089; Post-hoc Sidak’s test, P=0.0002, N = 5 mice in each group). These findings demonstrate that LHb activity may play a direct role in regulating an animal’s ability to return to approach-like behaviors after an unexpected aversive outcome.

Disclosures: J. Chandra: None. **J. Muir:** None. **E. lewis:** None. **N. Mejia:** None. **C.K. Kim:** None.

Poster

PSTR237: Neural Circuits I

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Program #/Poster #: PSTR237.06/M13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: F31DA060615

Title: The role of dopamine inhibition in punishment resistant reward seeking behavior

Authors: *N. MWENDA[1], J. L. SEILER[1], T. N. LERNER[2];
[1]Northwestern Univ., Chicago, IL; [2]Neurosci., Northwestern Univ., Chicago, IL

Abstract: Decision-making is an important cognitive function that animals use to seek life-preserving rewards like food while avoiding dangers like predation. Understanding how animals balance between risk and reward is important, with implications for disorders like substance use disorder (SUD), in which decision-making is impaired and animals pursue rewards in inappropriately high-risk situations. In studying when animals pursue sucrose rewards despite the risk of punishment (“punishment-resistant reward-seeking”), the Lerner Lab has found that dopamine (DA) signaling in the dorsomedial striatum (DMS) plays a crucial role. Punishment-resistant mice have increased phasic DA activity in the DMS on rewarded nosepokes and dips in DA activity on unrewarded nosepokes . However, the idea that these DA dips causally contribute to punishment resistance has not been explicitly tested. Using optogenetic, we found that mice are more likely to be punishment resistant if they receive inhibitory stimulation in DA terminals during unrewarded nosepokes. We also investigated if the behavioral changes we see when inhibiting DA terminals translate to changes in plasticity in the orbitofrontal cortex (OFC) to DMS circuit. Previous studies have shown that the OFC-DMS plasticity is important for punishment resistant behavior, therefore having a better understanding of OFC-DMS circuitry will provide insight into the decision-making circuitry that is impaired in SUD.

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Poster

PSTR237: Neural Circuits I

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Program #/Poster #: PSTR237.07/M14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH 1U01NS126050

Title: Model-based habit: attachment of model-free action values to hippocampal place representations

Authors: *S. WANG[1], R. GRGURICH[2], H. T. BLAIR, IV[3];
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Abstract: Model-based (or goal-directed) strategies select actions based on forward-looking predictions (derived from mental models) of future outcomes, and are thus sensitive to revaluation of anticipated outcomes. Model-free (or habitual) strategies select actions based upon their estimated value in the present state, and are thus insensitive to outcome revaluation. Here we investigated whether model-free action selection can occur when a mental model (cognitive map) is needed to discriminate the current state. Rats performed a navigation task in darkness on a plus maze surrounded by a 2x2 m square track (田); automatic doors controlled access to maze segments. A preferred reward (evaporated milk) was dispensed at SW & SE corners; plain water was dispensed at NW & NE corners. Distinct textures were laid in each corner. Rats began each trial by running from the maze center to the west (W: left→milk, right→water) or east (E: right→milk, left→water) choice point. Rats learned to select milk from both choice points in 7-10 days when choice points were laid with distinct floor textures (perceptual state discrimination, PSD) or 20-30 days when choice points were perceptually identical (inertial state discrimination, ISD). Trained rats were then water deprived; while thirsty, they ran the task in extinction (revaluation; REV) or with milk/water delivery (incentive learning; INC), or received a 2-bottle preference test in the home cage (BOT). REV rats persisted selecting corners previously rewarded with milk despite preferring water in their thirsty state (confirmed by post-session 2-bottle test), consistent with model-free choice. By contrast, INC rats selected corners rewarded with water but resumed selecting milk when tested 24h later under non-thirsty conditions; after another 24h, INC rats were retested under thirst in extinction and shifted to selecting corners previously rewarded with water, consistent with model-based choice. BOT rats preferred water over milk in the 2-bottle test, then selected milk on the maze 24h later under non-thirsty conditions; after another 24h, BOT rats were tested under thirst in extinction and persisted selecting corners previously rewarded with milk, consistent with model-free choice. Findings did not differ for PSD vs ISD rats, both of which showed impaired choice after hippocampal inactivation. Results suggest that REV & BOT rats relied upon a model-based representation (hippocampal cognitive map) to discriminate the current state (W vs E choice point), yet based their choice to turn left or right on model-free action values assigned in that state, implying that mental models can play a causal role in model-free decision making.

Disclosures: S. Wang: None. **R. Grgurich:** None. **H.T. Blair:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.08/M15

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NHMRC Ideas Grant APP2011633

Title: Plasticity and functional characterisation of hypothalamic inputs to the paraventricular thalamus

Authors: S. R. GILCHRIST[1,2], E. CHOI[3], G. P. MCNALLY[3], ***J. POWER**[1];
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Abstract: Most interactions with the world around us are forms or appetitive or aversive behaviour, driving us towards beneficial outcomes and away from harmful ones with many circumstances requiring evaluation of both risk and benefit. Recently, the paraventricular thalamus (PVT) has been implicated in shaping these motivated behaviours to current needs. A major input to the PVT is the hypothalamus, which is known to respond to changes in hunger states and promote appetitive behaviours. Here we functionally map inputs from the lateral hypothalamus (LH) to the PVT in male and female Long Evans rats using optogenetics, whole-cell patch-clamp recordings, retrograde viral tracing, and in situ hybridisation. The LH inputs were found to provide a mix of excitatory and inhibitory drive to the PVT. Interesting, while 4.4% of neurons in the lateral hypothalamus expressed markers for glutamatergic (VGLUT2) and GABAergic (VGAT) transmission, 25% of LH neurons projecting to the PVT co-expressed VGLUT2 and VGAT. Acute food deprivation altered hypothalamic inputs, decreasing excitatory transmission evidenced by a reduction in the AMPA:NMDA ratio, in the PVT neurons from food deprived subjects**.** These results suggest that the capacity of the PVT to adapt behaviour in response to changes in needs is guided in part by plasticity at LH inputs.

Disclosures: S.R. Gilchrist: None. **E. Choi:** None. **G.P. McNally:** None. **J. Power:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.09/M16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R00
One Mind Foundation
Brain Research Foundation

Title: To eat or not to eat: Deciphering Insula Cortex Circuits Governing Appetitive and Aversive Non-Homeostatic Feeding Behaviors

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Abstract: Feeding is a fundamental, innate behavior regulated by a homeostatic set point within our bodies. However, maladaptive feeding behaviors such as obesity and anorexia nervosa can emerge when this homeostatic system is disrupted. While most research has traditionally focused on the bottom-up perspective of food intake, acknowledging the importance of social context, cues, and experiences is crucial for a comprehensive understanding. Recent imaging studies point to the insular cortex (INS) as a key player in food perception and hedonic/reward processing. Specifically, neurons expressing nitric-oxide synthase 1 (INSNos1) within the INS have been identified as essential for non-homeostatic food intake. To delve deeper into these mechanisms, I employed chemogenetics and discovered that silencing INSNos1 neurons reduces overconsumption but unexpectedly enhances taste aversion. Notably, this manipulation didn't impact anxiety, memory, or homeostatic feeding, suggesting a specific role in non-homeostatic behaviors. My findings propose that Nos1 neurons may encode the salience of food in the environment, influencing non-homeostatic feeding without a broad impairment of function. To explore the long-term consequences, I investigated the metabolic changes resulting from Nos1 neuron manipulation. Utilizing the Caspase3 model and metabolic chambers, I observed significant effects on food intake, physical activity, and sexual dimorphism. To uncover the intricacies of the salient circuit, I employed tracing and calcium recording techniques. The data, including bulk calcium recordings and miniscope single-cell calcium recordings, suggest that Nos1 neurons play a crucial role in aversive taste learning and may contribute to detecting stimulus salience based on internal states and previous experiences. Understanding the upstream and downstream neural circuitry involved, I utilized monosynaptic rabies tracing and in situ hybridization/immunohistochemistry. Insights from these experiments indicated that INSNos1 neurons receive inputs from the amygdala (BLA) and hypothalamus, regions known for regulating aversive and internal state information, respectively. While the role of INSNos1 neurons in non-homeostatic feeding behaviors has become clearer, further experiments are needed to comprehend the intricate projections during non-homeostatic learning. Future studies will focus on elucidating precise mechanisms and functional implications of these projections. This approach holds promise in explaining causal relations between structure and function, simulating lesions or diseases, and predicting dynamics.

Disclosures: M. Olvera-Caltzontzin: None. **S. Stenzel:** None. **A. Kanakam:** None. **A. Bermudez Ibarra:** None. **S.A. Stern:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

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Program #/Poster #: PSTR237.10/M17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: EU Grant - MSCA 953327
MRC grant MR/T033320/1

Title: Understanding serotonin: gated Deep Neural Networks reveal a unified model across its role in learning, neurodevelopment and psychedelics

Authors: *G. OCANA-SANTERO[1,2], A. M. PACKER[1], T. SHARP[3], S. J. B. BUTT[1], S. SARAO MANNELLI[4], A. SAXE[4];
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Abstract: Serotonin is a neuromodulator that has been associated with a plethora of roles, including emotion, learning, sleep, reward and aversive signalling based on its dynamics and optogenetic studies. Despite recent research on serotonin several aspects are still not captured by existing theories, in particular the dynamics and role of serotonin during learning are still poorly understood. The recent emergence of the field of neuroAI opens the door to understand neuronal processes by modelling them with biologically-inspired architectures, allowing for an analytical understanding of their second order effects. In this sense, gated neural networks - in which the activation of the neurons is modulated by a gate - provide a naturalistic implementation of neuromodulation. This work combines theoretical results using artificial neural networks and experiments using optogenetics, in order to understand the role of serotonin in learning in a noisy environment. We develop an analytically solvable theoretical model in the teacher-student framework based on a two-layer gated neural network trained via online stochastic gradient descent. During training, the student network has to learn a N-to-one dimensional mapping from a teacher, despite perturbation coming from the environment with an opposing rule and variable input variance. In our model serotonin acts by modulating the gates according to saliency of the stimuli, and has an instrumental role in learning in the presence of noise. Then, we train mice expressing an opsin in serotonergic neurons in an analogous dual-teacher air puff discrimination task while monitoring serotonin and calcium dynamics with genetically-encoded fluorescent sensors (GCaMP6s and g5-HT3.0). Namely, animals learn that only one of two perpendicularly oriented air puffs is associated with reward, while ignoring some misleading trials of varying stimulus pressure, in which this rule is inverted. Overall, our model explains the effects of serotonin in the learning of this task. We further expand the model and experiments to reconcile results from the field of serotonin research, namely neurodevelopment and the effects of psychedelics. Altogether, we propose that the role of serotonin can be understood as input gating with second order effects in neuronal encoding and learning regularisation. We use this mechanistic modelling to reconcile diverse, and sometimes seemingly opposing (e.g., reward and

punishment encoding), results in the field of serotonin research. Finally, our results highlight the potential of gated deep neural networks as plausible models to understand the complex role of neuromodulators in the brain throughout life.

Disclosures: G. Ocana-Santero: None. **A.M. Packer:** None. **T. Sharp:** None. **S.J.B. Butt:** None. **S. Sarao Mannelli:** None. **A. Saxe:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.11/M18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 2RF1 AG050518

Title: Semaglutide modulates the activity of specific neuronal populations in adult rats

Authors: *C. D. WOHLFELD, A. M. BLAS, M. A. FRICK, J. L. WOODRUFF, J. R. FADEL; Pharmacology, Physiol., and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: GLP-1 agonists have seen a recent expansion in clinical use and have provoked considerable research interest. Beyond glycemic control and weight loss, preclinical evidence suggests multiple neuroprotective and anti-inflammatory effects facilitated by GLP-1 agonists. Concomitantly, dozens of clinical trials have emerged to assess GLP-1 agonist effectiveness in various neurodegenerative diseases. Despite emerging interest in therapeutic use, the mechanisms by which GLP-1 agonists function in the CNS are inadequately characterized. Some research findings are confounded because peripherally administered GLP-1 agonists exhibit limited distribution throughout the mammalian brain. Additionally, knowledge of which cell populations and neuroanatomical regions that are involved in central effects is limited. The effects of GLP-1 agonists on feeding and other integrative physiological functions suggest the involvement of hypothalamic regulatory centers such as the lateral hypothalamus. A prime candidate for mediating GLP-1 responses in this area is the orexin/hypocretin system, which projects to many areas of the brain including the basal forebrain cholinergic system, habenula, and locus coeruleus. Here, our goal was to begin to elucidate the potential mediators of GLP-1 agonist effects on neural functions. Young adult (3–4-month-old) male and female Fisher 344/Brown Norway F1 hybrid rats received an acute intraperitoneal administration of the GLP-1 agonist, semaglutide (0.12 mg/kg) or vehicle. Two hours later, the animals were euthanized, and their brain processed for immunohistochemical detection of c-Fos, a marker of neuronal activation. Dual labeling was performed to further examine the phenotype of semaglutide-activated neurons in brainstem, diencephalic, and basal forebrain regions. Semaglutide treatment elicited significantly increased activity in hypothalamic orexin neurons and cocaine and amphetamine-related transcripts neurons in the habenula. In the brainstem, there was a trend towards decreased activity in noradrenergic neurons in the locus coeruleus, and no effect in

dopaminergic neurons in the substantia nigra or ventral tegmental area. In the basal forebrain, there was a trend towards increased activity in cholinergic neurons in the medial septum in the semaglutide-treated group. These anatomical regions and cell populations are components of neural circuits that regulate feeding, metabolism, sleep, cognition and behavior, and immune function. Further inquiry into the neural mechanisms of GLP-1 agonist neuroprotective effects is needed as these drugs become more broadly prescribed.

Disclosures: C.D. Wohlfeld: None. **A.M. Blas:** None. **M.A. Frick:** None. **J.L. Woodruff:** None. **J.R. Fadel:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

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Program #/Poster #: PSTR237.12/M19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH-NIDA 5T32DA043469
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Dr. Edyth H. Schoenrich Scholarship Fund

Title: Real time assessment of nicotine seeking and avoidance behavior

Authors: *R. R. MANSOURI-RAD[1], C. I. MCQUILLEN[2], W. MARZILLI[2], D. S. MCGEHEE[3];
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Abstract: Tobacco smoking is a leading cause of preventable death worldwide, but the problem persists due to nicotine's addictive properties. Along with these reinforcing properties, nicotine also has aversive effects, particularly at higher doses, which smokers avoid through titration of their nicotine intake. Mice also behaviorally reduce nicotine intake at high doses, but it remains unclear whether this behavior is true aversion or adjustment of preferred consumption levels. To investigate nicotine self-administration patterns in nicotine-naïve male mice, we implemented a real-time two-bottle choice lick-o-meter device to continuously monitor consumption of two different flavored drinking solutions (n=7). We paired each mouse's initially preferred flavor solution with a high concentration of nicotine (100 ug/mL). Over 4 days of testing we observed avoidance of the flavored nicotine solution with subsequent avoidance of that flavor for four days following the removal of nicotine from the solution. These data demonstrate the aversive effects of nicotine and associated flavor cues. We then monitored nicotine consumption patterns of nicotine-naïve mice across a range of concentrations (0-100 µg/ml; n=6). Escalating the dose resulted in decreased consumption of the nicotine solution, consistent with our previous test of nicotine aversion. However, examination of nicotine intake revealed similar levels of consumption across all concentrations, suggesting dynamic behavioral adaptation through

reduction in lick duration, lick frequency, and fluid intake. These findings support appetitive nicotine consumption in an escalating dose paradigm. The interpeduncular nucleus (IPN) plays a key role in regulating nicotine intake, but the in vivo dynamics of the IPN's response to nicotine are not yet understood. We utilized fiber photometry and the intracellular Ca 2+ indicator GCaMP6s expressed pan-neuronally in the IPN to record nicotine induced changes in IPN neuronal activity in freely-behaving nicotine-naïve male mice. We administered both an aversive high dose (1.5 mg/kg i.p.) of nicotine and a reinforcing low dose (0.5 mg/kg i.p.) 24 hours apart in a counterbalanced order (n=9). We observed a dose dependent increase in IPN activity in response to nicotine and observed an unexpected IPN recruitment even with the reinforcing nicotine dose. Together these data suggest that aversive signaling from the IPN competes with nicotine's rewarding effects across a wide dose range to establish self-administration behavior.

Disclosures: R.R. Mansouri-Rad: None. **C.I. McQuillen:** None. **W. Marzilli:** None. **D.S. McGehee:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.13/M20

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA/NIH: R01 DA050165
Attilio and Olympia Ricciardi Research Fund

Title: Striatal cholinergic interneurons relay insulin's actions on ACh and DA dependent nutrient sensing

Authors: *J. C. PATEL[1], R. M. FEELEY[1], V. KHACHATURYAN[1], M. FERNANDES[1], P. WITKOVSKY[1], B. GAMALLO-LANA[2], A. C. MAR[2], M. E. RICE[1];
[1]Neurosurg., [2]Neurosci. and Physiol., NYU Grossman Sch. of Med., New York, NY

Abstract: The actions of insulin as a satiety signal in the hypothalamus is well-established. However, insulin receptors are also found in other brain regions, including the striatum. We previously reported that insulin amplifies evoked dopamine (DA) release in *ex vivo* striatal slices from male rats via a local mechanism involving enhanced excitability of striatal cholinergic interneurons (ChIs) and increased activation of nicotinic acetylcholine receptors (nAChRs) on DA axons. Here we examined the actions and consequences of insulin in the striatum of mice of both sexes. Through analysis of RNA-seq data in DropViz, we found that in striatum, the highest expression of InsR-mRNA is in ChIs compared to other striatal neurons and glia. Moreover, immunohistochemical staining in mouse striatal sections show ChIs express InsR protein. Patch-clamp recordings from non-labeled or tdTomato-labeled ChIs show that physiological concentrations of insulin (30 nM) increase action potential firing in ChIs from male mice during depolarizing current injection, mirroring our previous result in male rats. In females, the effect of

insulin was less pronounced. Strikingly, in contrast to control mice, enhanced ChI excitability and enhanced DA release induced by insulin was lost in mice of both sexes that lack forebrain choline acetylcholinesterase (ChAT-KO mice) and thus the ability to synthesize ACh. To determine the behavioral consequences of insulin's actions on striatal ChIs and the interplay between ACh and DA in ingestive reward signaling, we examined nutrient sensing in ChAT-KO mice and their littermate controls. Mice were given 1 h free access to a solution containing either 6% glucose or equally sweet 0.25% saccharin on alternating days in their home cages. As predicted, glucose consumption escalated over 3-4 paired sessions in control males, whereas escalation of saccharin consumption was limited. In ChAT-KO mice, there was no significant preference for glucose *versus* saccharin over the same time period, although total consumption of fluids was unaltered. Similar trends were seen in females, albeit not as robust. Together, these data show how insulin signaling via InsRs on ChIs enhances ChI excitability to boost striatal DA release across species. This local mechanism appears to play a role in nutrient sensing and supports our hypothesis that insulin acts as a nutrient reward signal.

Disclosures: J.C. Patel: None. **R.M. Feeley:** None. **V. Khachaturyan:** None. **M. Fernandes:** None. **P. Witkovsky:** None. **B. Gamallo-Lana:** None. **A.C. Mar:** None. **M.E. Rice:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.14/M22

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Anatomical mapping of striatal input to projection-defined entopeduncular nucleus subtypes

Authors: *D. REINA-GUERRA, M. WALLACE;
Anat. and Neurobio., Boston Univ., Boston, MA

Abstract: The basal ganglia are critically important for an animal's ability to select actions on an ongoing basis. The Entopeduncular Nucleus (EP) is a relatively understudied source of basal ganglia output which may be at least partially homologous with the primate globus pallidus interna (GPi). The topography of inputs to and outputs from the EP have been addressed, but never in the same animal, precluding a cohesive assessment of how individual EP neurons or clusters thereof fit into the larger context of striatum-EP-output circuits. To selectively label cells in the EP as a function of overlapping input and output, I inject anterograde monotranssynaptic viral vectors with Flp recombinase and retrograde tracers with Cre recombinase into the input and outputs of the EP respectively, in Cre/Flp reporter mice. In this way, Flp is delivered to the EP from its pre-synaptic inputs, and Cre is delivered to the EP via viral infection of its axons in EP targets, causing cells with both Cre and Flp recombinases to fluoresce. Brain slices are imaged and registered to 3D volumes, and the spatial organization of fluorescently labeled EP neurons is assessed along the rostral-caudal, dorsal-ventral, and medial-lateral axes. Reporter

mice or or wild type controls (C57BL/6) of both sexes receive tracer injections between 45 and 60 days of age and are sacrificed three to four weeks following injection. Revealing the topography of circuits through the EP will guide the use of optical techniques to target and monitor these circuits in vivo during behavioral experiments which force action selections.

Disclosures: D. Reina-Guerra: None. **M. Wallace:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

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Program #/Poster #: PSTR237.15/M23

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant 5K00MH134248-04
NIH Grant R01MH133608

Title: The Contribution of Norepinephrine in the Lateral Habenula to Action Selection During a Probabilistic Switching Task

Authors: *J. M. MARTINEZ, M. L. WALLACE;
Anat. and Neurobio., Boston Univ., Boston, MA

Abstract: The process of selecting an action and evaluating requires the basal ganglia (BG) and extended circuits such as the lateral habenula (LHb). The LHb is a photogenically conserved and genetically ancient brain nuclei found in all vertebrates. Despite the critical function of the LHb in action selection/outcome evaluation, the contributions of specific neuron subtypes within the LHb to these processes in both health and disease remain poorly understood. Previously, we defined genetically distinct neuronal subtypes within the LHb using whole transcriptome single-cell sequencing. Further, we have developed a probabilistic switching task allowing for observation of LHb circuit dynamics *in vivo* when used in conjunction with genetically encoded biosensors expressed in neurons and fiber photometry. Norepinephrine (NE), a neuromodulatory neurotransmitter whose CNS projections originate solely from the midbrain locus coeruleus, innervates large portions of the brain including the LHb. While NE is known to play a role in arousal, stress, attention, and memory, the exact nature and contributions of NE to behaviors and their underlying neural circuitry remains underexplored. In the LHb, NE is known to contribute to states of both arousal and anxiety. Additionally, we have found differential expression of adrenergic receptors (ARs) in LHb neuronal subtypes. Using our probabilistic switching task in cre-expressing mouse lines targeting various LHb neuronal populations in combination with calcium sensors (jCaMP) and norepinephrine sensors (GRAB NE), we show NE release carries reward information to the LHb *in vivo* in a behaving mouse during our task bilaterally. Further we examined the activity of three specific subpopulations of LHb neurons (Sst, Kcnc1, and Prokr2 +) while performing the probabilistic switching task using genetically encoded calcium sensors jRCaMP or jGCaMP and adrenergic receptor inhibitors. Together, these data shed light

on the function of LHb neuron populations in action selection while performing switching behaviors and the contributions of NE to these behaviors.

Disclosures: J.M. Martinez: None. **M.L. Wallace:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.16/M24

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant MH133608

Title: Anatomical and physiological properties of lateral habenula neuronal subtypes

Authors: *M. B. CORNIQUEL, E. KRAFT, J. M. MARTINEZ, M. L. WALLACE;
Anat. & Neurobio., Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA

Abstract: The lateral habenula (LHb) is an epithalamic structure primarily associated with negative reward prediction error and the processing of aversive stimuli. This region's impact on behavior is likely tied to its strong projections to midbrain and hindbrain nuclei, modulating the release of dopamine, serotonin, and noradrenaline. Projections to these targets may vary topographically across the subnuclei of the LHb. Recent single-cell RNA sequencing and in-situ hybridization studies indicate that distinct neuronal subtypes may underlie differential targeting of downstream regions. These subtypes appear regionally distinct and vary in their expression of ion channels, G protein-coupled receptors, and neurotransmitter receptors. Existing transgenic cre-lines (i.e. Sst, Kcnc1, Prokr2) allow for the targeting of these LHb neuronal subtypes and represent a promising route for further exploration of existing anatomical, physiological, and behavioral differences. We use mice from Sst-cre, Kcnc1-cre, and Prokr2-cre transgenic lines, aged 50-90 days at the start of the experiment, and induce cre-dependent fluorescent protein expression within the LHb via viral injection. Mice are then sacrificed three to four weeks following injection. Variations in axonal projection are determined by registering brain slices to the Allen Brain Atlas and measuring the density of fluorescently labeled axonal projections within each anatomically designated region of interest. Whole-cell patch clamp electrophysiology targeting fluorescently labeled cell bodies of each neuronal subtype is used to assess differences in physiological properties, including the frequency and magnitude of synaptic inputs, rheobase, and action potential amplitude. Advancing our knowledge regarding these genetically defined LHb subtypes will inform future work including targeting these circuits for monitoring or manipulation in vivo.

Disclosures: M.B. Corniquel: None. **E. Kraft:** None. **J.M. Martinez:** None. **M.L. Wallace:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.17/M25

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01MH133608

Title: Effects of chronic restraint on two-armed bandit task performance and activity of genetically distinct lateral habenula neuronal subtypes in mice

Authors: *C. NOWAK, M. WALLACE;
Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Stress-related disorders affect over 40 million individuals in the US annually and are a significant risk factor for numerous mental illnesses, including major depression. Chronic stress is known to affect reward-motivated behavior and change activity patterns (increase the probability of "burst firing") in the lateral habenula (LHb), a region implicated in the etiology of depression. Single-cell sequencing has recently identified that of the four major neuron types in the LHb, only those in the oval/medial and marginal LHb contain the low-voltage-sensitive T-type calcium channels necessary for burst firing. We used two transgenic mouse lines to target specific cell types of the LHb that showed differential expression of ion channels capable of burst firing (Kcnc1-Cre, marginal neurons; Sst-Cre, HbX neurons). We evaluated effects on reward-motivated behavior using the two-armed bandit task (2ABT), a freely moving behavioral task that requires water-deprived mice to evaluate action-outcome relationships trial-by-trial to achieve the highest number of water-rewarded trials. We performed chronic restraint (CR) and examined anhedonia, a reduced ability to experience pleasure, in age- and sex-matched mice (age p>60). Additionally, we measured the calcium and serotonin dynamics in the LHb during the 2ABT using dual-color fiber photometry before, during, and after CR. Once the performance criterion for 2ABT is met, the animals perform the 2ABT for 6 weeks. At week 3, animals are randomly assigned to the control or CRS group for 2 weeks, where they continue to perform the 2ABT on days they are stressed. Following week six of 2ABT performance, the stress and anhedonic states of the animals are evaluated through the Sucrose Preference Test (SPT), Elevated Plus Maze, and Open Field Test. Performance on the 2ABT and bulk fluorescence are compared across the three time points (pre-, during, and post-stress). Preliminary data (n=8) suggests CR mice show a decrease in probability of choosing the more highly rewarded port, increase in switching ports, and decreased number of trials and rewards each session during stress. Additionally, CR mice demonstrate a decreased preference for sucrose on the SPT.

Disclosures: C. Nowak: None. **M. Wallace:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.18/M26

Topic: I.03. Anatomical Methods

Support: National Institute on Drug Abuse R01DA056829
National Institute of Diabetes and Digestive and Kidney Diseases R01DK128475

Title: Projection-tags enable multiplex projection tracing and multi-modal profiling of projection neurons

Authors: *L. YANG[1], F. LIU[2], H. HAHM[2], T. OKUDA[2], X. LI[2], Y. ZHANG[2], V. K. SAMINENI[2];
[1]Washington Univ. in St. Louis Neurosci. PhD Program, St. Louis, MO; [2]Washington Univ. in St. Louis, St. Louis, MO

Abstract: Single-cell multiomic techniques have ignited immense interest in developing a comprehensive multi-modal map of diverse neuronal cell types and their brain wide projections. However, investigating the spatial organization, transcriptional and epigenetic landscapes of brain wide projection neurons, as well as their functional relevance, is hampered by the lack of efficient and easily adoptable high-throughput neuroanatomical tools. Here we introduce Projection-TAGs, a retrograde AAV platform that allows multiplex tracing of projection neurons using RNA barcodes, which act as the projection identifier. By using Projection-TAGs, we performed multiplex projection tracing of the mouse cortex and high-throughput single-cell profiling of the transcriptional and epigenetic landscapes of the cortical projection neurons. We leveraged Projection-TAGs to obtain a snapshot of activity-dependent recruitment of neuronal circuits and their molecular features in the context of a specific stimulus. Due to its flexibility, usability, and compatibility, Projection-TAGs hold promise for widespread application in constructing a comprehensive multi-modal map of brain neuronal cell types and their projections.

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Poster

PSTR237: Neural Circuits I

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Program #/Poster #: PSTR237.19/M27

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIAAA R01AA024434
Smith Family Award for Excellence in Biomedical Research

NIAAA NRSA F32AA029595
Brown University Undergraduate Teaching and Research Award

Title: Investigating the relationship between neuronal morphology and alcohol preference in Drosophila

Authors: *M. WANG, J. S. HERNANDEZ, N. J. MEI, R. AZANCHI, K. R. KAUN; Neurosci., Brown Univ., Providence, RI

Abstract: Alcohol Use Disorder (AUD) is a condition that involves compulsive alcohol administration despite negative consequences. However, the relationship between individual behavior and neuron morphology in conjunction with ethanol preference is still unclear. *Drosophila melanogaster* is an effective model to study behavioral and morphological differences because we can systematically identify and study individual neurons and use sophisticated genetic tools to localize regions of interest in the fly brain. Additionally, it is possible to generate large sample sizes using *Drosophila* due to its short breeding cycle. We developed an operant assay (OPEraNt Behavioral Assay for Reinforcement Response; OPEN BARR) to examine how flies choose to engage with intoxicating doses of volatilized ethanol. Flies were allowed to self-administer 75% volatilized ethanol for 15 minutes per day; one cohort (n=66) of flies did so for one day and another cohort (n=67) self-administered for three days. Flies were separated based on how much time they spent self-administering ethanol, with high preferring flies (HP) spending the most amount of time, and low preferring flies (LP) spending the least amount of time. HP flies display increases in time spent self-administering ethanol over days whereas LP flies reduce ethanol self-administration, and this is dependent on activity of the cholinergic α′3 mushroom body output neuron (MBON). We expressed myristoylated GFP in this neuron and measured its pre- and postsynaptic projections and arborization patterns in HP (n=15) and LP (n=15) flies. We hypothesized we would find morphological differences in the number of boutons and the number and length of branches of cholinergic α′3 MBON between HP and LP flies allowed to self-administer ethanol for three days. Overall, this work demonstrates that leveraging *Drosophila* genetic tools in an operant assay will elucidate the relationship between individuality in alcohol preference and morphology changes in neurons key for encoding alcohol preference. Ultimately, understanding how alcohol alters neuronal morphology could help us understand the transition from alcohol use to abuse.

Disclosures: M. Wang: None. **J.S. Hernandez:** None. **N.J. Mei:** None. **R. Azanchi:** None. **K.R. Kaun:** None.

Poster

PSTR237: Neural Circuits I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR237.20/M28

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

Support: Howard Hughes Medical Institute

Title: A complete neurotransmitter atlas of C. elegans males and hermaphrodites

Authors: *C. WANG[1], B. VIDAL IGLESIAS[1], S. SURAL[1], C. M. LOER[2], G. AGUILAR[1], D. M. MERRITT[1], I. TOKER[1], M. VOGT[3], C. CROS[4], O. HOBERT[1];
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Abstract: Mapping neurotransmitter identities to specific neurons is key to understanding how information flows in a nervous system. It also provides valuable entry points for studying the development and plasticity of neuronal identity features. In the *C. elegans* nervous system, neurotransmitter identities have been largely assigned by expression pattern analysis of neurotransmitter pathway genes that encode neurotransmitter biosynthetic enzymes or transporters. However, many of these assignments have relied on multicopy transgenic reporters that may lack relevant *cis*-regulatory information and therefore may not provide an accurate picture of neurotransmitter usage. In this study, we present the most extensive nervous system-wide map of neurotransmitter usage to date (**Figure 1**). We analyzed the expression patterns of 16 CRISPR/Cas9-engineered knock-in reporter strains for all main types of neurotransmitters in *C. elegans* (glutamate, acetylcholine, GABA, serotonin, dopamine, tyramine, and octopamine) in both the hermaphrodite and the male. Our analysis reveals novel sites of expression of these neurotransmitter systems within neurons, glia, and non-neuronal cells. The resulting expression atlas defines neurons that may be exclusively neuropeptidergic, substantially expands the repertoire of neurons capable of co-transmitting multiple neurotransmitters, and identifies novel neurons that uptake monoaminergic neurotransmitters. Furthermore, we also observed unusual co-expression patterns of monoaminergic synthesis pathway genes, suggesting the existence of novel monoaminergic transmitters. In summary, our comprehensive study provides an unprecedented characterization of neurotransmitter expression patterns in *C. elegans.* It paves the way for a better understanding of neuronal communication in both sexes of this nematode, elucidating principles that may extend to neurotransmitter dynamics in other organisms.

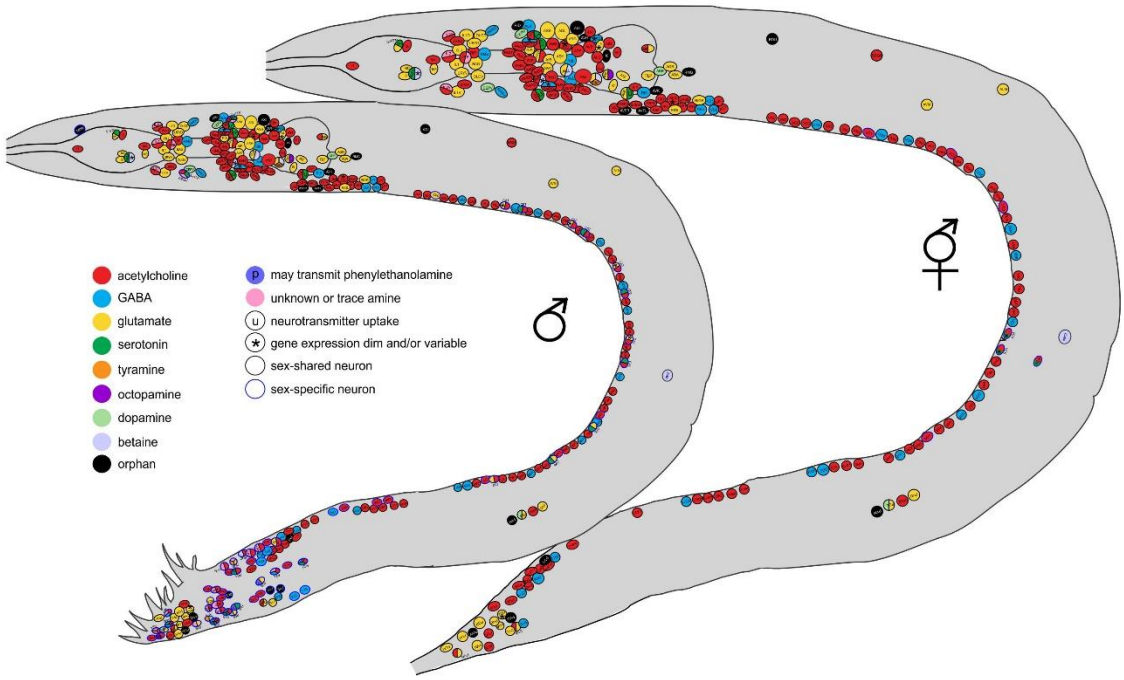

Figure 1. a complete neurotransmitter atlas for both sexes of *C. elegans*

Disclosures: C. Wang: None. **B. Vidal Iglesias:** None. **S. Sural:** None. **C.M. Loer:** None. **G. Aguilar:** None. **D.M. Merritt:** None. **I. Toker:** None. **M. Vogt:** None. **C. Cros:** None. **O. Hobert:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.01/M29

Topic: G.04. Emotion

Support: NIH UL1TR003015/KL2TR003016
Virginia Tech Institute for Society, Culture, and Environment Scholars Grant
Virginia Tech Whole Health Consortium Grant

Title: Exercise via mother-child dance improves mental health, immune function, and interbrain synchrony of mothers with moderate to severe levels of stress, depression, or anxiety

Authors: *M. AYCHMAN[1], N. TASNIM[2], S. DIESEL[1], C. GOLDING[1], G. E. HODES[3], J. C. BASSO[4];
[1]Human Nutr., Foods, and Exercise, Virginia Technol., Blacksburg, VA; [2]Translational Biol., Med., and Hlth., Virginia Technol., Blacksburg, VA; [3]Neurosci., Virginia Technol., Blacksburg, VA; [4]Human Nutr., Foods & Exercise, Virginia Technol., Blacksburg, VA

Abstract: Purpose: 1 in 5 mothers experience a mental health disorder, which has been associated with behavioral/emotional problems in offspring. Immune dysregulation (e.g., upregulated proinflammatory cytokine production) may underlie these issues and may be passed from mother to child. Exercise has been inversely correlated with depressive and anxious symptoms, and physical activity via dance may provide additional opportunity for social connection. Therefore, we hypothesize that exercise such as dance may help improve mental health, interbrain synchrony, and immune function. Methods: This study investigated the effect of exercise via a mother-child Brain-Compatible Dance Education curriculum on behavior and immune markers of inflammation in mothers with moderate to severe levels of stress, depression, or anxiety. Mother-child dyads were randomly assigned to participate in two weeks (5x per week, 45 minute session) of either exercise (mother-child dance) (n=11) or free play (n=6). Mothers answered self-reported mental health metrics before and after the experience. Participants provided saliva samples and their brain activity was recorded via hyperscanning electroencephalography during various interactive experiences. Results: Preliminary results indicate that exercise significantly increased positive affect ($t(15)=2.107$, $p=0.026$) and decreased parental stress ($t(14)=-1.849$, $p=0.043$) and depression (Beck Depression Inventory $t(14)=-1.791$, $p=0.047$; DSM 5 Cross-Cutting Symptom measure $t(11.580)=-1.995$, $p=0.035$). Salivary interleukin-6 (IL-6), a proinflammatory cytokine, was moderately positively correlated with maternal depression ($r=0.333$, $p=0.088$), decreased significantly during the intervention ($t(11)=2.312$, $p=0.041$), and was significantly correlated with the child's trauma symptoms ($r=0.697$, $p<0.001$), indicating a connection between the immune system and depression. Resting brain state recordings displayed a significant increase in synchronous beta, gamma, and theta oscillations in the frontotemporal region - responsible for emotion and behavior - between mother and child, indicating enhanced interbrain synchrony after the intervention ($p<0.05$, analyzed using HyPyp, significance determined as ±2 SD away from average imaginary coherence). Future analyses will focus on the influence of exercise on various neuroimmune markers associated with depression and their relationship to brain changes. Conclusion: These initial results demonstrate a positive effect of exercise on mothers with moderate-high levels of stress, depression, or anxiety and their children regarding the immune system, behavior, and brain.

Disclosures: M. Aychman: None. **N. Tasnim:** None. **S. Diesel:** None. **C. Golding:** None. **G.E. Hodes:** None. **J.C. Basso:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.02/M30

Topic: G.04. Emotion

Support: NIH UL1TR003015/KL2TR003016

Title: Getting Grounded: Investigating the influence of arts engagement on behavioral and brain dynamics

Authors: C. GOLDING[1], N. TASNIM[2], M. AYCHMAN[3], S. DIESEL[3], A. LUCIDON[4], A. SPEARMAN[4], ***J. BASSO**[1];
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Abstract: Motivation/problem statement: Art engagement is known to positively influence cognitive and emotional states, yet its precise effects on brain activity remain understudied. To explore brain dynamics during interactions with art installations, we employed mobile, hyperscanning electroencephalography (EEG). These art installations were hosted by Grounded, an organization based in Memphis, TN that focuses on using art as "a vehicle for growth, trauma release, and transformation." Our study focused on elucidating the neural correlates of arts engagement and its potential therapeutic implications in communities afflicted by violence. Despite anecdotal evidence suggesting the healing power of art, rigorous scientific studies on its behavioral and neural mechanisms are lacking. Methods/Approach: To assess psychosocial responses to the art installation, we utilized validated self-reported metrics administered before and after the experience (e.g., Depression, Anxiety, Stress Scale-21; Watts Connectedness Scale). To assess neural dynamics, we recorded brain activity using BrainVision LiveAmp 32 EEG devices before, during, and after art engagement as well as during post-engagement reflections in n=10 individuals. Results: Preliminary findings indicate that arts engagement significantly decreased depression ($t(9)=2.529$, $p=0.032$) and stress ($t(9)=6,862$, $p<0.001$) and significantly increased positive affect ($t(9)=-6.086$, $p<0.001$), mindfulness (body subscale, $t(9)=-3.769$, $p=0.004$), flow states (dispositional flow ($t(9)=-7.020$, $p<0.001$); core flow ($t(9)=-5.111$, $p<0.001$)), and social connectivity ($t(9)=-4.440$, $p=0.002$). At the neural level, preliminary investigations indicate that engagement with art increases the peak frequency in alpha activity (8-12 Hz) as well as the power in beta (12-30 Hz) and low gamma (30-45 Hz) activity. Additionally, we found that arts engagement increased interbrain synchrony ($p<0.05$, analyzed using HyPyp and significance determined as ±2 SD away from average imaginary coherence) among alpha, beta, and low gamma oscillations. Conclusion/Implications: Art engagement induces dynamic shifts in brain states towards heightened attention and cognitive engagement. These findings underscore the therapeutic potential of art in fostering cognitive stimulation and emotional well-being. Moreover, increased interbrain synchrony suggests the socio-emotional resonance of collective art experiences. Future investigations will explore the relationship between behavioral and neural outcomes.

Disclosures: C. Golding: None. **N. Tasnim:** None. **M. Aychman:** None. **S. Diesel:** None. **A. Lucidon:** None. **A. Spearman:** None. **J. Basso:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.03/M31

Topic: G.04. Emotion

Support: iTHRIV Scholars Program UL1TR003015
iTHRIV Scholars Program KL2TR003016
Virginia Tech Institute for Creativity, Arts and Technology

Title: Carving out creativity: Measuring behavioral and brain outcomes of the stone carving experience

Authors: *S. P. DIESEL[1,2], N. TASNIM[3], M. AYCHMAN[4], C. GOLDING[4], A. GARRASTEGUI SEGARRA[5], L. MCNAIR[6], J. C. BASSO[4];
[1]Virginia Technol., Blacksburg, VA; [2]Human Nutrition, Foods & Exercise, Virginia Tech, Blacksburg, VA; [3]Translational Biol., Med., and Hlth., Virginia Technol., Blacksburg, VA; [4]Human Nutr., Foods & Exercise, Virginia Technol., Blacksburg, VA; [5]Sch. of Neurosci., Virginia Technol., Blacksburg, VA; [6]Inst. for Creativity, Arts, and Technol., Virginia Technol., Blacksburg, VA

Abstract: Stone carving is a widespread and longstanding artistic practice across human cultures that offers a unique lens to explore its behavioral and neural impacts on modern humans. Despite its ancient roots and continued practice in modern day, limited research has examined its effects on mental health, social connectivity, and brain activity. This study seeks to fill this gap by investigating the effects of stone carving on psychological outcomes and intra- and inter-brain synchrony. Adult participants from southwest Virginia engaged in a series of stone carving sessions, during which their psychological state and brain activity were assessed through validated neuropsychological assessment scales (e.g., Positive and Negative Affect Scale; Watts Connectedness Scale; State Mindfulness Scale) and EEG hyperscanning, respectively. A pre-test/post-test experimental design without a control group was utilized, involving participants of varied stone carving expertise (n=20). Neuropsychological assessments were measured before and after the intervention. Neural recordings were performed during resting states before and after the intervention as well as during: 1) controlled deep breathing; 2) synchronous riffling (i.e., rhythmic carving); 3) visualization of their future work; 4) focused stone carving; and 5) group critique. For power analyses, log power was averaged across all 32 channels for each frequency band across each experience. A repeated measures ANOVA permutation test (of 800) with False Discovery Rate (FDR) correction was applied. Preliminary behavioral findings indicate that stone carving increased positive affect ($t(19)=-2.848$, $p=0.010$), mindfulness of bodily sensations ($t(19)=-2.244$, $p=0.037$) and mental events ($t(19)=-2.809$, $p=0.011$), and social connectedness ($t(19)=-4.689$, $p<0.001$). At the neural level, stone carving induced a significant increase in theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), and low gamma (35-45 Hz) frequencies during resting brain states. Additionally, during the stone carving experience, and specifically compared to synchronous riffling, power significantly increased across all frequencies. Additional analyses will focus on the effects of stone carving on intra- and inter-

brain synchrony. Collectively, these findings suggest that the artistic practice of stone carving acutely enhances socioemotional mental health and alters brain dynamics. Future analyses will focus on comparisons between intervention modules, comparing creative versus repetitive movements, group versus individual activities, and correlating the behavioral to the brain outcomes.

Disclosures: S.P. Diesel: None. **N. Tasnim:** None. **M. Aychman:** None. **C. Golding:** None. **A. Garrastegui Segarra:** None. **L. McNair:** None. **J.C. Basso:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.04/M32

Topic: G.04. Emotion

Support: National Center for Advancing Translational Science of the National Institutes of Health Award UL1TR003015/ KL2TR003016.
Elon CATL Teaching and Learning Grant

Title: Choreography creation is associated with increased dispositional flow and changes in beta neural activity in experienced dancers

Authors: *N. TASNIM[1], M. AYCHMAN[1], S. DIESEL[1], C. GOLDING[1], C. LIU[2], J. C. BASSO[3];
[1]Virginia Technol., Blacksburg, VA; [2]Elon Univ., Elon, NC; [3]Human Nutr., Foods & Exercise, Virginia Technol., Blacksburg, VA

Abstract: Flow is a psychological state of being fully engaged with a task. Dancers have subjectively reported experiencing heightened flow state during performance, but it is unknown how flow relates to brain physiology. Our goal was to examine the brain activity of trained dancers and its relationship to flow, performance, and the creative process associated with choreography creation. We recruited 6 female trained dancers (Ages: 19-22, Mean: 20) who co-choreographed a 2-3 minute solo with co-author Liu for 2 months. The choreography and rehearsal process ended with a final performance. At the beginning of the 2-month rehearsal process, the middle of the rehearsal process, and the final performance, brain activity from dancers was recorded using a 32-channel mobile electroencephalography (EEG) system (LiveAmp 32, Brain Products GmbH, Gilching, Germany). Brain activity was measured during a baseline activity, planning of choreography, general dance during rehearsal, and performance of a dancer's final solo. Validated behavioral and mental health questionnaires were administered to dancers before their 2-month rehearsal process and after the final performance. Scores were statistically compared using Wilcoxon Signed Rank Tests on paired samples. Dispositional flow (Dispositional Flow Scale) associated with dance increased after the 2 month rehearsal process (before 2-month rehearsals [median: 134, IQR: 19.5]; after final performance [median: 150, IQR:

12.2]; p = 0.0313; r = 0.899). Dancers' positive affect (Positive and Negative Affect Schedule) also increased (before [median: 39, IQR: 6]; after [median: 42.5, IQR: 6.25]; p = 0.0263; r = 0.953). Power spectral densities averaged across all 32-channels were calculated for different experiences that occurred during each session. We found a peak in whole-brain beta activity (12-30 Hz) associated with performance of dancers' solos. Dancers performed their solos during the middle rehearsal and performance, with higher power ($p<0.05$) observed around 14-15 Hz during the final performance in comparison to the middle rehearsal (paired permutation t-test, 800 permutations, false discovery rate correction). Brain activity was recorded while dancers looked at a crosshair (3-5 minutes) at the start of the rehearsal/performance for a pre-dance baseline assessment. Peak in whole-brain alpha (8-12 Hz) was observed at 10 Hz before the beginning and middle rehearsals, however, a more prominent peak was observed at 8 Hz before the final performance, suggesting a different mindset before performance. Our results suggest that flow in dance is associated with focused concentration as seen through increased beta activity.

Disclosures: N. Tasnim: None. **M. Aychman:** None. **S. Diesel:** None. **C. Golding:** None. **C. Liu:** None. **J.C. Basso:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.05/M33

Topic: G.04. Emotion

Support: JSPS KAKENHI Grant Number 23KJ2001

Title: Neural mechanism of musical pleasure elicitation by prediction error - An EEG study

Authors: *F. UENO[1,2], S. SHIMADA[1];
[1]Meiji Univ., Kawasaki, Japan; [2]Japan Society for the Promotion of Science, Tokyo, Japan

Abstract: Why does music fascinate people? It is postulated that internal predictive models, which is learned from previous listening experiences, is used while listening to music to predict how the next acoustic feature will change, and that the prediction error (surprise) when the actual sound deviated from the prediction will activate the brain's reward system and elicit musical pleasure. In this study, we analyzed the relationship between musical pleasure and 'surprise' which is calculated objectively from the musical information. We also investigated the brain activity that reflects musical pleasure. First, we selected 8 pieces of music (clip1~8) with the highest "musical surprise" information content (IC). The IC (for melody/harmony) is the objective measure of musical surprise, calculated using a variable-order Markov model (IDyOM). Next, we recorded EEG for 32 subjects during listening to the selected music. Subsequently, they were asked to report the continuous time courses of the degree of subjective pleasure evoked during music listening, on a scale of 1 to 4. They were also asked to report the time courses of the subjective surprise as well. Finally, they were asked to answer the Barcelona

Musical Reward Questionnaire (BMRQ). Analysis was performed on 24 subjects (23.3 ± 3.25 years old, mean ± SD), due to the exclusion of 8 subjects with a BMRQ score of less than 65. Pearson's correlation coefficients were obtained for the time-courses of subjective pleasure and surprise (ICs and subjective surprise). There was a significant positive correlation between subjective pleasure and ICs in 4 musical pieces (clip2(subjective pleasure-IC(melody)): $r = 0.37$, $p < 0.001$; clip4(subjective pleasure-IC(melody)): $r = 0.394$, $p < 0.001$; clip6(subjective pleasure-IC(harmony)): $r = 0.407$, $p < 0.001$; clip7(subjective pleasure-IC(melody)): $r = 0.336$, $p < 0.001$). Next, the EEG analysis was performed on clip2 with 18 subjects (23.1 ± 3.14 years old, mean ± SD) (six subjects were excluded due to technical problems). Here, we calculated the time-course of power values in the θ band (4-7 Hz), and fitted them to the time-course of subjective pleasure (general linear model analysis). Results showed significant fit for electrodes located in the superior temporal gyrus (STG) and/or postcentral gyrus (PoG) (FT8: $t(29) = 2.67$, $p = 0.012$; C3: $t(29) = 2.33$, $p = 0.026$). These results suggest that objective ICs of surprise based on musical information of melody and harmony predicts musical pleasure, which is related to the θ band activity in STG and/or PoG.

Disclosures: F. Ueno: None. **S. Shimada:** None.

Poster

PSTR238: The Influence of Art on Cognition, Neural Function, and Wellbeing

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR238.06/M34

Topic: G.05. Mood Disorders

Support: Minnesota Futures Grant, University of Minnesota
5R25DA057802-02
5T32NS105604-04

Title: Entropy of fMRI signals in the default mode network before and after a 2-week creative arts intervention for adolescents with depression

Authors: *E. F. ABEBAW[1], D. J. ROEDIGER[2], M. E. DIMAGGIO-POTTER[3], L. PADILLA[4], B. MUELLER[2], S. YUE[5], A. ABDALLA[2], C. DURKIN[2], Y. TANIGUCHI[6], B. KLIMES-DOUGAN[7], K. CULLEN[2];
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Abstract: Studying the complexity of brain signals may have promise for understanding brain-based disorders such as depression. Sample entropy measures the unpredictability and complexity of a signal and can be applied to fMRI data to estimate the complexity of neural

functioning. However, few studies have investigated the role of entropy in adolescent depression. Prior research has implicated the Default Mode Network (DMN) in depression. The current study investigated sample entropy of resting-state fMRI signals in the DMN in thirty-nine adolescents with depression before and after they completed Creativity Camp, a 2-week creative arts intervention. We hypothesized that DMN sample entropy would change after the intervention and that changes in DMN sample entropy would be correlated with adolescent ratings of depression. Each adolescent participated in eight four-hour sessions centered around creative exploration, creation, and reflection. We administered fMRI scans and self-report clinical questionnaires before and after the intervention. The Children's Depression Inventory 2 (CDI-2) was used to measure adolescent ratings of depression. Sample entropy was calculated on the resting-state time series from each vertex in the brain signals. We then calculated the average value for all vertices within 24 key nodes of the DMN. Analyses included (1) measuring the pre-post change in both CDI-2 and DMN sample entropy using paired t-tests; (2) measuring the correlation between DMN sample entropy at each time point; and (3) measuring the correlation between change in depression with change in sample entropy. Results showed a significant decrease in adolescent CDI-2 scores pre-to-post intervention ($t(38) = -3.33$, $p = 0.002$). We found a non-significant change in DMN sample entropy pre-to-post intervention ($t(23) = 1.81$, $p = 0.08338$) and a trending correlation between change in CDI-2 scores and DMN sample entropy pre-post ($t(23) = -1.91$), $p = 0.0685$). Our findings suggest that sample entropy in the DMN may be impacted by changes in depression.

Disclosures: E.F. Abebaw: None. **D.J. Roediger:** None. **M.E. DiMaggio-Potter:** None. **L. Padilla:** None. **B. Mueller:** None. **S. Yue:** None. **A. Abdalla:** None. **C. Durkin:** None. **Y. Taniguchi:** None. **B. Klimes-Dougan:** None. **K. Cullen:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.01/M35

Topic: G.04. Emotion

Support: STI 2030—Major Projects (2021ZD0200401to A.W.R.)
The National Natural Science Foundation of China (U20A20221, 819611280292)
The Key Research and Development Program of Zhejiang Province (2020C03004)
The Fundamental Research Funds for the Central Universities

Title: Mesoscale functional connectivity of medial pulvinar to cingulate and insular cortex in macaque monkey revealed by INS-fMRI

Authors: Y. FENG[1,2], S. YAO[2,3], N. SHI[1,2], M. LIU[1,2], ***J. WANG**[2,3], A. W. ROE[1,2,3];
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Science, Zhejiang Univ., Hangzhou, China; [2]Zhejiang Univ. Interdisciplinary Inst. of Neurosci. and Technol., Hangzhou, China; [3]MOE Frontier Sci. Ctr. for Brain Sci. and Brain-Machine Integration, Sch. of Brain Sci. and Brain Medicine, Zhejiang Univ., Hangzhou, China

Abstract: Motivation: In primates, the pulvinar is a large thalamic nucleus involved in vision and attention. Of its three major subregions, the lateral pulvinar (PL) and inferior pulvinar (PI) support vision-guided functions; the medial pulvinar (PM) serves to integrate attention, sensation, and emotion (Froesel et al. 2021). Here, we investigate the connectivity of PM to limbic cortex. Our previous work, using INS-fMRI, showed that functional connections of PL and PI with visual cortex are topographically organized at mesoscale (Yao et al. 2023). We hypothesize that, similar to visual cortex, there is a mesoscale topography of PM to emotion-related cortex. Specifically, we use INS-fMRI to examine the mesoscale connectivity to two prominent limbic areas, the cingulate and insular cortex (Romanski et al. 1997; Guedj et al. 2020). **Method:** We used a mesoscale functional connectivity mapping approach (INS-fMRI: Infrared Neural Stimulation in 7T fMRI) in macaque monkey (Xu et al. 2019). INS evokes neural response at the fiber tip via short pulse trains of light (1875nm, 200 Hz, 200 μm optical fiber), which leads activation at connected sites, resulting in a brain-wide network mapped with 7T fMRI. We conducted INS stimulation at 6 sites in PM of two anesthetized monkeys and evaluated the significant BOLD responses in ipsilateral cingulate and insular cortex. **Results:** The functional connectivity of PM to cingulate cortex was patchy (patches largely < 2mm in size) and distributed in both area 24 (ACC) and area 23 (PCC), each of which contains a limbic motor body map (Morecraft et al., 2007). In ACC, each of the face, arm, leg regions contained non-overlapping activations from each of the 3 stimulation sites in PM of each monkey, suggesting an integrative connectivity from PM to ACC. In PCC, patches were less organized but were also spatially separated. A similar patchy functional connectivity of PM to insular cortex was observed, distributed through the granular Ig, dysgranular Id, and agranular Ia subdivisions. This connectivity had an integrative organization and appeared to be related to distinct interoceptive functional regions within the insular (Evrard, 2019). **Conclusion:** We find functional connectivity of emotion-related connectivity of PM is organized at mesoscale. Unlike the point-to-point topographic organization between PI/PL and visual cortex, PM's connectivity with cingulate and insular cortex exhibits an organization reflecting local integration of inputs from PM. This suggests an organizational architecture of connectivity underlying emotion-related circuits in the brain.

Disclosures: Y. Feng: None. **S. Yao:** None. **N. Shi:** None. **M. Liu:** None. **J. Wang:** None. **A.W. Roe:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.02/M36

Topic: G.04. Emotion

Support: NIH Grant MH067924
Staunton Farm Foundation

Title: Nuclei-specific functional maturation of fronto-amygdala circuitry through adolescence: longitudinal insights from 7 Tesla fMRI

Authors: *A. OJHA[1], V. SYDNOR[2], W. FORAN[2], S. F. SORRELLS[3], F. J. CALABRO[4], B. LUNA[5];
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Abstract: Fronto-amygdala circuitry, crucial for cognitive and emotional processing, undergoes protracted development throughout adolescence. Previous studies show mixed findings as to the shape and direction of neurodevelopmental trajectories of fronto-amygdala maturation, which may be in part due to the amygdala's diverse nuclei, which have unique functions and developmental trajectories.
We leveraged 7T high-resolution neuroimaging data to identify amygdala nuclei and their functional connectivity across prefrontal (PFC) regions in 156 10-25-year-olds, assessed longitudinally (221 visits), during rest (8min) and when engaged in a cognitive task (with task effects regressed out). We use generalized additive mixed models (GAMMs) to capture non-linear age-related developmental trajectories and applied Bonferroni corrections to control for multiple comparisons. Connections that showed changes with age were further interrogated using cognitive domain mapping to identify their specific function.
The functional connectivity of most amygdala nuclei to PFC regions did not show a significant association with age. Only connectivity between the cortico-amygdala transition area (CAT) and the subgenual/ventral anterior cingulate cortex (ACC) as well as with the lateral PFC, showed age related effects reflected in a U-shaped developmental trajectory. Seed-based connectivity indicated that this circuitry is associated with monitoring internal states (pain, eating, and anxiety). Finally, U-shaped CAT connectivity with the dorsolateral PFC and ventral ACC was associated with higher externalizing features while low externalizing showed increases with age. Together, results suggest that maturation of fronto-amygdala connectivity is largely in place by adolescence except for functional connectivity between the CAT nucleus and PFC showing a trough in adolescence possibly underlying immaturities in the ability to monitor internal states, which may be more predominant with increasing normative externalizing features. Overall, this connectivity strengthens into adulthood supporting greater executive integration of CAT processing supporting mature processing of internal states.

Disclosures: A. Ojha: A. Employment/Salary (full or part-time):; University of Pittsburgh. **V. Sydnor:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **W. Foran:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **S.F. Sorrells:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **F.J. Calabro:** A. Employment/Salary (full or part-time):; University of Pittsburgh. **B. Luna:** A. Employment/Salary (full or part-time):; University of Pittsburgh. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIMH.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.03/M37

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH Grant 1-R01-MH-113574-01-A1

Title: Linking context processing to PTSD symptoms and hippocampal activity

Authors: *G. S. VOGT[1,2], J. SHEYNIN[3], I. LIBERZON[3,4];
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Abstract: Altered contextual processing has been hypothesized to underlie memory abnormalities in posttraumatic stress disorder (PTSD). Specifically, altered pattern separation (PS) and pattern completion (PC), two memory processes involved in context processing, might contribute to impaired recall of fear extinction memories reported in these patients. Further, the hippocampus plays a critical role in processing context and abnormal hippocampal activity may be linked to PS and PC deficits. Here we examine the relationship between PS and PC performance, PTSD symptoms, and hippocampal activity. One hundred and seventeen participants (39% female, mean age (SD) = 32 (11.7) years), including sixty-seven healthy controls and fifty participants with PTSD, underwent a functional MRI scan during which they performed two tasks: (1) Mnemonic Similarity Task (MST) to distinguish previously viewed items from novel items or lures (i.e., items similar to, but distinct from previously viewed items); and (2) Context Discrimination and Recognition Task (CDRT) whereby they identified the type of room presented ("office" vs. "living room") from an ambiguous set of available details. PS performance was derived from the MST and PC performance was derived from the CDRT. Participants also completed the PCL-5 to assess PTSD symptoms and analysis was conducted on the entire, combined sample. PCL-5 scores were negatively correlated with discrete recognition (PC) scores on the CDRT ($r = -.224$, $p = .015$) but not with MST PS scores ($r = -.136$, $p = .14$). In further examination, PCL-5 scores were found to be positively associated with errors in which images containing similar, but novel items were misidentified as previously viewed contexts ($r = .268$, $p = .003$), but not with accuracy of memory for previously viewed contexts. Finally, a hierarchical linear regression found that PCL-5 scores predicted these errors in PC performance ($\beta = .21$, $t = 2.07$, $p = .04$) when controlling for age. Findings indicate that greater PTSD symptoms are associated with greater impairment of recognition performance via an increased number of errors in misidentifying similar, yet novel contexts, suggesting an imbalance towards PC of ambiguous contexts occurs in individuals with PTSD. Neuroimaging results will also be presented linking recognition performance to hippocampal function.

Disclosures: G.S. Vogt: None. **J. Sheynin:** None. **I. Liberzon:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

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Program #/Poster #: PSTR239.04/M38

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH grant MH125615

Title: Fear conditioning prompts sparser representations of conditioned threat in primary visual cortex

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Abstract: Threat detection and valuation is an important function of the visual system. It has been proposed that repeated exposure to threatening stimuli alters sensory responses. Our previous study has found that neural representations of conditioned threat in the primary visual cortex become sparser with aversive learning. We examined this issue further in this study. Simultaneous fMRI and EEG data were recorded from 18 participants viewing the random appearance of two Gabor patches with the 45-degree Gabor patch (CS+) occasionally paired with a loud scream (US) (25% reinforcement rate) and the 135-degree Gabor patch (CS-) never paired with the US. Applying the MVPA decoding method to fMRI data in a sliding trial window fashion, we found that (1) decoding accuracy between CS+ and CS- was significantly higher than chance level in all visual areas throughout the conditioning session, (2) as aversive learning progressed, the number of voxels contributing to the representation of CS+ decreased in primary visual cortex but not in other visual areas, and (3) the number of voxels contributing to the representation of CS- remained the same. Analyzing the concomitantly recorded EEG data, we found that (1) the latency of the event-related potential evoked by CS+ became progressively shorter with aversive learning and (2) the latency of the event-related potential evoked by CS- became progressively longer. These results confirmed that the neural representation of conditioned threat became sparser in the primary visual cortex and suggested that the sparsified representation facilitated the detection and evaluation of threat.

Disclosures: L. Cui: None. **A. Keil:** None. **M. Ding:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

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Topic: G.01. Fear and Aversive Learning and Memory

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Title: Distinct contribution of the visual thalamus to processing different phases of threat learning in humans

Authors: *M. BADARNEE[1], Z. WEN[2], M. MILAD[2];
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Abstract: It has been long proposed that visual threat might be processed via the thalamo-subcortical and cortical pathways. The distinct thalamic contribution to this model has not been tested in humans, especially in the context of early emotional learning where the CS-US association is thought to be created. We analyzed the BOLD signal in the pulvinar's subnuclei and lateral geniculate nucleus (LGN) during conditioning, extinction recall, and renewal in 293–412 subjects that underwent a two-day threat conditioning and extinction paradigm while in the fMRI. We used repeated measures ANOVA to analyze trial-by-trial signals during early learning (4 trials). We observed a signal consistent with an associative learning pattern within the anterior pulvinar; a gradual differential increase in BOLD signal from trial one onwards that is specific to the CS+ ($F_{1,292}$=20.42, p<0.001; CS+>CS- $p_{trial\ 1}$=n.s., $p_{trial\ 2}$<0.001). LGN showed increased arousal response in the first trial and dropped in the following trials ($F_{1,292}$=11, p=0.001; CS+>CS- $p_{trial\ 1}$<0.001, $p_{trial\ 2}$=n.s.). This functional pattern is consistent with a transmitter of threatening information and/or sensory processor rather than a learner. The two reigns contributed to extinguishing the CS+ (Anterior pulvinar: $F_{2.9,933.9}$=6.3, p<0.001. LGN: $F_{2.7,875.6}$=11.29, p<0.001) but still showed increased signal to CS+ in recall (Anterior pulvinar: $F_{1,411}$=6.22, p=0.013; CS+>CS- $p_{trial\ 1}$<0.01, $p_{trial\ 2}$<0.05. LGN: $F_{1,411}$=6.82, p=0.009; CS+>CS- $p_{trial\ 1}$<0.005, $p_{trial\ 2}$=n.s.) and renewal (Anterior pulvinar: $F_{1,317}$=8.57, p=0.004; CS+>CS- $p_{trial\ 1}$<0.001, $p_{trial\ 2}$=n.s.; LGN: $F_{1,317}$=5.82, p=0.016; CS+>CS- $p_{trial\ 1}$<0.001, $p_{trial\ 2}$=n.s.). The anterior pulvinar showed increased connectivity with the vmPFC, amygdala, and hippocampus during conditioning (t-values 2.43–2.60, all p_{FDR} < 0.05) and with the vmPFC during renewal (t=3.47, p_{FDR} < 0.05). These findings shed some light on the unique contributions of the thalamo-subcortical vs. cortical pathways in processing visual threats in humans. The anterior pulvinar might contribute to creating the CS-US association, whereas the LGN behaves as a transmitter of the CS-US information and/or contributes to processing skin-related information. The increased connectivity of the anterior pulvinar with the vmPFC, amygdala, and hippocampus might indicate its role in encoding emotional learning: The contextual information is most likely processed in the hippocampus, the negative emotional valence of the CS+ is encoded in the amygdala, and the integration of this information might be encoded in the vmPFC for regulation and future responses.

Disclosures: M. Badarnee: None. **Z. Wen:** None. **M. Milad:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

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Program #/Poster #: PSTR239.06/M40

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R21MH133055

Title: Causal mapping of dorsomedial prefrontal cortex for regulation of the threat response using stereoelectroencephalography in humans

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Abstract: The adaptive ability to manage emotions appears to rely on fronto-limbic brain network function, but direct causal testing of human medial brain circuits underlying emotion learning and regulation processes remain scarce. Correlative studies have implicated a dorsomedial prefrontal cortex (dmPFC)-amygdala network in learning-related regulation of the emotional response to threat. Adapting correlative assessments for direct causal testing of dmPFC function will fill a critical gap in understanding neural circuitry underlying flexible regulation of threat responses. 5 patients participated in recording and stimulation testing during Pavlovian fear conditioning during stereoelectroencephalography (sEEG) monitoring for refractory epilepsy. Psychophysiological measures assessed learning-related changes in skin conductance response (SCR) to threat (100-dB white noise; 0.5s). During an initial acquisition phase (75 trials), threats were presented alone (unpredictable threat) or co-terminated with a 6s warning cue (tone700Hz; predictable threat). In a subsequent test phase (20 trials), inhibitory stimulation pulses (20 Hz, 2 mA) were delivered 2s prior to threats for predictable-test, but not predictable-sham trials. Patient CT and MRI scans were normalized in standard space to select the nearest electrode to a pre-determined dmPFC region (MNI: x=-4, y=40, z=25) based on prior work. After replicating typical learning-related changes in SCR during acquisition (predictable < unpredictable threat SCR), we hypothesized dmPFC stimulation in the test phase would disrupt anticipatory regulation (predictable-sham < predictable-test SCR). A paired samples t-test confirmed reduced SCRs to predictable (m= 1.03 µS) vs unpredictable (m= 2.39 µS) threat during the acquisition phase ($t = 3.7$, $p < 0.01$). During the test phase, linear mixed effects (LME) models for 2 participants yielded a main effect of trial type ($Fs \geq 7.5$, $ps < 0.05$). Posthoc tests revealed effects were driven by increased SCRs to unpredictable threats ($ps < 0.05$), but no difference between predictable-sham and predictable-test ($ps > 0.25$). Remaining LMEs (n=3)

failed to reach criteria for significance (Fs ≤ 1.2, ps ≥ 0.3). The current study demonstrates learning-related changes in SCR during sEEG recording in humans. However, the dmPFC stimulation test results suggest inhibiting regulation may critically depend on additional factors (e.g., grey matter site, pulse duration). This adaption of a correlative brain-behavior assessment for direct mapping of medial brain networks marks a crucial step towards testing casual inferences about human's ability to manage emotions.

Disclosures: R.J. Chatfield: None. **C.T. Eickstead:** None. **A. Vennel:** None. **A.R. Nolan:** None. **A. Bolaram:** None. **B.C. Cox:** None. **A.D. Fobian:** None. **J.F. Magnotti:** None. **D.C. Knight:** None. **J.P. Szaflarski:** None. **A.M. Goodman:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.07/N1

Topic: G.04. Emotion

Support: NIH Grant R01MH113238
Duke University Medical Center Grant KL2 TR002554

Title: Insula functional connectivity during retrieval and reappraisal of negative memories in transdiagnostic emotion dysregulation

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Abstract: Background: Emotion dysregulation is a transdiagnostic problem that is associated with negative effects on psychiatric outcomes. In a sample of adults with emotion dysregulation who completed a neurostimulation intervention (rTMS), we previously found increased functional connectivity between prefrontal cortex (PFC) and insula to be both a marker of cognitive emotion regulation and a marker of active rTMS treatment compared to sham. However, it is unclear whether insula connectivity patterns during cognitive regulation vary between clinical and non-clinical groups. Methods: 33 adults with emotion dysregulation and at least one DSM-V disorder and 60 adults with no psychiatric history completed an emotion regulation paradigm in a 3T GE scanner as part of separate studies. Participants were prompted to either cognitively reappraise or passively feel their emotional responses to cued negative and neutral autobiographical memories. Left and right insula connectivity seeds were derived from activation during the Reappraise Negative vs. Feel Negative contrast. Data were analyzed using generalized psychophysiological interaction models implemented in FSL using the Cue Negative vs. Cue Neutral and Reappraise Negative vs. Feel Negative contrasts. Results: Healthy

participants did not display increased insula connectivity with the PFC, or any other region, when reappraising vs. feeling negative affect. During cueing of negative vs. neutral memories, we found increased insular connectivity with the inferior parietal lobule ($z = 3.2 - 4.5$, $p < .03$) in both groups, with the dlPFC ($z = 3.6$, $p = 0.03$) in the clinical group, and with the vlPFC ($z = 3.7 - 4.3$, $p < 0.05$) in the control group. Conclusion: In contrast to our findings in individuals with emotion dysregulation, healthy participants do not show increased insula-PFC connectivity during cognitive reappraisal of negative affect. Therefore, these groups can be distinguished by their insula-PFC connectivity patterns during active cognitive regulation, which has important implications for neuroscience-based intervention development. In both groups, we found increased connectivity between the insula and the inferior parietal lobule during recall of negative vs. neutral memories, which may reflect either reconstructive emotional retrieval processes or the initial, automatic engagement of cognitive control circuitry to downregulate distress. However, during negative memory recall, the insula engages different PFC subregions depending on clinical status. Further research is needed to parse the role of dlPFC and vlPFC subregions during recall of negative memories in the context of emotion regulation.

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Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

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Topic: G.03. Motivation

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McDonnell Center for Systems Neuroscience
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American Epilepsy Society

Title: Dynamic cortico-limbic networks underlie approach-avoidance conflict in humans

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Abstract: Choosing to approach or avoid actions or stimuli that represent both rewarding and aversive outcomes is characteristic of everyday decisions and can be accompanied by feelings of anxiety. In humans, excessive avoidance is a feature of generalized anxiety disorder, PTSD, and agoraphobia. Research in rodents has identified a neural circuit, mediated by theta-band activity, between the ventral hippocampus (HC), the amygdala (AMY) and prefrontal cortex (PFC) that provides a mechanism for control of approach/avoidance behavior in anxiogenic contexts. Human fMRI research has identified similar regions, but with a greater focus on the differential contributions of PFC subregions, such as the anterior cingulate (AC), orbitofrontal cortex (OFC) and dorsolateral PFC (dlPFC). In humans, circuit-level activity in these regions has been challenging to study, given inherent limitations in the resolution of non-invasive techniques. Here we tested for the presence and relevance of a theta (3-8Hz) circuit across these regions using 15 adult presurgical epilepsy patients who performed a novel, continuous-time approach-avoidance conflict decision-making task inspired by the arcade game Pacman (240 trials). We found the decision to move towards the center of the corridor was associated with potential gains (eating “dots”, resulting in points) and potential losses (ghost attack, resulting in loss of the Pacman life). We find evidence of a cortico-limbic circuit mediated by theta coherence, wherein theta power in the HC, AMY, OFC, and AC is elevated during approach, but drops during avoidance (linear mixed effects models predicting theta power using approach/avoidance, $p_{FDR\text{-}time} < 0.01$ for at least 500 ms for each region). The OFC acts as a hub in this circuit, with high coherence across multiple regions, whereas the HC is predominantly connected to the AMY, and the AC is primarily connected to the dlPFC. Importantly, the degree of connectivity between the OFC and the other regions is predictive of how long participants continue their approach, with more synchronous activity leading to longer approaches (linear mixed-effects models predicting electrode pair theta synchrony using avoidance times, Std. Beta: 0.05 [95% CI:0.05-0.06]). Finally, under increased threat, the system dynamically switches to one characterized by a sustained increase in high-frequency activity (70-150Hz) in the dlPFC. The results provide evidence for a distributed cortico-limbic circuit, mediated by theta oscillations, underlying approach-avoidance conflict. Characterizing this network has implications for the development of novel therapies for treatment-resistant anxiety disorders.

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Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.09/N3

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01MH124761

Title: Intracranial neurophysiological signatures of fear and anxiety in the human hippocampal-amygdala circuit during virtual reality fear conditioning

Authors: *A. JANG[1,2], J. GILL[2,3], M. VALLEJO[2], R. MUSTAPHA[2], J. BAHAM[2], H. N. ZUBAIR[4], S. HILLER[2], J. A. SCHNEIDERS[2], D. BATISTA[2], M. JENKENS-DRAKE[2], C. ORAGWAM[4], B. BARTHOLOMEW[9], U. TOPALOVIC[2], M. SEEBER[2], M. STANGL[10], C. S. INMAN[11], M. S. FANSELOW[5], M. CRASKE[5], A. ADHIKARI[5], R. KOEK[6,12], J.-P. LANGEVIN[7,13], N. A. SUTHANA[2,8,7,5];
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Abstract: Anxiety disorders involve fear responses that are excessive and disproportionate to the situation. Understanding how the human brain processes fear and how this may go awry in anxiety disorders is crucial for developing effective therapies. Rodent models of anxiety suggest that the formation and regulation of fear responses to the environment require a complex interplay between the amygdala and hippocampus. It is hypothesized that the hippocampus forms a representation of the environmental context while the amygdala ties this context to emotions. However, it is unclear whether these findings can be translated to humans due to the challenges of obtaining direct recordings of these subcortical brain regions. This project aims to investigate the hippocampal-amygdala neural mechanisms governing the acquisition and extinction of contextual fear responses in humans. We recorded intracranial EEG (iEEG) activity in seven participants with treatment-resistant epilepsy with the chronically implanted Responsive Neurostimulation (RNS) device in the hippocampus and amygdala while they completed a fear conditioning experiment. To simulate real-world fear responses to different environmental contexts, we designed a virtual reality (VR) experiment where participants ambulated in different virtual environments (e.g., library, museum, grocery store). Occasionally, the lighting of the environment changed colors, where one of the colors (conditioned stimulus, CS+; e.g., green light) was followed by an aversive, unconditioned stimulus (US, e.g., sudden appearance of a giant spider). The other color (CS-; e.g., blue light) did not lead to the US. After this CS-US association was learned, participants entered a new environment where neither of the colored lights led to the US, facilitating fear extinction. We found an increase in iEEG theta power and theta-gamma phase-amplitude coupling in the amygdala along with increased skin conductance and heart rate for CS+ trials when compared to CS- trials. These fear-based activities abated after the participants underwent fear extinction. Using representational similarity analysis of iEEG power, we found that activity patterns within the hippocampus, but not the amygdala, have significantly increased similarity among trials of identical contexts (e.g., library-library) when compared to trials of different contexts (e.g., library-museum). Together, our results suggest functionally distinct roles of the amygdala and hippocampus in contextual fear learning.

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Poster

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Program #/Poster #: PSTR239.10/N4

Topic: G.01. Fear and Aversive Learning and Memory

Support: BCS-2001025

Title: Sleep signatures of emotional memory consolidation from young adulthood through middle age

Authors: *K. SANDERS[1], X. NIU[1], M. UTAYDE[1], E. A. KENSINGER[2], J. PAYNE[1]; [1]Univ. of Notre Dame, Notre Dame, IN; [2]Psychology and Neurosci., Boston Col., Chestnut Hill, MA

Abstract: Emotional information is remembered better than neutral information and better than the neutral context in which it is experienced. Prior research suggests that consolidation processes, especially those that occur during sleep, underlie this selective memory effect. In young adults, emotional memory consolidation has been linked to the amount of time spent in Rapid Eye Movement (REM) sleep; but much less is known about what EEG signatures of REM sleep predict later emotional memory. Furthermore, both emotional memory and sleep architecture change with age; however very few studies have examined sleep signatures of emotional memory in middle-aged adults and a few prior studies suggest that slow-wave sleep, instead of REM sleep, may predict emotional memory consolidation in older populations. In the current study, healthy adults aged 18-59 rated the emotionality of scenes consisting of an emotional object (e.g. a car crash) or neutral object (e.g. an intact car) on an always neutral background (e.g. a city street). Approximately 12 hours later, after a typical day awake or a night of sleep, participants completed a surprise memory test where they saw objects and backgrounds separately and rated whether the item was the same, similar, or new compared to the scenes they had previously encountered. Participants in the sleep condition had their brain activity recorded with EEG while they slept in the lab. Preliminary results replicated prior research showing that participants remember emotional objects at the expense of memory for the paired neutral backgrounds. Although increased age was associated with decreased memory performance and reduced time in both REM and slow-wave sleep, age did not interact with the memory trade-off effect. Furthermore, REM sleep predicted larger emotional memory trade-off effects due to a positive correlation with memory for the negative objects while SWS predicted a smaller trade-off effect due to a positive correlation with memory for the paired backgrounds. This study furthers our understanding of how both memory and sleep change with age and suggest a

relatively preserved emotional memory trade-off effect despite changes in both memory and sleep.

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Poster

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Title: Frequency-dependent audibility modulates alpha activity responses to perceived emotions in music.

Authors: *J. LEE;
Hallym Univ. Col. of Med., Anyang, Korea, Republic of

Abstract: Frequency-dependent audibility modulates alpha activity responses to perceived emotions in music. People with hearing loss complain of listening to music, and it is even more challenging to perceive the emotions of music. Although a large body of studies are focused on the perception of musical emotions in people with hearing loss, the underlying cortical mechanisms for the perception of musical emotions in people with hearing loss are unclear. In this study, to investigate the effect of audibility, we measured cortical activity response to the emotional perception of music. Normal hearing group (NH), high- (HFsim), and low- (LFsim) frequency simulated hearing loss groups were created by using original stimuli and applying low-, and high-pass filtering (1000 Hz cutoff) to musical stimuli, respectively. A total of 48 healthy participants were randomly assigned to three groups (16 people / group). Fifteen musical stimuli developed in our lab were used for the study. The pre-evaluated stimuli were composed of five melodies, and each melody was expressed differently according to emotions including happiness, sadness, and neutrality. During 64-channel EEG recording, participants listened to the randomly presented stimuli binaurally via two speakers followed by ratings of arousal and valence (dimensional model). A total of 300 trials were conducted 20 times repeatedly for 15 stimuli. The NH group had lower ratings for arousal than the LFsim group and for valence than the LFsim and NH groups. To examine the effect of frequency-dependent filtered music, we performed a time-frequency analysis comparing three groups. The lack of high-frequency audibility significantly enhanced the alpha power during the perception of three emotions. To examine the effect of dimensional responses to the perception of emotion, we selected trials according to the normalized rating criteria of arousal and valence (high or positive >= 0.3, low or negative < -0.3, middle -0.3<= and < 0.3). As a result, alpha power of low arousal reveals a prolonged period with significant points, indicating extended periods of differences between

groups while minimal variability in the perception of low valence among groups.The activation of alpha was more evident in sad than happy condition, and in arousal than valence, which conditions showed no different ratings in HFsim group compared to other groups. These results suggest that the lack of high-frequency auditory information might require more cognitive load in the process of perceiving emotions. Also, audible spectral information, especially high-frequency audibility, affects alpha activity during the perception of musical emotion.

Disclosures: J. Lee: A. Employment/Salary (full or part-time):; Hallym University College of Medicine (full time).

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.12/N6

Topic: G.04. Emotion

Support: Aligning Science Across Parkinson's Grant

Title: Integrated representations of threat and action control in the human frontal pole

Authors: *J. STASIAK, M. LI, S. T. GRAFTON, R. C. LAPATE;
Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The frontal pole (FP) is thought to be a critical hub supporting the integration of action-goal information with internal signals (Mansourri et al., 2017; Roelofs et al., 2023; Badre & Nee, 2018). Recent evidence indicates that FPl has privileged access to amygdalar projections compared to other LPFC regions (Folloni et al., 2019), thereby putatively supporting the integration of emotional and action-relevant information for cognitive control (Lapate et al. 2022). To test whether representations of emotional information and action-relevant goals are differentially integrated along distinct regions of the LPFC, we analyzed the data of a threat-of-shock paradigm using a multivariate representational similarity approach (Kriegeskorte and Kievit, 2013). Participants (n=67) tracked a prolonged (18s) countdown to shock administration, which could be avoided by making successful, time-sensitive motor responses at the end of the countdown period. We orthogonally manipulated shock intensity and controllability, such that in 'controllable' trials, participants made a motor response to try to avoid an electric shock; in 'uncontrollable' trials, a motor response was performed, but a shock was always received. During threat anticipation, the amygdala represented threat intensity (p=0.01). In contrast, FPl held integrated, conjunctive representations of threat intensity and action controllability (p=0.002), which were abolished during the action epoch-giving rise to threat-only coding in FPl (p=0.01). Critically, conjunctive representations of threat and controllability were absent in mid-LPFC, and significantly stronger in FPl versus mid-LPFC-thereby suggesting representational specificity along distinct LPFC regions for cognitive control in emotional contexts. Collectively, these findings support a functionally specific representational role for FPl in goal-directed action

to include the integration of threat intensity and volitional control to contextually shape action goals.

Disclosures: J. Stasiak: None. **M. Li:** None. **S.T. Grafton:** None. **R.C. Lapate:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.13/N7

Topic: G.04. Emotion

Support: NIMH Grant R01-MH134000

Title: Functional organization of lateral prefrontal cortex during time-emotion integration

Authors: *M. LI[1], R. WANG[2], J. WANG[2], R. C. LAPATE[2];
[1]Psychological and Brain Sci., Univ. of California, Santa Barbara, Goleta, CA; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The capacity to respond to dynamic emotional events in a time-and-context-sensitive manner is paramount to emotional wellbeing. Extant research underscores a critical role for the lateral prefrontal cortex (LPFC) in the temporally organized control of behavior, but mechanistic studies of LPFC function in emotion are lacking. Here, we examined LPFC's functional organization during the representation of time-emotion integrated control signals. Participants viewed negative and positive images over a 12-s period, which yielded predominantly positive vs. negative emotional sequences with varying amounts of temporal evidence (Δ_{time}: 1200ms vs. 1800ms). Participants were asked to indicate the predominant emotional valence after each sequence, based on a colored contextual cue (orange vs. purple) that indicated the mapping between predominant valence and action goal (left vs. right) and signaled an action preparation epoch, which was followed by action execution. Behaviorally, we found that greater temporal evidence benefitted time-emotion integration performance in both positive-longer and negative-longer sequences. During action preparation, emotional valence and temporal evidence signals were linearly decodable from the anterior LPFC (including frontal pole, FP), and contextual cue rule/color and action-goal signals were linearly decodable from mid-LPFC. Critically, temporally-integrated emotional signals in FP informed mid-LPFC contextual cue representations: Greater emotional-valence decoding in FP during emotional-sequence processing predicted greater contextual cue decoding in mid-LPFC during action preparation. Moreover, stronger decoding of emotional valence, temporal evidence, and contextual cue decoding in LPFC predicted higher time-emotion integration accuracy. Further, LPFC representations predicted behavior in a time-and-valence-dependent manner: During action preparation, higher emotional-valence decoding predicted better temporal integration for positive-longer (vs. negative-longer) sequences, whereas temporal-evidence decoding predicted better performance for stronger (vs. weaker) temporal-evidence trials. Collectively, these

findings provide novel insights into the role of LPFC in representing temporally-organized control signals during dynamic emotional experiences, and suggest a rostro-caudal axis of time-emotional integration for context-sensitive action.

Disclosures: M. Li: None. **R. Wang:** None. **J. Wang:** None. **R.C. Lapate:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.14/N8

Topic: H.03. Decision Making

Support: R01MH095894
R01MH108627
R37MH109728
R21AG073958
R01MH118203
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R01NS12305

Title: Predicting entrepreneurial pitch success using behavioral, textual, and EEG neuroforecasting.

Authors: *J. WONG[1], M. L. PLATT[2], J. YUN[3];
[1]Earlham Col., Richmond, IN; [2]Neuroscience, Perelman Sch. of Med., [3]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Less than 2% of US capital is attracted by women-owned businesses. Closing this gap would be facilitated by a deeper understanding of how entrepreneurial pitches engage investor interest and convert them to funding. Traditional methods primarily rely on self-reported subjective assessments, which may not fully capture how a pitch shapes investor decisions in real time. Here, we bridge this gap by applying a neuroforecasting approach that integrates self-reports, textual sentiment analysis, and neural responses via EEG to forecast the outcomes of entrepreneurs' pitches. We conducted an EEG experiment with 28 subjects who watched 14 Shark Tank pitches, then, analyzed EEG frontal alpha asymmetry (FAA)-a biometric measure of positive motivation-from power spectral density and neural synchrony (intersubject correlation)-a measure of shared attention, emotion, and mindset-from time-frequency representations. We also employed natural language processing to analyze transcripts for various sentiments. After each pitch video, participants self-reported their interest in obtaining further information, willingness to invest, perception of the investment's quality, and expected financial returns. We also measured individuals' tendencies toward dynamic capability (seeking and sensing investment opportunities) via a post-study survey. We first used hierarchical linear models to predict investor interest, with predictors including self-reported pitching content quality,

investors' dynamic capabilities, and FAA (averaged whole span of each pitch). We found that all measures significantly predicted investor interest. Importantly, incorporating FAA and dynamic capability measures into the model significantly improved predictions, even controlling for age and gender. However, adding the joyful sentiment from text analysis did not increase variance. Second, the FAA predicted out-of-sample investors' interest (N=497) within the first 10 seconds of pitching, thus providing a biomarker of early impression and interest. Moreover, peak neural synchrony around 50 seconds into the pitch, when entrepreneurs were typically describing their product solutions, significantly predicted real-world deal outcomes. Thus, integrating EEG data with self-reported measures provides a robust predictive approach to evaluating and refining pitches to maximize successful outcomes. Our findings build upon and extend recent work in neuroforecasting predicting pitch success using portable EEG with a small sample, thus offering a practical and scalable solution for entrepreneurs to refine their presentation strategies and successfully secure a deal.

Disclosures: J. Wong: None. **M.L. Platt:** None. **J. Yun:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.15/N9

Topic: G.01. Fear and Aversive Learning and Memory

Title: Imagery rescripting attenuates the return of generalized fear through spontaneous recovery

Authors: *S. MITRA[1], M. K. ASTHANA[2];
[1]Humanities and Social Sci., Indian Inst. of Technol. Roorkee, Roorkee, India; [2]Dept. of Humanities & Social Sci., Indian Inst. of Technol. Roorkee, Roorkee, India

Abstract: Standard extinction (SE) is the most widely implemented intervention for reducing conditioned and generalized fear; however, the possibility of fear return still remains. Hence, novel interventions may be used to augment the efficiency of SE. Imagery rescripting (ImRS), based on the premise of UCS devaluation, encompasses the devaluation of the cognitive representation of the UCS in a positive direction. Imaginal extinction (IE), based on the principles of UCS expectancy evaluation, involves vivid imagination of the conditioned stimuli during extinction. In the current study, we compared the efficacy of ImRS over IE and SE in attenuating the return of generalized fear. Seventy-two healthy individuals (M=18.83 years, SD=0.44) were randomly assigned to ImRS, IE, or SE groups. On day 1, the participants underwent the fear acquisition and generalization phases. On day 2, the intervention and generalization testing phases were conducted. On day 3, 15 days after acquisition, the return of fear due to spontaneous recovery was assessed. Skin conductance responses (SCR), expectancy, and valence ratings were outcome measures of conditioned and generalized threat-based fear responses. We found that the ImRS group had a significantly reduced differential SCR in the generalization testing [$F(2,71) = 4.767$, $p = 0.011$] and spontaneous recovery [$F(2,71) = 3.686$,

p= 0.030] phases. Further, the mean expectancy of CS+ in the ImRS and IE groups, and IE and SE groups varied in both extinction [F (2,71) = 4.513, p= 0.014] generalization testing [F (2,71) = 10.88, p < 0.001] phases. Moreover, the CS valence of the ImRS group was significantly higher than the IE and SE groups in the spontaneous recovery phase [F (2,71) = 4.228, p= 0.019]. Our study is the first known to compare the efficacy of ImRS and IE in attenuating the return of generalized fear response. We found that ImRS effectively attenuated the return of fear response. Further, the CS valence increased in a positive direction on Day 3. However, the rate of extinction was slower, and the generalized fear response was sustained for the IE group. The findings suggest that distinct mechanisms may be involved in ImRS and IE. ImRS may require a higher level of cognition, inducing novelty due to introducing the positive narrative. ImRS resulted in a competition between the two memories during retrieval, following a dynamic 'higher route'. On the contrary, the fear responses were sustained in the IE group, showing the possibility of a slow and stagnant 'lower route'. Hence, ImRS may be a more effective intervention for attenuating the return of generalized fear.

Disclosures: S. Mitra: None. **M.K. Asthana:** None.

Poster

PSTR239: Motivation and Emotion: Brain Imaging, EEG, and Human Behavior Studies

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR239.16/N10

Topic: G.01. Fear and Aversive Learning and Memory

Title: Exploring the conditioned pleasant and unpleasant auditory stimuli in the acquired equivalence paradigm

Authors: *V. MAITHANI[1], M. K. ASTHANA[2];
[1]Humanities and Social Sci., Indian Inst. of Technol., Roorkee, Roorkee, India; [2]Dept. of Humanities & Social Sci., Indian Inst. of Technol. Roorkee, Roorkee, India

Abstract: Acquired Equivalence is a form of conditioning where two dissimilar stimuli, followed by the same consequence, form an association such that any change in the consequence of one stimulus may also transfer to the other. When A-B and C-B associations are formed, the presentation of A or C produces an internal representation of B. If another association of A-D is formed, an A-(B)-D link is generated. When C alone is presented, B, a common mediator between A and C, generalises D to C and creates a C-(B)-D association, facilitating the acquired equivalence. The current study assessed the acquired equivalence in conditioned pleasant and unpleasant stimuli, from pleasant to unpleasant and vice versa, using auditory stimuli. The study was divided into Experiments 1A and 1B. Experiment 1A was conducted to select auditory stimuli across valence and arousal dimensions for Experiment 1B. Two auditory stimuli with the highest pleasant and the lowest unpleasant valence ratings were selected as unconditioned stimuli (UCS). Experiment 1B consisted of 27 participants (M=22.44, SD=3.52). Four geometrical shapes (square, rhombus, triangle, and star) were selected as conditioned stimuli (CS). The

experiment was divided into habituation, acquisition, and transfer-testing phases. The acquisition phase consisted of stages 1 and 2. In Stage 1, two shapes were paired with an unpleasant UCS (U1, U2) and the other two shapes with a pleasant UCS (P1, P2). Stage 2 included a reversal of UCS, i.e., one of the shapes previously paired with the unpleasant UCS (U1) was paired with the pleasant UCS and vice versa (P1). Valence and arousal ratings were recorded for differences in the transfer-testing phase. The ratings assessed whether a transfer of unpleasant to pleasant conditioning and vice versa occurs in 4 shapes from stage 1 (24 trials) to stage 2 (12 trials). A paired samples t-test indicated significant difference in U1 (t=-6.170, p=0.000), U2 (t=-2.460, p=0.021) and P1 valence ratings (t=5.308, p=0.000). The findings indicate a significant transfer of valence for shapes undergoing direct reversal (U1 and P1) and indirect transfer of valence from unpleasant to pleasant CS (U2), thus establishing an acquired equivalence effect in an auditory conditioning paradigm. The results suggest that if a previously unpleasant CS undergoes a new association with a pleasant UCS, due to acquired equivalence, the other associated CS also undergoes a similar transformation in valence from unpleasant to pleasant. The findings can help devise interventions for anxiety disorders to reduce the unpleasantness of a stimulus by conditioning the other associated, approachable stimulus with a pleasant consequence.

Disclosures: V. Maithani: None. **M.K. Asthana:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.01/N11

Topic: G.05. Mood Disorders

Support: NIH Grant 5T32AG071745
NIH Grant 5R21MH118749
Georgetown University Professional Development Grant
Georgetown University Biomedical Graduate Education Fellowship
Source Research Foundation Grant

Title: Behavioral deficits in $5HT_{2A}^{-/-}$ mice

Authors: *K. DIAL, S. DEASY, K. GILBERT, M. AMONTREE, K. CONANT; Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Psychedelics are experiencing renewed interest due to their substantial improvement in treating clinical endpoints in multiple mental health disorders over currently available therapeutics. However, the mechanisms by which psychedelics work to treat affective disorders are not yet fully elucidated. In this study, we utilized mice lacking the serotonin (5HT)-2A receptor, the receptor responsible for the psychedelic experience, to understand how it is involved in regulating behavior. Male and female 5HT2AR knockout (-/-) mice on the Balb/c background and Balb/c wild type (WT) mice (15 wk, N=40) were subjected to a battery of

behavioral tests. Behaviors were scored automatically by Any-Maze software or recorded and scored by a blinded colleague. Sex differences and statistically significant increases in anxiety, depression, and obsessive-compulsive behaviors were observed in $5HT_{2A}R^{-/-}$ mice (analyzed by Student's T-test or ANCOVA with Bonferonni correction as appropriate). Mice spent less time in the open in the Elevated Plus Maze (EPM), Elevated Zero Maze (EZM), and Open Field Test (OFT), and spent more time grooming in the Sucrose Splash Test (SST), indicating higher levels of anxiety compared to controls. $5HT_{2A}R^{-/-}$ mice built lower quality nests than WT Balb/c mice, indicating higher apathy. Finally, $5HT_{2A}R^{-/-}$ mice buried more marbles in the marble burying test than WT mice and shredded more of their food than their WT counterparts, indicating higher obsessive-compulsive behavior. Taken together, these results suggest that the 5HT2A receptor is crucial in regulating behaviors associated with depression, anxiety, and obsessive-compulsive disorders, making it a good therapeutic target in these illnesses.

Disclosures: K. Dial: None. **S. Deasy:** None. **K. Gilbert:** None. **M. Amontree:** None. **K. Conant:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.02/N12

Topic: G.05. Mood Disorders

Support: 2023 NARSAD Young Investigator Grant, Brain and Behavior Research Foundation. Grant Number: 31547
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PID2022-141700OB-I00
MCIN/AEI/10.13039/501100011033

Title: Changes in ventromedial prefrontal cortex - dorsal raphe nucleus activity are implicated in the depressive phenotype induced by α-synuclein in female mice

Authors: *M. SANCHO ALONSO[1,2,3,4], M. VILA-MARTÍN[1], L. JIMÉNEZ[1], U. SARRIÉS-SERRANO[5,3,4], L. MIQUEL RIO[5,3,4], V. PAZ SILVA[6,2,4], C. SAVARELLI BALSAMO[1], A. TERUEL SANCHIS[7], V. TERUEL-MARTI[1], A. BORTOLOZZI[5,3,4];
[1]Dept. of Human Anat. and Embryology, Univ. of Valencia, Valencia, Spain; [2]Institute of Biomedical Research of Barcelona (IIBB-CSIC), Barcelona, Spain; [3]Biomedical Research Networking Center for Mental Health (CIBERSAM), Madrid, Spain; [4]Systems Neuropharmacology Research Group, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain; [5]Inst. of Biomed. Res. of Barcelona (IIBB-CSIC), Barcelona,

Spain; [6]Biomed. Res. Networking Ctr. for Mental Hlth. (CIBERSAM), Madrid, Spain; [7]Facultat de Biologia, Univ. de València, Burjassot, Spain

Abstract: Parkinson's disease (PD) is a neurodegenerative disease classically characterized by motor dysfunction. In addition to the motor symptoms, the non-motor symptoms such as depression and anxiety, are gaining increasing clinical attention. More than 40% of PD patients also suffer from depression, with a higher incidence in women than in men. However, there is no effective clinical treatment for PD-related depression. Abnormalities in the serotonergic (5-HT) system are often associated with mood disorders, and aggregates of α-synuclein (α-Syn) have been reported in 5-HT raphe nuclei of patients with PD and depression. Here, we propose that the ventromedial prefrontal cortex (vmPFC)-dorsal raphe nucleus (DR) circuit is a critical component underlying depressive/anxiety disorders in PD due to its reciprocal connectivity. Female mice overexpressing the human mutant A53T α-Syn (h-α-Syn) in DR 5-HT neurons were studied. Several behavioral tests were performed to assess anxiety and depressive phenotype. Neuronal activity and local field potential (LFP) of infralimbic (IL) and prelimbic (PL) cortices were assessed in awake head-fixed mice using a multi-channel probe and a virtual reality corridor. Changes in synaptic markers and h-α-Syn levels were also assessed. Overexpression of h-α-Syn in DR 5-HT neurons leads to anhedonia and anxiety-like behaviors, which is reversed by ketamine (10 mg/kg, i.p.). At the same time, changes in SV2A, synaptophysin, MAP-2 and BDNF levels were found in PL and IL, which may contribute to anxiety/depressive symptoms. In addition, alterations in LFP and neuronal activity were detected in both PL and IL, which may contribute to anxiety/depressive symptoms. Overall, this study suggests that overexpression of h-α-Syn in 5-HT neurons alters the functionality of the vmPFC-DR circuit in female mice, resulting in a depressive/anxious phenotype.

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Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.03/N13

Topic: G.05. Mood Disorders

Support: UNAM-DGAPA IA2015723
Graduate Scholarship CONAHCyT to JAP, GLO and SGR

Title: Hippocampal neurogenesis is necessary for the resilience to stress in the offspring of enriched mothers.

Authors: J. ARROYO-PEREZ[1], G. LOPEZ-OROPEZA[2], S. GARCIA-RIOS[2], P. BALLESTEROS-ZEBADUA[3], P. DURAN[4], ***A. MARTINEZ-CANABAL**[5];

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Abstract: Adult offspring of environmentally enriched parents show enhanced adult hippocampal neurogenesis. The generation of newborn neurons facilitates emotional resilience to stress by decreasing the expression of depressive and anxiety-like behaviors. We hypothesized that adult offspring of enriched dams would show resilience to chronic unpredictable mild stress (CUMS) by presenting lower levels of depressive and anxiety-like behaviors in a mechanism mediated by hippocampal neurogenesis. Therefore, we assessed whether adult offspring from enriched (EE) and non-enriched (NoEE) BALB/c females showed differences in depressive and anxiety-like behaviors before and after 21 days of CUMS exposure. We found less immobility in the Forced Swimming Test to assess depressive-like behaviors in EE offspring after CUMS exposure. Likewise, we found less expression of anxiety-like behavior through the Elevated Plus Maze, in which EE offspring spent more time within open arms. The EE offspring showed consistently higher levels of hippocampal proliferation and rates of immature neurons than NoEE offspring, which interestingly correlated with behavioral evaluations. To establish a causal relationship between neurogenesis levels and the observed resilience, we exposed EE mice offspring to focal X-irradiation (2 sessions of 7.5 Gy) before CUMS exposure to induce neurogenesis ablation. Notably, irradiated EE offspring showed more immobility and less time in open arms than the sham group, showing that neurogenesis is necessary for the intergenerational resilience effect of environmental enrichment.

Disclosures: J. Arroyo-Perez: None. **G. Lopez-Oropeza:** None. **S. Garcia-Rios:** None. **P. Ballesteros-zebadua:** None. **P. Duran:** None. **A. Martinez-Canabal:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.04/N14

Topic: G.05. Mood Disorders

Support: T32 DA 55569-2

Title: The effects of a high-fat diet on emotion-like behaviors in female mice

Authors: *B. M. GONZALEZ OLMO[1,2,3], M. E. BOCARSLY[4,2,3];
[1]Pharmacology, Physiol. & Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ; [2]Rutgers Brain Health Institute, Piscataway, NJ; [3]Rutgers Addiction Research Center, Piscataway, NJ; [4]Pharmacology, Physiol. and Neurosci., Rutgers NJMS, Newark, NJ

Abstract: Mental health disorders, including anxiety and depression, are a worldwide concern with growing prevalence. Interestingly, mental health disorders have been shown to increase the likelihood of emotional eating and the associated obesity. Further, research indicates a strong relationship between obesity and negative emotions. Clinical studies have shown that females are more sensitive to interpersonal and emotional stress than males, and they are more vulnerable to emotional eating than males. For this study, we fed adult female C57BJ/J mice (n=10/group) either a low-fat, standard chow diet or a 60% high-fat diet (HFD) for four weeks. Body weights were taken every day. We tested anxiety-like behavior with open field-test and elevated zero maze. We tested for depressive-like behavior with the tail suspension test and sucrose anhedonia test. Lastly, we collected brain tissue of the amygdala (known as the processing center for emotions) to measure dopamine receptors mRNA expression. Our results demonstrated that body weight gain increased more rapidly in mice fed HFD compared to the chow-fed mice. We did not find any significant differences in the anxiety-like behaviors with a chronic exposure of HFD consumption. However, we did find that HFD-fed mice showed more indications of depressive-like behavior compared to the chow-fed group. Lastly, we found dopamine D1 receptor mRNA (DRD1) was reduced in the HFD-fed mice compared to the chow group. In conclusion, our study demonstrates that the chronic exposure of HFD affects female mice in depressive-like behaviors and not in anxiety-like behaviors. Furthermore, we were able to show that these HFD-induced depressive-like behaviors could be mediated via DRD1 within the amygdala.

Disclosures: B.M. Gonzalez Olmo: None. **M.E. Bocarsly:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.05/N15

Topic: G.05. Mood Disorders

Title: Depression-like behavior is comorbidity in a model of heart failure with preserved ejection fraction induced by high-fat diet and L-NAME.

Authors: *R. A. MALDONADO;
Inst. for Obesity Res., Insituto Tecnologico y de Estudios Superiores de Monterrey, Monterrey, Mexico

Abstract: Heart failure with preserved ejection fraction (HFpEF) is a syndrome with a 35% 2-year rate of hospitalization and 14% 2-year mortality. HFpEF patients have a prevalence of depression 20%. Several studies report that HFpEF and depression, share proinflammatory cytokines, specifically, IL-33 plasma concentration presents a positive correlation with left ventricle diastolic disfunction and depressive symptoms. In addition, downregulation of IL-33 in the amygdala prevents the development of LPS-induced depression. However, there is a lack of focus in the diagnosis of depression in HFpEF patients, resulting in an under-recognition of depression in 50% of the cases, that may be due to the absence of benefit from standard

pharmacotherapy. Thus, we propose that a mice model of HFpEF develops depression-like behavior and have an increases expression of IL-33 in the amygdala. To achieve this, we employ eight-week-old male C57BL/6 mice that were randomly assigned to the following groups. HFpEF group: mice fed with *ad libitum* HFD (60% Fat) and drinking water with L-NAME (0.5g/L) for 12 weeks, control: mice fed with *ad libitum* chow diet and tap water for 12 weeks. To determine cardiac hypertrophy, we measure the left ventricle cardiomyocyte cross-sectional area and, to identify the presence of depression we employ the open field test (OF), sucrose preference (SP) and novelty suppressed feeding (NSF). To determinate inflammation at the amygdala, we measure IL-33, IL-10, IL-6 and IL-1β expression by qPCR. Also, we measure GFAP gene expression as an indirect marker of gliosis. Finally, knowing that cholesterol homeostasis is critical for normal brain function, we quantify the ABCA1 expression. We found that the HFpEF group presents a 26% increase cardiomyocyte area, a reduction of 11% (SP), a latency to eat of 53 min (NSF) and spend 30% less time at the center and spend 20% more time at the edges of the arena in the OF test. Finally, the HFpEF group present an increased expression of ABCA1, GFAP and IL-33 as well as a downregulation of IL-10 in the amygdala. An exposure to HFD+ L-NAME for 12 weeks promotes cardiac hypertrophy and depression-like behavior accompanied by an increased expression of IL-33 in the amygdala.

Disclosures: R.A. Maldonado: None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.06/N16

Topic: G.04. Emotion

Title: Effects of a high-sodium diet on anxious behavior, body weight, food intake, water intake, and adiposity in male and female rats

Authors: *H. MURPHY[1], C. WIDEMAN[2];
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Abstract: Given the prevalence of anxiety and dietary increases of sodium intake in the U.S., exploration of the correlation between anxiety and a high-sodium diet is relevant. Although sodium is a vital nutrient needed for various physiological functions, many individuals who ingest more than the recommended amount develop health problems and increased anxiety. Numerous studies on anxiety in rats have utilized the elevated plus maze (EPM), which has sheltered areas (closed arms) and exposed areas (open arms) to indicate the presence or absence of anxiety. In the current study, a high-sodium diet was utilized and an EPM was employed to evaluate the consequences of this diet on anxiety. It was hypothesized that rats fed a high-sodium diet would exhibit increased anxiety when compared with control rats and gender differences would be found in body weight, food and water intake, and adiposity in treated animals. 12 male

and 12 female Long-Evans rats were equally divided into experimental and control groups. A one-week habituation period consisted of a 12-hour dark/12-hour light cycle, and body weight and food and water intake were measured daily. During the experimental period, experimental rats of each gender consumed a high-sodium chloride diet which contained 4% more NaCl than the control diet. At the onset of the dark period each rat was placed into the EPM, and movement was recorded on a computer for a five-minute period 2 days before the end of the habituation week and 2 days before the end of the third experimental week. At the end of the experiment, mesenteric and renal fat were measured. During habituation, all rats spent more time in closed arms compared to open arms. By experimental week 3, male control rats spent more time in the closed arms, while the experimental rats spent more time in the open arms. For female rats in experimental week 3, both control and experimental rats spent more time in the open arms. A gender-based difference in body weight was evident as male control rats weighed more than their female counterparts, and male experimental rats exhibited higher weights than female experimental rats. For water intake, experimental rats of both genders drank significantly more water than control rats. Both experimental groups ate more than control groups, and male rats ate more than female rats. Mesenteric fat was greater in male control rats than experimental rats. There was no difference in mesenteric fat between female control and experimental rats. Similarly, male control rats had significantly more renal fat than experimental rats with no difference in female groups. Physiological changes induced by a high-sodium diet did not produce increased anxiety in either gender.

Disclosures: H. Murphy: None. **C. Wideman:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.07/N17

Topic: G.05. Mood Disorders

Support: DoD #PR230701

Title: Assessment of Comorbidities Between Depression and Cardiovascular Dysfunction in Socially Defeated Mice

Authors: *A. NGUYEN[1], C.-W. LIU[1], H. LE[1], H. CHO[2], A. BARNEY[1], J. PARK[3], W. LEE[1], D. FERGUSON[1], H.-D. KIM[1];
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Abstract: Depression is a major cause of disability worldwide and significantly contributes to the incidence of suicide and suicidal attempts. It often coexists with cardiovascular diseases, which are similarly linked to chronic stress. Research over several decades has demonstrated a close relationship between cardiovascular functions and brain activity, suggesting a potential

interplay between mental health and heart health. Comorbidities of cardiovascular diseases and psychiatric disorders, especially depression, have been observed in both patient populations in clinics, however, the causal relationships remain unclear. In this study, we explore the hypothesis that neurovascular dysfunctions within the brain's reward circuitry contribute to both mood disorders and cardiovascular impairments. Using a well-validated preclinical depression model, chronic social defeat stress, we identified structural and genetic changes in the neurovascular components in the nucleus accumbens, a key brain reward region, of depressed mice Concurrently, we observed cardiovascular irregularities, such as increased heart rate and aortic dysfunction, in affected mice. These findings hint at a connection between neurovascular dysfunctions in the brain's reward circuitry and the onset of mood disorders and cardiovascular dysfunctions, underscoring the need for further research to uncover underlying mechanisms and develop comprehensive treatments.

Disclosures: A. Nguyen: None. **C. Liu:** None. **H. Le:** None. **H. Cho:** None. **A. Barney:** None. **J. Park:** None. **W. Lee:** None. **D. Ferguson:** None. **H. Kim:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Topic: G.05. Mood Disorders

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Title: Sex-dependent impact of a high-fat diet in anxious/depressive-related behavior and neuroplasticity/inflammatory markers.

Authors: J. SENSERRICH GUERRERO[1], C. SAMUELLI[1], M. SERRANO[1], E. CASTRO[1,2], A. DIAZ[1,2], A. ADELL[3,1,2], A. PAZOS[1,2], *F. PILAR-CUELLAR[1,2];
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Abstract: Major depressive disorder (MDD) is a neuropsychiatric disease that affects two women for every man diagnosed. The mechanisms underlying the etiopathogenesis of depression are still not clear, therefore, different hypotheses have been formulated. One of the latest hypothesis suggests that a stress-induced inflammatory response is associated with a reduction in the monoaminergic availability and with the activation of microglia inducing glutamate-mediate excitotoxicity in the central nervous system. Another inflammatory factor is the Western diet. Moreover, the prevalence of depression in people with obesity is twice higher than in non-obese people, being more evident in women than in men. Here we have evaluated the effect of a high-fat diet (HFD) on behavior, and neurochemical and molecular markers associated with

depression/anxiety, in both male and female mice. C57BL/6 mice (n=8-10), 6 weeks old, were given an HFD (60% fat) for 7 weeks. Both male and female HFD mice presented an anxious-like phenotype assessed by the central time in the open field test and the latency to feed in the novelty-suppressed feeding test. Only HFD female mice showed depressive-like behavior, such as anhedonia in the sucrose preference test and behavioral despair in the tail suspension test. The HFD male mice showed lower mTOR pathway activation in the hippocampus and no changes in BDNF levels. Female HFD mice presented a trend towards increased eIF2α, a marker of endoplasmic reticulum stress, which has been associated with depression. Plasma IL-6 and IL-10 levels were lower in HFD female mice, while HFD male mice only showed a reduction in IL-10 plasma levels. The tryptophan metabolism was also evaluated in the hippocampus and prefrontal cortex in this model. In the hippocampus, HFD male mice presented lower tryptophan levels and an increase in the kynurenine/tryptophan ratio. HFD female mice showed lower kynurenine and tryptophan levels, suggesting a differential regulation in this area depending on the gender. No significant differences were observed in the prefrontal cortex. Our results confirm the potential effect that a high-fat diet can exert on depression and anxiety. Furthermore, there is sexual dimorphism in the behavioral effect and the mechanisms involved, highlighting the importance to use preclinical female animal models for a deeper knowledge of sex bias.

Disclosures: J. Senserrich Guerrero: None. **C. Samuelli:** None. **M. Serrano:** None. **E. Castro:** None. **A. Diaz:** None. **A. Adell:** None. **A. Pazos:** None. **F. Pilar-Cuellar:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Topic: G.05. Mood Disorders

Support: Betty Huse, M.D. Charitable Foundation
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University of Wisconsin-Milwaukee

Title: Exploring the Role of histone acetylation in Adolescent Depression

Authors: S. ZIMMERMAN, G. B. DREW, P. C. BENDIS, *P. GEORGIOU;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Major depressive disorder affects approximately 13% of adolescents in the United States. Although classical antidepressant treatments effectively reduce depressive symptoms in adolescents, low remission rates and potential adverse effects, such as suicide-related thoughts, underscore the need for developing novel pharmacotherapies for treating adolescent depression. During adolescence, in both humans and mice, there is a surge in steroid gonadal hormones, which impact their behavior and brain functions. Stress in the form of victimization and bullying

are prevalent among adolescents and, when combined with increased gonadal hormones, are associated with adolescent depression and suicidality. We hypothesized that the increased risk of depression in adolescents is due to the combinatorial effects of increased stress along with the increased production of gonadal hormones in both sexes. To explore this hypothesis, we have conducted complex bioinformatics analyses and drug repurposing screenings for drugs and compounds that can reverse or mimic the molecular signatures of adolescent depression using RNAseq data from the human dorsolateral prefrontal cortex. We demonstrated that histone deacetylase inhibitors can reverse the molecular changes occurring in adolescents with depression, suggesting an increase in histone acetylation that affects gene expression. Interestingly, gene ontology analysis for biological processes revealed significant downregulation in cholesterol metabolic and biosynthetic processes in adolescent patients compared to adult patients. Cholesterol is the precursor for most steroid gonadal hormones. Correspondingly, pathway enrichment analysis indicated downregulation in steroid biosynthesis. These findings suggest that increased histone acetylation may lead to downregulation in steroid gonadal hormone synthesis during adolescence, potentially elevating the risk of developing depression. To validate these findings, we subjected adolescent male and female mice to acute foot-shock stress and assessed the development of anhedonia, social preference deficits, and anxiety-like behavior using the sucrose preference, social interaction, and open field tests, respectively. Adolescent stressed mice displayed social interaction deficits and anxiety-like behaviors, effects that were reversed by the administration of a histone deacetylase inhibitor prior to the acute stress. These findings suggest that targeting these systems could represent a novel strategy for treating adolescent depression.

Disclosures: S. Zimmerman: None. **G.B. Drew:** None. **P.C. Bendis:** None. **P. Georgiou:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Title: Dusp1 (MKP-1) is necessary for the development of stress-induced depressive-like behavior but not for learned helplessness behavior

Authors: *M. SCHOTT[1], M. THOUAYE[2], S. DORIDOT[3], S. LIMA[1], V. VEDARTHAM SRINIVASAN[4], C. FILLINGER[5], T. SERCHOV[6], I. YALCIN-CHRISTMANN[7], M.

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Abstract: Predicted to be the first cause of the global burden of disease by 2030, Major Depressive Disorder (MDD) is a common debilitating pathology of varied etiologies involving complex molecular pathways in many brain areas. Throughout the years, the use of animal models has highlighted that depending on the paradigm used, behavioral symptoms associated with depression can be mediated by different molecular mechanisms and may notably vary depending on the species, strain or the sex of the subjects. The dual-specificity phosphatase 1 (DUSP1), also known as mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), has been previously identified as a crucial player in the development of depressive-like symptoms induced by paradigms of chronic stress, chronic pain, chronic social defeat and withdrawal from chronic methamphetamine treatment in rodents. In this study, we use both male and female C57BL/6J mice to address the importance of DUSP1 in the development of behavioral consequences of two models of helplessness-like behavior and a model of chronic stress. The models used in this study are (1) a classical model of learned helplessness based on inescapable footshocks, (2) a model of chronic despair induced by repeated forced swim on 5 days and (3) a model of chronic variable stress based on the repetition of 3 stressors for 21 days. In those 3 models and both sexes, we used qPCR to study Dusp1 induction in relevant brain regions, namely the anterior cingulate cortex (ACC), the nucleus accumbens (NAc), and the dorsal and ventral hippocampus (dHPC and vHPC). The contribution of Dusp1 to behavioral outputs was then addressed using Dusp1-deficient mice. We found that, contrary to the depressive-like consequences of a chronic stress, Dusp1 is not critical to the development of helpless-like behaviors in the learned helplessness model and the repeated forced swim model. Overall, this study highlights differences in the molecular mechanisms involved in the development of different helpless-like vs. depressive-like symptoms in murine models.

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Poster

PSTR240: Animal Models of Mood Disorders

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Topic: G.05. Mood Disorders

Support: National Natural Science Foundation of China 82271576
National Natural Science Foundation of China 82071541)

Title: Deficiency of Autism Susceptibility Gene Trio in Cerebellar Purkinje Cells Leads to Depression

Authors: *J. WANG[1,2], D. ZHANG[3], J. LI[4], K. GAO[5];
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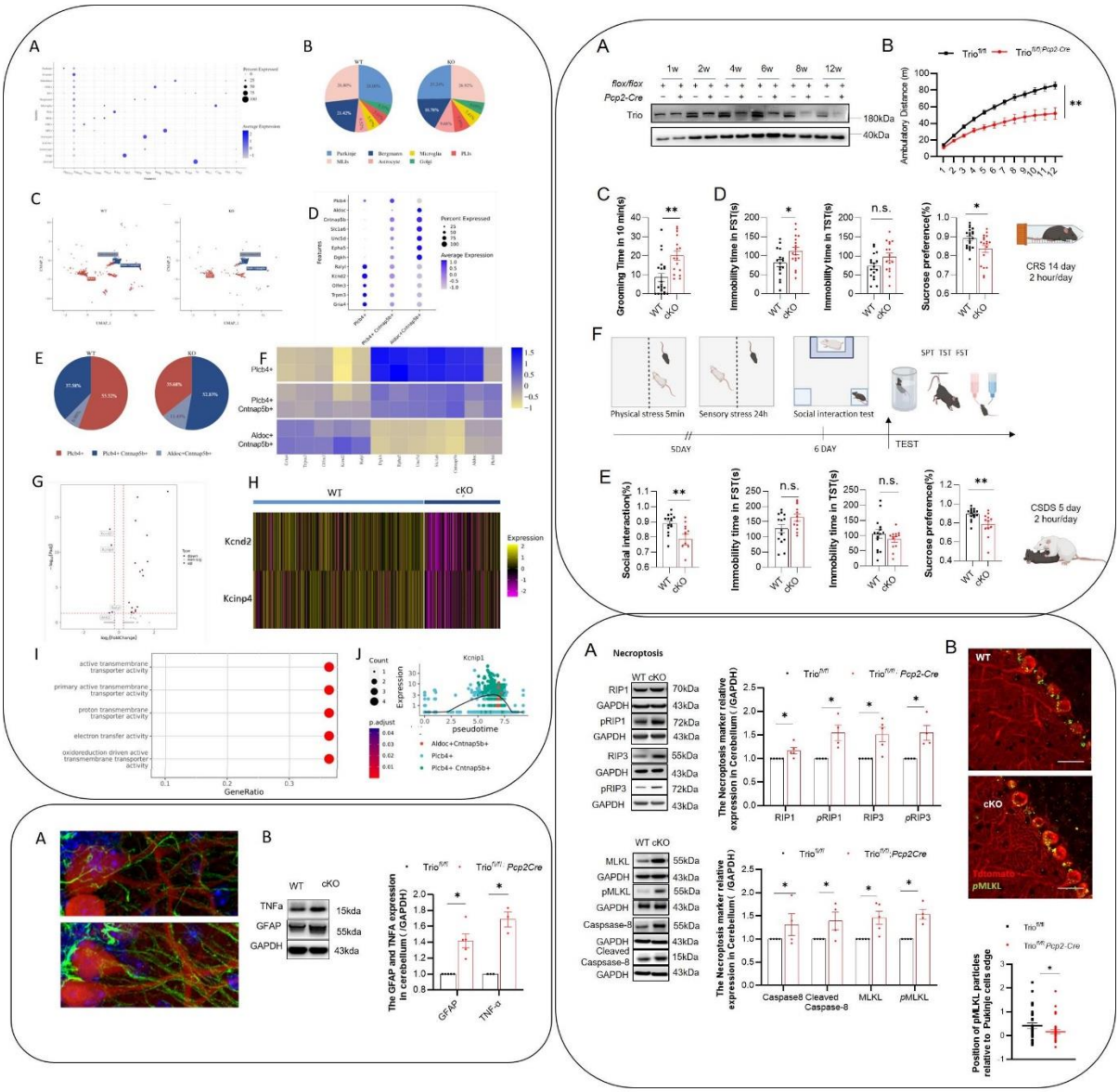
Abstract: Cerebellar dysfunction is linked to psychiatric disorders, including autism (ASD) and depression. Gene Trio has been implicated in multiple neurological conditions, notably ASD. Adults diagnosed with ASD increased risk for developing mental health comorbidities, with anxiety and depression. However, the physiological and pathological dimensions of these associations require further elucidation. Here, Results of single-cell sequencing of cerebellar tissues in Trio deletion in Purkinje cells (PCs) mice (10 weeks) indicate a reduction in PCLβ4+ PCs, alongside abnormalities in K+ channel protein pathways and an increase in glial cells. 12-week-old individuals are associated with abnormal neuroexcitability and susceptibility to depression. Our research suggests that Trio deletion triggers AIF1-mediated mitochondrial stress and pMLKL-mediated necroptosis in PCs, leading to prolonged cell death. This prolonged cell death escalates into chronic inflammatory stress, characterized by increased TNF-α expression, activation of astrocyte. Furthermore, the upregulation of pMLKL expression, induced by a virus, activates similar apoptotic processes and inflammation in PCs in 12 week.Therefore, Trio deletion in PCs induces necroptotic apoptosis and inflammation, resulting in neuronal hyperexcitability and disrupting psychomotor behaviors in adults. This study underscores the complex interplay between genetic factors and neural cell function, further illuminating the pathophysiological pathways that potentially contribute to psychiatric disorders in the context of ASD.



Disclosures: J. Wang: None. **D. Zhang:** None. **J. Li:** None. **K. Gao:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.12/N22

Topic: G.05. Mood Disorders

Support: P20GM103430

Title: Cognitive impairments across the lifespan in a mouse model of bipolar disorder

Authors: *S. C. SOARES[1], D. ARRUDA[2], E. FRAATZ[3], V. R. HEIMER-MCGINN[1]; [1]Roger Williams Univ., Bristol, RI; [2]Roger Williams Univ., Portsmouth, RI; [3]Roger Williams Univ., Middletown, RI

Abstract: Cognitive deficits are a core symptomatic category of bipolar disorder (BD). They are impaired across mood states, present throughout the lifespan, and observed in unaffected first-degree relatives. In this study, we characterized cognitive behaviors across the bipolar lifespan using the ClockΔ19 line, a mouse model with a disrupted circadian clock that displays a BD-like phenotype, including hyperactivity and 24-hour mood cycling. The use of a mouse model is beneficial as it will allow us to investigate the underlying molecular mechanisms linking circadian genes to cognition in BD. We included female mice to address potential sex differences as well as heterozygous mice to model first-degree relatives. Specifically, we compared male and female homozygous ClockΔ19 mice (+/+) to heterozygous (+/-) and wildtype (-/-) mice at two timepoints, adolescence (~6 weeks) and adulthood (~6 months). We assessed cognitive flexibility using the attentional set-shifting task (AST), and recognition memory using the novel object recognition (NOR) and novel object location (NOL) tasks. In AST, preliminary results (n=4 per group) indicate potential sex differences in reversal learning: for males, +/+ appeared to be impaired compared to -/-, while for females, +/- (but not +/+) appeared to be impaired. Both impairments appeared to be sustained into adulthood and intra-dimensional shifts were not impaired at either timepoint. In NOR and NOL, object recognition was impaired for male +/+, while location recognition was impaired for female +/+. In adulthood, male NOR deficiencies appeared to be sustained, while female NOL deficiencies were no longer evident. Overall, preliminary results suggest that there are sex-specific deficiencies in several cognitive domains in +/+ compared to -/- in adolescence and that some of these appeared to be sustained into adulthood (AST, NOR), while others appeared to recover (NOL). None appeared to worsen over time. If these observed preliminary trends continue to persist with an increase in sample size, the ClockΔ19 transgenic mouse model can further be used to investigate the underlying pathophysiology of BD across the lifespan. In the future, characterizing cognitive behaviors and its underlying molecular mechanisms could provide insight into new treatments for patients affected by cognitive impairments in BD.

Disclosures: S.C. Soares: None. **D. arruda:** None. **E. Fraatz:** None. **V.R. Heimer-Mcginn:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.13/N23

Topic: G.05. Mood Disorders

Support: URI/NIH 5p20GM103430-20

Title: Clock∆19 reversal learning deficits translate to humans bipolar disorder

Authors: *D. J. ARRUDA[1], V. R. HEIMER-MCGINN[2], S. SOARES[3];
[1]Roger Williams Univ., Portsmouth, RI; [2]Psychology Dept., Roger Williams Univ., Pawtucket, RI; [3]Roger Williams Univ., Bristol, RI

Abstract: Cognitive deficits in bipolar disorder (BD) are understudied, undertreated, and may be predictive of disease outcome. Deficits in cognitive flexibility are trait symptoms of BD and manifest in problem solving, multitasking, and planning. They affect approximately 60% of people with BD and to a lesser extent, first degree relatives. There is evidence that reversal learning performance, in particular, may help define BD endophenotypes. Since they are evident as early as adolescence, they could also be useful diagnostically. Validating this behavior in a mouse model will advance our understanding for future study of this impairments underlying mechanisms. The *Clock*∆19 mouse model has a loss of function in the *clock* gene, which genomic data suggest is disrupted in people with BD. This model also has phenotypic traits characteristic of BD such as 24-hour manic mood cycling and hyperactivity but lacks extensive study of its cognitive flexibility. The current study assesses reversal learning in adolescent mice in an attentional set-shifting task (AST) comparing genotype (wild type, heterozygous, and homozygous) and sex (males and females) in *Clock*∆19 mice. Preliminary data (n=8 per group) indicate that there may be reversal learning deficits in the male homozygous group as well as in the female heterozygous group. Specifically, trends suggest that the male homozygous group requires more trials to criterion to complete the rule reversal phase of the AST, while the female heterozygous group appears to commit more errors in this phase. These findings, showing a specific impairment in reversal learning and not in intra-dimensional shift are consistent with the clinical pediatric literature. Sex differences and cognition in first-degree relatives, however, are not well defined in the clinical literature, so our results will elucidate the effect of these characteristics. In addition, since *Clock*∆19 mice have mutations in the *clock* gene, our results suggest that circadian rhythm disruptions contribute to the cognitive impairments seen in BD patients and their first-degree relatives. If preliminary trends persist with increased sample size, this model will facilitate investigations of the underlying pathophysiology of cognitive flexibility in BD. In the future, characterizing cognitive behaviors and its accompanying molecular mechanisms could provide therapeutic targets for BD-related cognitive impairment.

Disclosures: D.J. arruda: None. **V.R. Heimer-Mcginn:** None. **S. Soares:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Program #/Poster #: PSTR240.14/N24

Topic: G.05. Mood Disorders

Support: URI/NIH 5P20GM103430-20

Title: Sex-specific recognition memory impairments in adolescent CLOCKΔ19 mouse model of bipolar disorder

Authors: *E. R. FRAATZ[1], S. SOARES[1], V. R. HEIMER-MCGINN[2];
[1]Roger Williams Univ., Bristol, RI; [2]Psychology Dept., Roger Williams Univ., Pawtucket, RI

Abstract: Cognitive deficits are a trait symptom of bipolar disorder (BD) and are predictive of disease outcome and quality of life. However, they remain understudied and undertreated, largely due to difficulties developing animal models that reproduce the mood fluctuations that characterize BD. One emerging model is the ClockΔ19 transgenic mouse line, which exhibits regular mood cycling between manic and euthymic behavior over 24 hours. Of interest, visuo-spatial recognition and emotion recognition are impaired in BD. In this study, we examine recognition memory using novel object recognition (NOR) and novel object location (NOL) tasks. We compare adolescent male (M) and female (F) homozygous (HOM) ClockΔ19 mice to both heterozygous (HET) and wildtype (WT) mice with a preliminary sample of 9 F-HOM, 6 M-HOM, 8 F-HET, 8 M-HET, 8 F-WT, and 9 M-WT. In NOL, preliminary data suggests female ClockΔ19 HOM mice have impaired location recognition compared to WT, whereas male HOM appear unaffected. In contrast, HET mice of both sexes appear to have impaired location recognition compared to WT. In NOR, preliminary data suggests the HOM group, regardless of sex, has impaired object recognition compared to WT. These investigations are currently being extended to social recognition and longitudinal measures of object and location recognition. Our findings are consistent with other circadian models, including an environmental mouse model (Short Day), and a Bmal1 genetic model, which speaks to the emerging role of circadian rhythms in BD-related cognition. Finally, since these other circadian studies have not included females, and considering sex differences in recognition remain unclear in BD patients, our current results and future studies will help clarify how potential sex differences relate to BD endophenotypes. In the future, this model will be useful for developing sex-specific treatments for cognition.

Disclosures: E.R. Fraatz: None. **S. Soares:** None. **V.R. Heimer-Mcginn:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.15/N25

Topic: G.05. Mood Disorders

Support: University of Richmond School of Arts and Sciences

Title: Altered cognitive, emotional and neuroplastic indices in a rat model of enhanced anticipation of appetitive events.

Authors: *S. HARTVIGSEN[1], J. SHATALOV[1], B. WIXTED[2], J. GU[2], K. G. LAMBERT[3]; [1]Dept. of Psychology, Univ. of Richmond, Richmond, VA; [2]Psychology, Univ. of Richmond, Richmond, VA; [3]Psychology and Neurosci., Univ. of Richmond, Richmond, VA

Abstract: Depression, a mood disorder characterized as prolonged periods of melancholy, affects 322 million people worldwide. Symptoms manifest themselves across multiple domains, including emotional dysregulation, cognitive deficits, maladaptive stress responses and alterations in neural functioning and neuroplasticity. Despite the multifaceted pathology of depression and the alarmingly high number of individuals suffering from this psychiatric disease, the efficacy of existing treatments for depression is woefully low. Thus, there is a need to identify novel behavioral interventions to increase emotional resilience against the onset of depression-like symptoms. Our lab is interested in understanding how chronic enhanced anticipation of appetitive events can shape emotional resilience, cognition and neuroplasticity. To explore this, our lab compared the effects of chronic enhanced appetitive event anticipation (Unpredictable Positive Experience Response; “UPER”) training on sucrose preference, persistency behavior, performance in a spatial foraging task (Dry Land Maze; “DLM”), anxiogenic behavior and neuroanatomical markers. We hypothesized that compared to rats receiving unanticipated appetitive events (Enriched; “ENR”) and handled control rats that didn’t receive appetitive events (“CTRL”), the UPER rats would display reduced anxiety, increased cognitive performance on a foraging task, reduced anhedonia and increased neuroplasticity. We found a main effect of training on anxiogenic behavior, as UPER rats spent more time in the center of the DLM ($p=0.0003$) and consumed more food in a novel area ($p=0.002$). There was a significant training by sex interaction in cognitive performance on the DLM ($p=0.006$), as male CTRLs took the longest to approach a previously baited well. We found training to affect search strategy during the foraging task ($p=0.003$), as UPERs surveying fewer wells (average of 3 wells) before consuming treats whereas CTRLs displayed a more hesitant strategy, waiting until an average of 16 wells were surveyed before consumption. An interaction between training and sex was seen in a task of persistence whereby UPER trained males displayed increased persistence to attain an unattainable reward but CTRL males displayed lower persistence than CTRL females ($p=0.004$). Semiquantitative assessment of hippocampal (CA2) expression of brain derived neurotrophic factor (BDNF) unexpectedly found CTRL rats had a higher expression of BDNF than ENRs and UPERs ($p=0.025$). Overall, these results suggest UPER training may shape changes to the emotional, cognitive, behavioral and neuroplastic systems also affected by depression.

Disclosures: S. Hartvigsen: None. **J. Shatalov:** None. **B. Wixted:** None. **J. Gu:** None. **K.G. Lambert:** None.

Poster

PSTR240: Animal Models of Mood Disorders

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Program #/Poster #: PSTR240.16/N26

Topic: G.05. Mood Disorders

Title: Modeling depression in Rats: Chronic corticosterone administration as a tool for antidepressant efficacy assessment

Authors: *L. BRUHN MADSEN[1], S. FLUNKERT[2], E. AUER[2], L. BREZNIK[3], M. DAURER[3], M. PROKESCH[3], A. FANTINA-WOBLISTIN[3];
[1]Scantox, Ejby, Denmark; [2]Neuropharm., Scantox Neuro GmbH, Grambach, Austria; [3]Scantox Neuro GmbH, Grambbach, Austria

Abstract: MODELING DEPRESSION IN RATS: CHRONIC CORTICOSTERONE ADMINISTRATION AS A TOOL FOR ANTIDEPRESSANT EFFICACY ASSESSMENT Lone Bruhn Madsen[2], Ewald Auer[1], Aaron Fantina-Woblistin[1], Livia Breznik[1], Magdalena Daurer[1], Stefanie Flunkert[1], Manuela Prokesch[1]*[1]Scantox Neuro GmbH, Neuropharmacology, Grambach, Austria[2]Scantox Denmark ApS, Ejby, Denmark*
Depression is a prevalent mental illness that affects approximately 280 million people worldwide. It is characterized by extended periods of depressed mood or loss of interest in activities that once provided pleasure. The etiology and pathogenesis of depression is still poorly understood; however, it is often preceded by prolonged periods of stress. In this study, we examined the effects of chronic treatment with corticosterone, a rodent´s stress hormone, on depressive-like symptoms in rats. We also investigated whether the observed changes could be reversed by the selective serotonin reuptake inhibitor fluoxetine, which demonstrated good efficacy in clinical trials. Since there are various protocols available, we compared two protocols that were previously shown to produce robust changes in behavior.96 Wistar rats were allocated to six experimental groups. Each group was treated daily with corticosterone or vehicle for a total of 30 days, either through drinking water or subcutaneous injection. After 21 days of treatment, animals were tested for behavioral deficits that are typical for depression followed by *ex vivo* evaluations of different brain tissues for stress- and serotonin-related changes. Our results provide an extensive characterization of the corticosterone-induced depression model on different readouts that focus on typical symptoms of depression. To our knowledge, this is the first study directly comparing two protocols of corticosterone administration on behavioral and *ex vivo* readouts. Together with already published data, our study further supports the value of the corticosterone-induced depression model as a valuable tool to investigate the pathogenesis of depression and to test novel treatment candidates.

Disclosures: L. Bruhn Madsen: Other; Scantox. **S. Flunkert:** Other; Scantox Neuro. **E. Auer:** Other; Scantox Neuro. **L. Breznik:** Other; Scantox Neuro. **M. Daurer:** Other; Scantox Neuro. **M. Prokesch:** Other; Scantox Neuro. **A. Fantina-Woblistin:** Other; Scantox Neuro.

Poster

PSTR240: Animal Models of Mood Disorders

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Program #/Poster #: PSTR240.17/N27

Topic: G.05. Mood Disorders

Support: NRF of Korea Grant 2018R1A6A1A03025221

Title: Estrogen deficiency induces anxiety and depression-like behaviors via caspase-1-mediated neuroinflammation

Authors: *D. BAEK;
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Abstract: The high risk of depressive disorders in postmenopausal women is an emerging medical issue, but no pathophysiological mechanism remains largely unknown. Based on the hypothesis that caspase-1, activated by inflammasome complexes after estrogen production decreases, affects anxiety and depressive behaviors, we investigated the effects and underlying mechanisms of caspase-1 using an ovariectomized (OVX)-induced mouse model. Along with behavior tests (anxiety and depression), microglial, astrocytes, and neuronal activity were measured in brain regions (hippocampus, hypothalamus, raphe nucleus, and habenula). OVX-induced wild-type (WT) mice exhibited overexpression of caspase-1 in brain regions, which induced anxiety- and depression-like behaviors (open field, forced swimming, tail suspension, and rota-rod tests), whereas loss of caspase-1 expression in Caspase-$1^{-/-}$ knockout mice no showed these behaviors. These behavioral alterations were associated with caspase-1-mediated neuroinflammation via microglia and astrocyte over-activation, as evidenced by CX_3CR1/Iba-1 and GFAP/LCN2 double-positive signals in the hippocampus, hypothalamus, raphe nucleus, and habenula. Additionally, OVX-induced WT mice treated with caspase-1 inhibitors not only resisted the development of anxiety and depression-like behaviors but also alleviated neuroinflammation caused by the overactivation of microglia and astrocytes. Our study suggests evidence shedding light on the fact that caspase-1 activation is a key mediator of depression in women after menopause.

Disclosures: D. Baek: None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.18/N28

Topic: G.05. Mood Disorders

Support: NIH AR077692
NIH T32GM133393

Title: An atopic dermatitis mouse model reveals potential utility for brain circuitry studies of atopic dermatitis-generated comorbid depression (ADCD).

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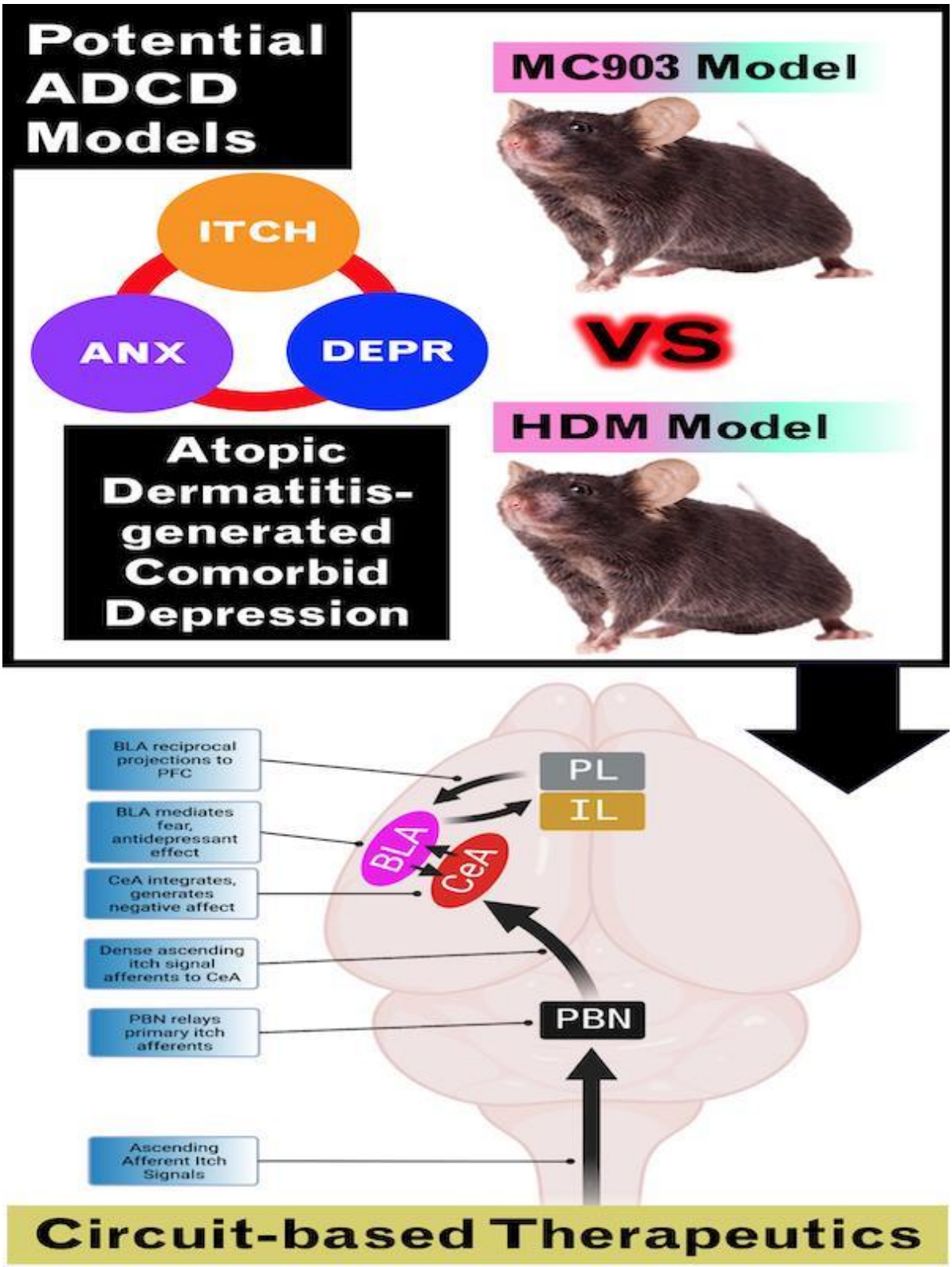
Abstract: Chronic atopic dermatitis (AD) affects millions globally and has an alarming propensity to generate comorbid chronic depression. Currently, no effective therapies exist specifically for AD-generated comorbid depression (ADCD), likely due to unknown itch- and affect-processing brain mechanisms and inadequate pre-clinical models. Hence, there is a critical need for a suitable pre-clinical research model that can be used to study ADCD's unique pathophysiology. In this investigation, we searched for pre-clinical models of ADCD by evaluating previously established house dust mite-induced (HDM model) and MC903-induced (MC903 model) AD mouse models for comorbid depression-like features. Using the elevated plus maze, forced swim test, scratching behavior assays, histology, and clinical skin scoring, we show that the HDM model lacks significant anxiety-like ($p = 0.858$, student's t-test) and depression-like behavior ($p = 0.0512$), but develops an AD-like skin pathology with strong scratching behavior ($p < 0.005$). By contrast, the MC903 model uniquely displays not only a more robust AD-like skin pathology, but also concomitantly significant scratching bouts ($p < 0.0001$), scratching duration, ($p = 0.047$), anxiety-like ($p = 0.0367$) and depression-like ($p = 0.0256$) behavior, and a greater state of stress ($p < 0.005$) indicated by serum corticosterone ELISA. Ultimately, our investigation reveals that both models faithfully recapitulate human AD pathology features, but only the MC903 model generated concomitant ADCD-like features. Using the MC903 model, future investigations will explore itch- and affect-processing brain regions for structural and functional hallmarks of the depression-like state and identify potential circuit-based therapeutic targets for treating this comorbidity.



Disclosures: I.P. McConnell: None. **B. Chimmiri:** None. **S.K. Mishra:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.19/N29

Topic: G.05. Mood Disorders

Title: Deletion of histidine phosphatase LHPP induces depression-like phenotypes in mice

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Abstract: Histidine phosphorylation (pHis), occurring on the histidine of substrate proteins, is a hidden phosphoproteome that is poorly characterized in mammals. LHPP (phospholysine phosphohistidine inorganic pyrophosphate phosphatase) is one of the histidine phosphatases and its encoding gene was recently identified as a susceptibility gene for major depressive disorder (MDD). However, little is known about how LHPP or pHis contributes to depression. Here, by crossing the Lhpp floxed mice (Lhppf/f) with the Nestin::Cre mice to generate genetic deletion of LHPP in central nervous system of mice, we observed the LHPP-deficient mice exhibited the depressive-like phenotypes including forced swimming test and sucrose preference deficits. We found that LHPP deficiency increased the levels of global pHis in brain, and we further used immunoprecipitation and quantitative mass spectrometry to study histidine phosphoproteomic and the potential substrates of LHPP. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of these differentially expressed proteins revealed changes in a variety of biological processes, especially energy metabolism in LHPP-deficient mice. These findings highlight the critical role of LHPP in the regulation of depression and provide novel insights into the involvement of histidine phosphorylation in the pathogenesis of MDD.

Disclosures: Z. Wang: None. **Y. li:** None. **W. Li:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.20/N30

Topic: G.05. Mood Disorders

Support: Neural Injury and Plasticity (NIP) Training Grant (T32NS041218-19)
Aging and Alzheimer's Research Training Program (AART) (T32AG071745-01)

Title: Ccr5 modulates behavior and mood-associated phenotypes in mice

Authors: *K. HUMMEL[1], K. CONANT[2];
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Abstract: Chemokine signaling, which is primarily thought to regulate immune cell migration, has become a growing topic of interest in disorders of the central nervous system. In particular, the CCL5/CCR5 axis has been strongly linked to cognition and memory, and is thought to play a crucial role in synaptic plasticity. Still, little is known about the role of the CCL5/CCR5 axis in plasticity-associated mechanisms of mood and behavior. In this study, we investigate the role of CCL5/CCR5 in anxiety and anhedonia using a constitutive CCR5 knockout (KO) mouse model. To this end, we use a combination of behavioral, physiological, and biochemical assays to identify molecular and phenotypical changes in CCR5 KO mice, compared to wild type controls. We used a battery of behavioral assays to study phenotype differences in anxiety and anhedonia, as well as cognition and memory. We also use *in vivo* EEG telemetry to measure cortical activity differences in CCR5 KO mice. Finally, we use immunofluorescence, as well as western blot and ELISA assays, to measure plasticity-associated perineuronal net changes in mood-associated brain regions, including the cortex and hippocampus. This study highlights the potential role of the CCL5/CCR5 axis in mood, and provides new insight to the role of chemokine signaling in anxiety and anhedonia associated behavior.

Disclosures: K. Hummel: None. **K. Conant:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.21/N31

Topic: G.05. Mood Disorders

Title: A neuro-immune circuit mediates cancer cachexia associated apathy

Authors: X. ZHU[1], S. STAROSTA[2], Q. CHEVY[3], A. SIEBELS[4], A. V. KRAVITZ[5], F. LUCANTONIO[6], P. OSTEN[7], M. PIGNATELLI[8], ***A. KEPECS**[9];
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Abstract: Cachexia, a severe wasting syndrome associated with inflammatory conditions, often leads to multi-organ failure and death. Patients with cachexia frequently experience extreme fatigue, apathy, and clinical depression. Distinguishing these psychological effects from end-of-life suffering is challenging without understanding how cachexia alters brain function. Inflammation is a critical factor in cancer cachexia, as pro-inflammatory cytokines and chronic inflammation are known to produce negative mood and loss of motivation, but the brain

mechanisms involved remain elusive. To explore the psychiatric symptoms caused by inflammation and the neural circuits implicated, we repurposed a mouse model of colon cancer. We demonstrated that cancer cachexia induces selective deficits in behavioral effort-sensitivity, the willingness to work for rewards. We uncovered a cytokine-sensing brainstem-to-basal ganglia circuit that controls motivation. This neural circuit detects elevated interleukin, an inflammatory cytokine, at the onset of cachexia and translates it into decreased mesolimbic dopamine, thereby increasing effort-sensitivity. By ablating the cytokine-sensing neurons or stimulating dopamine neurons, we were able to alleviate these motivational deficits, pointing to novel potential therapeutic targets. Our findings uncover a central neural circuit that senses inflammation and orchestrates behavioral changes, providing mechanistic insights into the connection between chronic inflammation and depressive symptoms.

Disclosures: X. Zhu: None. **S. Starosta:** None. **Q. Chevy:** None. **A. Siebels:** None. **A.V. Kravitz:** None. **F. Lucantonio:** None. **P. Osten:** None. **M. Pignatelli:** None. **A. Kepecs:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.22/N32

Topic: G.05. Mood Disorders

Support: EU H2020 MSCA ITN Project “Serotonin and Beyond” (N 953327)
the Next Generation EU- National Recovery and Resilience Plan, the Ministry of University and Research (n ECS 00000017 “Tuscany Health Ecosystem THE”, Spoke 8)

Title: Serotonergic control of circadian dopamine rhythmicity

Authors: N. BARSOTTI[1], **G. MADDALONI**[1,2], S. MIGLIARINI[1], S. NAZZI[1], M. PICCHI[1], F. ERRICO[3], A. USIELLO[4], *M. PASQUALETTI[1,5];
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Abstract: Physiology and behavior are structured temporally to anticipate daily cycles of light and dark, ensuring fitness and survival. Neuromodulatory systems in the brain - including serotonin and dopamine- exhibit daily oscillations in neural activity themselves and help shaping circadian rhythms by widely signaling wakefulness, arousal, and associated processes. Disrupted neuromodulation can cause circadian abnormalities that are thought to underlie several neuropsychiatric disorders, including bipolar mania and schizophrenia, for which mechanistic understanding is still lacking. Here we show that genetically depleting serotonin in *Tph2* knockout mice promotes manic-like behavioral traits and disrupts daily oscillations of the

dopamine biosynthetic enzyme tyrosine hydroxylase (TH) in midbrain dopaminergic nuclei. Specifically, while TH mRNA and protein levels in the Substantia Nigra (SN) and Ventral Tegmental Area (VTA) of wild type mice doubled between the light and dark phase, TH levels were high throughout the day in *Tph2* knockout mice, suggesting a hyperdopaminergic state. Analysis of TH expression in striatal terminal fields also showed blunted rhythms. Additionally, we found low abundance and blunted rhythmicity of the neuropeptide cholecystokinin (*Cck*) in the VTA of knockout mice, whose downregulation has been implicated in manic-like states in both rodents and humans. Altogether, our results point to a previously unappreciated serotonergic control of circadian dopamine signaling and propose serotonergic dysfunction as an upstream mechanism underlying dopaminergic deregulation and ultimately maladaptive behaviors.

Disclosures: N. Barsotti: None. **G. Maddaloni:** None. **S. Migliarini:** None. **S. Nazzi:** None. **M. Picchi:** None. **F. Errico:** None. **A. Usiello:** None. **M. Pasqualetti:** None.

Poster

PSTR240: Animal Models of Mood Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR240.23/N33

Topic: G.05. Mood Disorders

Support: Doctoral Training Scholarships - FRQS

Title: Lateral hypothalamus promotes compulsive-like behavior through disinhibition of serotonin cells

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Abstract: The dorsal raphe nucleus (DRN) holds most of the brain's serotonin (5-HT) cells. Dysregulation of 5-HT cells is linked to depression and obsessive-compulsive disorder, however, the mechanism connecting their pathophysiology and 5-HT is currently under debate. Here, we studied how the lateral hypothalamus (LHA), a key presynaptic partner of the DRN, controls DRN neuronal activity, and how it translates into behaviour. We adopted a transsynaptic anterograde strategy to target DRN neurons which receive input from LHA (DRN^{LHA} neurons) by injecting AAV1-cre in LHA and a cre-dependent AAV encoding eGFP in the DRN. All data were collected from C57BL/6 male and female mice aged 12-20 weeks. Combining our viral approach with immunostaining against a 5-HT marker, revealed that only 14.4%±1.7 of DRN^{LHA} were 5-HT cells, while the majority comprising 86.6% were non-5-HT (n = 7 mice). Whole-brain axon tracing showed that DRN^{LHA} neurons send dense reciprocal projections to the LHA, but also to subcortical areas in the forebrain and midbrain. To examine LHA-DRN functional connectivity, we combined optogenetics and ex vivo whole-cell patch-clamp recordings on acute

slices. Optostimulation of LHA axons (n = 33) induced excitatory postsynaptic currents (EPSCs) in 15%, inhibitory postsynaptic currents (IPSCs) in 27% in patched DRN^{LHA} cells, and both EPCSs and IPSCs in 58%. From these, the E/I ratio suggests a larger inhibitory component, indicating an overall inhibitory drive from LHA to the DRN. Next, we asked whether the DRN^{LHA} cells synapse locally onto 5-HT DRN neurons. Optostimulation of DRN^{LHA} cells (n = 12) induced either IPSCs (54%) or convergent EPSCs and IPSCs (46%) in 5-HT cells, with a greater inhibitory component, indicating that LHA disynaptically regulates 5-HT neurons through inhibiting local GABA interneurons. To examine their role in behavior, we chronically blocked synaptic transmission of DRN^{LHA} cells by expressing the tetanus toxin light chain (TeLC). TeLC mice demonstrate increased mobility, locomotion speed, stereotypical circling and nest shredding compared to controls (n = 21 mice), suggesting compulsive-like behavior. In the same mice, we observed increased cFos expression and decreased frequency of spontaneous IPSCs in 5-HT cells. These findings suggest that specifically blocking transmission in DRN^{LHA} disinhibits 5-HT DRN neurons. Overall, we showed that the LHA primarily regulates the DRN by inhibiting local GABAergic neurons, ultimately resulting in the disinhibition of 5-HT neurons. Chronic disinhibition promotes compulsive-like behaviors, demonstrating the potential role of LHA-DRN dysregulation in this pathology.

Disclosures: R. Sadretdinova: None. **C.D. Proulx:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.01/N34

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R37 #5R37DA051686
NIDA T32 #5T32DA007278

Title: Kappa opioid receptor system in the nucleus accumbens mediates escalation of cocaine consumption

Authors: *L. GORDON-FENNELL[1], R. D. FARERO[1], L. M. BURGENO[2], G. D. STUBER[3], A. G. SCHINDLER[4], C. I. CHAVKIN[5], L. S. ZWEIFEL[6], P. E. PHILLIPS[7];
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Abstract: The neural processes underlying the progression of substance use disorder (SUD) remain poorly understood - limiting current treatment options. The kappa opioid receptor (KOR) system is known to be activated by drug consumption, has been implicated in withdrawal and

negative affect, and interacts with the dopamine system in the NAc, making it a possible mechanism for the development of SUD. Here, we focus on the SUD-like phenotype of escalation of drug consumption using a long-access (6hr) cocaine self-administration paradigm in Wistar rats. To interrogate the necessity of the KOR system in the NAc during escalation, we utilized two approaches: pharmacology and CRISPR. After being trained on drug self-administration under short-access conditions (1hr), male rats were microinjected in the NAc with either a long-lasting KOR antagonist (nor-BNI, n = 14) or a vehicle control (n = 16) and then continued onto long-access self-administration. Control rats increased in their drug consumption across self-administration sessions ($p < 0.001$), demonstration escalation, whereas nor-BNI rats did not ($p = 0.18$), providing evidence that activation of the NAc KOR system is necessary for escalation. To delineate which antagonized KOR subpopulation produced this behavioral effect, we utilized CRISPR technology to knock-out oprk1 either in the NAc or ventral tegmental area (VTA) of male and female rats. Control rats were injected with a control CRISPR. Overall, oprk1 NAc KO (n = 16) and control (n = 22) rats increased in their drug intake across self-administration sessions ($p < 0.001$ and $p < 0.001$, respectively), demonstrating escalation, whereas oprk1 VTA KO rats (n = 14) did not escalate in their drug intake ($p = 0.33$). These data support that activation of KOR on VTA neurons, likely VTA terminals in the NAc, during drug consumption is necessary for the development of this SUD-like phenotype. To further implicate the necessity of KOR specifically on VTA terminals in the NAc, we are currently using a combinatorial approach with DIO-CRISPR and CAV2-Cre to knock-out oprk1 in NAc-projecting VTA neurons and observing the behavioral consequences. To better understand the temporal dynamics of KOR signaling, we are also currently measuring dynorphin levels in the NAc during cocaine self-administration using fiber photometry coupled with kLight. Uncovering the changes in neural systems throughout the development of SUD will inform future therapeutic approaches to SUD and the ongoing drug epidemics.

Disclosures: L. Gordon-Fennell: None. **R.D. Farero:** None. **L.M. Burgeno:** None. **G.D. Stuber:** None. **A.G. Schindler:** None. **C.I. Chavkin:** None. **L.S. Zweifel:** None. **P.E. Phillips:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.02/N35

Topic: G.09. Drugs of Abuse and Addiction

Support: F31 AA031178

Title: Nociceptive & cognitive symptoms of withdrawal are associated with increased cfos expression in rmtg-projecting lateral habenula neurons

Authors: *H. YANG[1], K. Y. BOSQUE CORDERO[2], J. R. SANCHEZ[3], E. J. GLOVER[4]; [1]Univ. of Illinois At Chicago, Schaumburg, IL; [2]Dept. of Psychiatry, Ctr. for Alcohol Res. in

Epigenetics, Chicago, IL; [3]Psychiatry Dept., Univ. of Illinois at Chicago, Chicago, IL; [4]Dept. of Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Individuals with alcohol use disorder (AUD) often struggle to maintain sobriety due to withdrawal symptoms that emerge during early abstinence including hyperalgesia and impaired decision making. Although widely reported, the precise mechanisms mediating these symptoms is unknown. To begin to explore this, we assessed withdrawal-induced changes in pain sensitivity and cognitive performance in a preclinical model of AUD. Separate groups of adult Long-Evans rats were tested for thermal and mechanical pain sensitivity or delay discounting for positive or negative outcomes. After baseline testing, rats were rendered dependent using a 14-day chronic intermittent ethanol (CIE) vapor exposure paradigm. Rats were retested after CIE exposure. Rats exhibited a significant withdrawal-induced increase in pain sensitivity during acute ($p=0.005$) and protracted withdrawal ($p=0.0077$) with sex differences in the time course and type of hyperalgesia observed. CIE exposure was also associated with significant impairments in delay discounting that differed depending on whether reward delivery was associated with positive or negative consequences. These data indicate that the rat CIE exposure model successfully recapitulates withdrawal symptoms observed in clinical populations. Withdrawal was associated with a significant increase in cFos expression in the rostromedial tegmental area (RMTg; $p<0.0001$) and also in RMTg-projecting lateral habenula (LHb) neurons ($p=0.029$) in a separate group of CIE exposed rats relative to controls. In combination with previous work linking RMTg and LHb activity with pain regulation and decision-making, these data suggest that the LHb-RMTg circuit may play an important role in mediating nociceptive and cognitive symptoms of withdrawal from chronic ethanol exposure.

Disclosures: H. Yang: None. **K.Y. Bosque Cordero:** None. **J.R. Sanchez:** None. **E.J. Glover:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.03/N36

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Z1A000611

Title: Discriminating the neurochemical actions of misused drugs on mouse accumbens dopamine dynamics using FSCV

Authors: M. HERSEY, M. K. BARTOLE, C. S. JONES, A. H. NEWMAN, ***G. TANDA**; NIH, NIDA IRP, Baltimore, MD

Abstract: An increasing number of individuals are affected by substance use disorders (SUD) and increased rates of drug overdose deaths creating a large global disease burden. There are currently limited options for FDA-approved pharmacological treatments for SUD. A better

understanding of the involvement of the dopaminergic neurochemistry underlying SUD would facilitate the development of efficacious therapeutic options to treat this disease. Administration of an acute dose of many addictive substances produces robust increases in nucleus accumbens dopamine (DA) via actions at the DA transporter (DAT) or direct/indirect actions on the dopaminergic system which are largely thought to account for the increased risk of misuse and dependence associated with these drugs. In this work, we employ fast scan cyclic voltammetry (FSCV) to probe DA dynamics in the nucleus accumbens shell (NAS) of male and female C57BL/6 mice after administration of drugs known to produce addiction/dependence like cocaine, methylphenidate, and fentanyl and expanding to other misused drugs. We previously found that administration of cocaine (3 and 10 mg/kg; i.p.) slowed NAS DA clearance in both male and female mice but produced more robust increases in evoked NAS DA in female mice. Administration of methylphenidate (3 and 10 mg/kg; i.p.) also produced a robust increase in evoked NAS DA as well as slowed DA clearance in both male and female mice. No statistically significant sex differences in DA dynamics were observed following methylphenidate administration. Administration of fentanyl (30 and 100 ug/kg; i.p.) produced limited changes in evoked NAS DA dynamics. In conclusion, by probing DA dynamics in naïve mice following the administration of misused drugs we aim to tease out the precise mechanism/s of action of these agents on the dopaminergic system, which may help focus research progressing toward the development of effective treatment options for SUD.

Disclosures: M. Hersey: None. **M.K. Bartole:** None. **C.S. Jones:** None. **A.H. Newman:** None. **G. Tanda:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.04/N37

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA051551
NIDA Grant DA044308
NIDA Grant DA049568

Title: Brain infiltrating $CD8^{+}$T cells as a key driver of opioid reward.

Authors: *J. P. SENS[1,2], E. G. PECK[1,2], M. H. DAWES[1,2], W. J. BROOKS[1], E. J. WISER[1], S. R. JONES[1], J. R. GLAUSIER[3], R. S. HOFFORD[1,2], D. D. KIRALY[1,2,4];
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Abstract: Opioid use disorder (OUD) is a chronic relapsing disorder characterized by compulsive drug seeking behaviors and continued opioid use despite negative consequences. Dysfunction of the immune system has long been observed in OUD, however, its contribution to the progression of the disease remains largely unknown. Herein, we have discovered aberrant $CD8^+$ T cell trafficking into the nucleus accumbens (NAc) following chronic opioid administration in mouse models and in human OUD post-mortem brain tissue. Transcriptional profiling of brain resident $CD8^+$ T cells from OUD mouse models and from human OUD post-mortem NAc tissue demonstrated converging alterations in molecular pathways, suggesting this pathophenotype is critical in human disease states. Immunological depletion of $CD8^+$ T cells significantly decreased opioid seeking behaviors in mouse models, namely conditioned placed preference and intravenous fentanyl self-administration. Fast-scan cyclic voltametric analysis of the NAc core exhibited marked alterations in dopamine release and reuptake mechanics of $CD8^+$ T cell deficient mice with prior opioid exposure. Single nucleus RNA sequencing (snRNAseq) of NAc from CD8+ T cell deficient mice revealed novel cellular populations highly responsive to $CD8^+$ T cell mediated signaling. These studies add important new behavioral and mechanistic insights into the role of $CD8^+$ T cells in OUD and provide important novel therapeutic avenues.

Disclosures: J.P. Sens: None. **E.G. Peck:** None. **M.H. Dawes:** None. **W.J. Brooks:** None. **E.J. Wiser:** None. **S.R. Jones:** None. **J.R. Glausier:** None. **R.S. Hofford:** None. **D.D. Kiraly:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.05/N38

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01DA054905

Title: The impact of perinatal fentanyl exposure on dopamine circuitry and behavior

Authors: *J. M. MCINERNEY[1], J. OLUSAKIN[2], M. LUJÁN[2], B. QAMAR[3], S. HAJIRNIS[4], A. KASHYAP[4], R. R. CAMPBELL[4], J. F. CHEER[2], M. LOBO[2];
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Abstract: Use of illicit substances has reached endemic levels in the United States with over 91,000 deaths due to drug overdose occurring in 2020. This has largely affected women of childbearing age as opioid use disorder has more than quadrupled among pregnant women in the past 20 years. Both clinical and preclinical studies show that opioid exposure during gestation can lead to developmental delays, cognitive impairment, anhedonia and reduced motivation in offspring. Opioids induce neuroadaptations in the mesolimbic dopamine pathway, which is

largely implicated in reward. The pathway originates in the ventral tegmental area (VTA) where dopamine neurons project to the nucleus accumbens (NAc) and other reward nuclei. Prolonged opioid use has been shown to reduce the soma size of VTA dopamine neurons and decrease dopamine output in the NAc. However, how perinatal opioid exposure, including the highly potent opioid fentanyl impacts the morphology and transcriptional landscape throughout the development of the mesolimbic dopamine pathway as well as the lasting effect on motivation requires further investigation. Using Imaris analysis software we produced 3 dimensional reconstructions of VTA dopamine neurons and found that perinatal fentanyl exposure (PFE) reduced the soma size of these neurons in juvenile mice compared to juvenile control subjects. Differential gene expression analysis also revealed downregulation of important genes for neuronal maturation and axonal guidance, including transcription factors critical for dopamine neuron development, in VTA of PFE juvenile mice compared to controls. Lastly, PFE mice exhibit reduced motivation in a spinning disc test during juvenile ages and altered cue evoked dopamine signal in the NAc at adult ages, the latter measured by fiber photometry during an operant task. These results indicate that perinatal fentanyl exposure and subsequent abstinence produce lasting morphological, transcriptional, and behavioral effects on the mesolimbic dopamine pathway.

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Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR241.06/N39

Topic: G.09. Drugs of Abuse and Addiction

Support: UMN Medical Discovery Team on Addiction
MJT Grant: NIH P30 DA048742

Title: Microglial synaptic engulfment following repeated acute exposure to fentanyl

Authors: *D. W. HART, M. ESGUERRA, M. THOMAS;
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Abstract: While substance use disorders take a significant toll on people and society, much is still not understood about the neurobiological underpinnings of these diseases. Recent work has highlighted the ability of opioids and other drugs to induce synaptic plasticity in the mesolimibic reward pathway in the brain. In particular, synaptic connections from the infralimbic cortex (IL) to the nucleus accumbens shell (NAcSh) change in strength throughout the cycle of abstinence and relapse. In addition, studies over the past decade have highlighted the role of microglia in shaping synaptic circuits through the pruning of synapses. This process was initially described to

occur homeostatically during development but has since been expanded to various disease models such as neurodegenerative disorders, neuropathic pain, and alcohol use disorder. However, the role of microglia in opioid-induced synaptic plasticity remains understudied. Therefore, we examined whether repeated acute exposure to fentanyl increased synaptic engulfment by microglia. Twelve C57BL/6J mice (6 male and 6 female) underwent fentanyl psychomotor sensitization. Following baseline testing, half the mice received intraperitoneal (i.p.) saline injections whereas the other half received 0.3mg/kg fentanyl (i.p.) for ten days. Following the i.p. injection, animals were allowed to freely roam an open arena for 30 minutes while their total distance traveled was recorded. Fentanyl-treated animals displayed immediate hyperlocomotion and exhibited sensitization by the end of the ten days of treatment. To characterize microglial morphology and synaptic engulfment, brain sections containing both IL and NAcSh from this cohort were triple-immunostained with the microglial marker Iba1, the lysosomal and phagocytic marker CD68, and the postsynaptic marker PSD95. Preliminary data shows that 3D reconstruction of high magnification confocal images with Imaris did not reveal gross differences in PSD95+ volumes within CD68+ structures in microglia within the NAcSh. However, we observed a decrease in total microglial volume in fentanyl-treated animals compared to saline treatment which could indicate a change in microglial state in response to the fentanyl exposure. In ongoing experiments, we aim to determine the microglial phenotype in the IL, investigate engulfment of presynaptic markers, as well as potential changes in microglial morphology after fentanyl exposure.

Disclosures: D.W. Hart: None. **M. Esguerra:** None. **M. Thomas:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.07/N40

Topic: G.09. Drugs of Abuse and Addiction

Title: Amphetamine and morphine reduce activity during acute withdrawal by similarly modifying bout organization in rats

Authors: *W. WHITE, I. M. WHITE;
Morehead State Univ., Morehead, KY

Abstract: In prior research, when male or female rats were given 2.0 mg/kg amphetamine or 5.0 mg/kg morphine, they had reduced activity 13-24 hours following treatment, and by 24 hours post-treatment they had engaged in 25% less activity, on average, than following saline. The reduced activity was a sign of acute withdrawal. Rats are typically active in bouts separated by intervals of inactivity. The primary purpose of this research was to identify how amphetamine and morphine changed bout organization to reduce activity. The research also assessed whether changes in bout organization differed by drug and sex. The subjects were 68 adult male (N=48) and female (N=20) Wistar rats. The activity of animals was monitored in open fields having

bedding, food, water, and a 12-12 hour light-dark cycle. Each animal received one or a series of tests. On day 1 of a test, rats were given saline, and 2 or 3 days later rats were given drug. Treatments were given at light onset, and activity was monitored for 24 hours following each treatment. Drug treatments were 2.0 mg/kg amphetamine or 5.0 mg/kg morphine. Distance moved 13-24 hours post-treatment was divided into 5-minute bins, and bout organization was quantified from this data. A bout began when an animal moved at least 50 cm during a 5-minute bin, and it ended with a bin followed by three consecutive bins having less than 50 cm moved. Amphetamine and morphine reduced activity 13-24 hours post-treatment primarily by reducing bout duration rather than bout number or amplitude. The modification in bout organization due to amphetamine and morphine appeared similar, and the modification following amphetamine appeared similar in males and females. Similarities suggested amphetamine and morphine modified bout organization via similar mechanisms.

Disclosures: W. White: None. **I.M. White:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.08/O1

Topic: G.09. Drugs of Abuse and Addiction

Support: NSFC Grant 82071498

Title: Cortico-thalamic ensembles and circuits drive the forgetting of drug memory

Authors: Z. LI[1], W. ZHENG[1], J. SHI[2], L. LU[3], ***Y. XUE**[4];
[1]Peking Univ., Beijing, China; [2]Natl. Inst. On Drug Dependence of Peking Un, Beijing, China; [3]Inst. Mental Hlth.,Peking Univ. Sixth Hospita, Beijing City, China; [4]Natl. Inst. on Drug Dependence, Peking Univ., Beijing, China

Abstract: Previous research has shown that the cue-induced memory extinction paradigm can effectively erase addiction memories and mitigate relapse risks. However, the neural underpinnings of this phenomenon remain elusive, impeding the development of more tailored and efficacious clinical interventions. In this study, we employed a rat model of methamphetamine addiction to elucidate the neuronal ensemble mechanisms underlying this behavioral paradigm. Fos mapping indicated that the infralimbic (IL) subregion of the prefrontal cortex is active during cue recall. Utilizing DREADD technology to inactivate this IL subregion reversed the effects of cue-induced extinction, whereas similar inactivation of the prelimbic (PrL) cortex did not impact the extinction process. Furthermore, using viruses engineered with the enhanced synaptic activity-responsive element (E-SARE) promoter to label neurons involved in cue-induced extinction, coupled with chemogenetic activation of these IL area neurons, successfully replicated the extinction effects. This intervention erased addiction memories without disturbing normal reward memory circuits. However, activating the neuron ensembles

recruited during the IL extinction process alone did not eliminate addiction memories. Combining E-SARE neuronal activity labeling and synaptotagmin synaptic tagging, revealed that the neuronal ensembles involved in cue-induced and traditional extinction paradigms project to distinct downstream neuronal circuits. Notable differences were observed in the amygdala, nucleus accumbens, dorsal striatum, and midline thalamic nuclei, accompanied by differential fos expression in these regions. Single-cell sequencing further demonstrated significant disparities in the molecular expression profiles and gene networks among neuron subgroups engaged by the two extinction paradigms. Further investigations highlighted that the cue-induced extinction paradigm activates projections from the IL to the intermediodorsal thalamic nucleus (IMD). Inhibiting this projection negated the effects of cue-induced extinction, while its activation mimicked these effects, effectively reducing relapse. These findings indicate the existence of specialized neuronal ensembles within the IL that mediate the effects of cue-induced extinction. Activation of the IL-IMD circuit appears crucial in reducing drug craving and preventing relapse, suggesting a potent neural target for enhancing addiction therapy outcomes.

Disclosures: Z. Li: None. **W. zheng:** None. **J. Shi:** None. **L. Lu:** None. **Y. Xue:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.09/O2

Topic: G.09. Drugs of Abuse and Addiction

Title: Dopamine signals are smaller in mice with a partial deletion of striatal D2 receptors despite enhanced acetylcholine transmission

Authors: *E. S. SWANSON[1], J. SHIN[2], L. G. ANDERSON[2], H. C. GOLDBACH[2], V. A. ALVAREZ[2];
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Abstract: Low availability of dopamine D2 receptors (D2Rs) in the striatum has long been hypothesized to predispose some individuals toward compulsive drug taking, but the mechanism by which this may enhance vulnerability to substance use disorders is not fully understood. Here we examine striatal microcircuitry in a mouse model of low D2R availability. In a cross between Adora2a-cre and *Drd2*$^{loxP/loxP}$ transgenic mouse lines, mice with a 30% reduction in striatal D2Rs were generated through a single-allele deletion of the *Drd2* gene that was targeted to spiny projection neurons. We used a combination of fast-scan cyclic voltammetry (FSCV) and fiber photometry to demonstrate that *ex vivo* electrical stimulation in brain slices, as well as direct *in vivo* optogenetic activation of dopamine axon terminals, evokes smaller striatal dopamine signals in mice with lower D2R expression. Although low-D2R mice exhibit reduced dopamine signals, subsequent brain slices recordings integrating FSCV and *ex vivo* slice photometry reveal that, relative to electrically-evoked dopamine release, simultaneous acetylcholine release from striatal

cholinergic interneurons is elevated. Furthermore, antagonism of nicotinic acetylcholine receptors located on dopamine terminals confirms that acetylcholine signaling can account for a greater component of striatal dopamine signaling in low-D2R mice. Electrophysiological and immunohistochemical analyses shed light on the functional role and distribution of cholinergic interneurons in these animals. Taken together, these results contribute to our understanding of striatal dopamine transmission is modulated by local acetylcholine release, and how this relationship is dysregulated by a known neurobiological marker of addiction.

Disclosures: E.S. Swanson: None. **J. Shin:** None. **L.G. Anderson:** None. **H.C. Goldbach:** None. **V.A. Alvarez:** None.

Poster

PSTR241: Addiction, Neuromodulation and Underlying Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR241.10/O3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA055825

Title: Exploration of the M1 muscarinic acetylcholine receptor for altering dopamine signaling in the nucleus accumbens

Authors: *E. S. GEISLER[1], C. VANCE[2], J. ELTIT[2];
[1]Pharmacol. and Toxicology, [2]Physiol. and Biophysics, Virginia Commonwealth Univ., Richmond, VA

Abstract: Stimulant use disorder (SUD), defined as the patterned use/abuse of psychostimulants including cocaine, is an epidemic that directly affects around 2.5 million people in the United States and does not have an FDA-approved pharmacotherapy. Mesolimbic dopamine signaling to the nucleus accumbens (NAc) is necessary for the maintenance of SUD. M1 muscarinic acetylcholine receptors (mAChRs) on medium spiny neurons (MSNs) show promise as pharmacological targets for the treatment of SUD. Based on previous literature, the administration of mAChR-selective compounds, in particular the bitopic M1 agonist-PAM VU0364572 (VU72) should attenuate cocaine-dependent increases in reward signaling by modulating M1 mAChRs located on NAc MSNs. This compound, however, has not been extensively characterized. Using the Ca^{2+} indicator fura-2, we further characterized this ligand for potency, efficacy, and functional selectivity in HEK cells stably expressing M1 and M5 cells. Following this, we used fiber photometry with the fluorescent sensor $GRAB_{DA2m}$ to determine the effects this compound has on dopamine levels and the alterations in signaling patterns in the NAc of awake, behaving animals. In cell culture, VU72 produces an EC_{50} of 84.9 nM (95% CI: 60.2 nM, 120 nM), compared to acetylcholine's (ACh) 2.45 nM (95% CI: 1.70 nM, 3.52 nM) and an E_{max} of 94.5% of acetylcholine (95% CI: 87.2, 101.9), suggesting that, as in previous literature, it is a moderate potency agonist at M1. As a PAM at 10 and 30 nM doses, there was a

significant main effect of acetylcholine dose ($F(5, 98) = 159.6$, $p < 0.0001$) and VU72 dose ($F(2, 98) = 3.398$; $p = 0.0374$) on Ca^{2+} fluorescence, as well as a significant interaction ($F(10, 98) = 2.285$; $p = 0.0188$). These sub-threshold doses of VU72 increase the response of low ACh concentrations without changing the EC_{50} or E_{max} of ACh in producing Ca^{2+} signals. In M5 cells, VU72 produced no activity as an agonist or antagonist when tested up to 10 μM, suggesting functional selectivity for M1 mAChRs. In preliminary *in vivo* experiments, administration of VU72 to drug-naïve Sprague-Dawley rats (n = 2) produced no apparent change in dopamine fluorescence compared to saline ($p > 0.05$) at any dose between 0.3 mg/kg - 10 mg/kg IP. These preliminary data suggest that VU72 is a selective agonist at the M1 receptor with very limited PAM effect at this receptor. VU72 does not alter dopamine signaling in the NAc when administered alone. Future studies would evaluate the effect of VU72 as a pretreatment to non-contingent cocaine administration on DA signals in the NAc.

Disclosures: E.S. Geisler: None. **C. Vance:** None. **J. Eltit:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.01/O4

Topic: G.09. Drugs of Abuse and Addiction

Support: Estonian Research Council grant PRG1473

Title: Dna methylation pattern and cytokine profile in the peripheral blood of substance use disorder patients

Authors: *K. ANIER, A. KALDA;
Univ. of Tartu, Tartu, Estonia

Abstract: Substance use disorder (SUD) is understood as a brain disorder with profound alterations at the structural, functional, and molecular levels in the human brain. Studies on rodents suggest that epigenetic modifications, such as DNA methylation and demethylation, are involved in inducing stable gene expression changes in the brain, which underlie addiction. However, the role of aberrant DNA methylation in the development of SUD in humans is unclear. Furthermore, currently no effective pharmacotherapy is available for stimulant-induced addiction and novel treatment options are greatly needed. We have previously shown that cocaine modifies DNA methylation throughout the brain and in peripheral blood cells of mice (Anier et al. 2018) and repeated treatments with either cocaine or amphetamine decreased the enzymatic activity of ten-eleven translocation enzymes (TETs) in human peripheral blood mononuclear cells (Anier et al. 2022). The aims of this study were to investigate: 1) the expression and activity of epigenetic DNA modifiers DNA methyltransferases (DNMTs) and TETs and 2) cytokine profile in the peripheral blood of SUD patients. A total of 50 male participants (aged 25-48), 16 patients with SUD and 16 healthy controls with matched

demographic and clinical data had nicotine dependence and 6 patients with SUD and 12 healthy controls without nicotine dependence (non-smokers) were enrolled in this study. We excluded subjects who had a serious physical illness, subjects with primary mental illness in the SUD group and those with mental illness in the control group. qPCR was used to measure the mRNA levels of DNMTs and TETs and cytokines. Enzyme activity of DNMTs and TETs and plasma levels of cytokines (IL-1β, IL-6, IL-10, TNF-α) were assessed with an ELISA-based assay. Our qPCR data showed that in peripheral blood of SUD patients (both nicotine dependence and non-smokers) were *DNMT1, DNMT3A, DNMT3B* and *TET1-3* mRNA levels significantly downregulated compared with respective control groups. These data were in line with DNMT and TET enzyme activity results, which also demonstrated that in peripheral blood of SUD patients were significantly decreased DNMT and TET activity compared with healthy controls. We also found that in peripheral blood and plasma of SUD patients (both nicotine dependence and non-smokers) were pro-inflammatory cytokines IL-1β and IL-6 significantly upregulated and TNF-α downregulated and anti-inflammatory cytokine IL-10 upregulated compared with healthy controls. In conclusion, our present study found significant differences in peripheral blood DNA methylation patterns and cytokine profiles between SUD patients and healthy controls.

Disclosures: K. Anier: None. **A. Kalda:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.02/O5

Topic: G.09. Drugs of Abuse and Addiction

Support: MPOWER

Title: Identifying genetic and genomic biomarkers for withdrawal signs and treatment responses in the saliva of infants with Neonatal Opioid Withdrawal Syndrome

Authors: *B. QAMAR[1], M. E. CORTES-GUTIERREZ[2], D. SMOLYAK[3], A. L. BEITELSHEES[4], S. A. AMENT[5];
[1]Univ. of Maryland Sch. of Med. Program In Mol. Med., Baltimore, MD; [2]Univ. of Maryland, IGS, Baltimore, MD; [3]Computer Sci., Univ. of Maryland, Col. Park, College Park, MD; [4]Med., Univ. of Maryland Sch. of Med., Baltimore, MD; [5]Dept. of Psychiatry and Inst. for Genome Sci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neonatal Opioid Withdrawal Syndrome (NOWS) is a condition in which infants born to mothers using opioids during pregnancy exhibit withdrawal signs including poor feeding, tremors, and high-pitched crying. The ongoing opioid epidemic has been associated with an escalating incidence of NOWS, leading to higher NICU admissions and poor health outcomes. Some infants with NOWS respond to non-pharmacological care such as rooming-in and breastfeeding, but many receive pharmacotherapy with opioids such as morphine or methadone.

Existing tools are insufficient to predict the severity of withdrawal signs or the response to pharmacotherapy, making the condition difficult to manage and likely worsening long-term outcomes. Our research program aims to optimize care for infants with NOWS through the development of predictive biomarkers in peripheral biofluids. We collected saliva samples (n~60) and buccal swabs (n~125) from infants with NOWS and healthy controls born at the University of Maryland Medical Center. We are using these samples to profile thousands of potential biomarkers, including human and microbial transcripts, genome-wide DNA methylation, and polygenic risk scores derived from the infants' genome sequences. Our analyses of these data will characterize genetic and genomic factors correlated with the severity of withdrawal signs and the response to pharmacotherapy. We will construct multivariate predictive models, integrating these genomic data with clinical variables derived from the infants' and mothers' electronic health records. Our long-term goal is to develop biomarkers and algorithms to optimize clinical care for opioid-exposed infants.

Disclosures: B. Qamar: None. **M.E. Cortes-Gutierrez:** None. **D. Smolyak:** None. **A.L. Beitelshees:** None. **S.A. Ament:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.03/O6

Topic: G.09. Drugs of Abuse and Addiction

Title: Risk Loci Associated with Internet Gaming Disorder in Adolescent

Authors: *Y. SUN[1], J. SHI[2];
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Abstract: Internet Gaming Disorder (IGD) is a psychiatric disorder that often occurs during adolescent development, but its genetic mechanism is unknown. We aim to assess the risk genetic loci of IGD in adolescents. This genome-wide association study (GWAS) is based on the Adolescent Brain Cognitive Development Study (ABCD study) data. Cross-sectional analyses were conducted using data from the third year of follow-up at the age of 12.91±0.64 years. The degree of IGD was assessed using the Video Game Addiction Questionnaire. GWAS was performed by controlling for age, sex, and genetic structure. A polygenic risk score (PRS) was used to explore the genetic association between substance use disorders and IGD. GWAS summary data was obtained from PGC, GWAS Catalog, and our previous addiction cohort. A total of 5865 participants (3143 male; 4712 female) and 4032260 SNP were included in the analysis. The GWAS identified rs376069954 located in EIF3IP1 (P = 2.65e-9) was significantly associated with the degree of IGD. IGD degree was positively correlated with alcohol use disorder (P = 0.057), cannabis use disorder (P = 0.0075), and smoking (P = 0.033), while negatively correlated with heroin addiction (P = 0.0011). Under the optimal model (P threshold

= 0.0002) methamphetamine addiction (P = 0.020) was positively correlated with IGD, but as the P-value thresholds increased methamphetamine addiction and IGD showed to be negatively correlated. Among psychiatric disorders, IGD was positively correlated with eating disorder (P = 2.9e-4), autistic disorder (P = 0.0013), insomnia (P = 0.0028), and attention deficit hyperactivity disorder (ADHD, P = 0.0055) and negatively correlated with obsessive-compulsive disorder (OCD, P = 0.00029). We would further verify these findings in our Chinese IGD cohort, and assess the genetic factors for the risk development trajectory of IGD.

Disclosures: Y. Sun: None. **J. Shi:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.04/O7

Topic: G.09. Drugs of Abuse and Addiction

Support: 1R01DA055823

Title: Multi-omic mapping of cell types in the human habenula

Authors: *K. MONTGOMERY, C. SOTO, H. DIVECHA, D. GONZALEZ-PADILLA, E. YALCINBAS, E. NELSON, R. ZHANG, A. CHANDRA, S. V. BACH, R. BHARADWAJ, J. E. KLEINMAN, T. M. HYDE, L. HUUKI-MYERS, L. COLLADO TORRES, K. R. MAYNARD; Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: The habenula (Hb) is a bilateral midline structure that is implicated in reward processing. Dysfunction of the Hb has been associated with neuropsychiatric and substance use disorders. The Hb is composed of two cellularly distinct subregions: the medial habenula (MHb) and the lateral habenula (LHb), which mediate different functions. Due to its small size, few studies have investigated the molecular anatomy of the human Hb and little is known about the transcriptomic signatures and spatial organization of LHb and MHb cell types in the human brain. Here we generated the first molecular map of the human Hb at single cell and spatial resolution. We performed single nucleus RNA-sequencing of post-mortem human Hb from 7 adult neurotypical control donors and identified seven distinct LHb and three distinct MHb neuronal populations. We defined unique marker genes for these populations and spatially mapped a subset of LHb and MHb cell types using RNAScope single molecule fluorescent in situ hybridization (smFISH). To identify novel patterns of spatial gene expression across the human Hb, we also performed the 10x Genomics Visium assay in adult neurotypical control donors. We identified data-driven subdomains across the anterior-posterior axis of the human Hb. In summary, we present the first single cell molecular atlas of the human Hb and provide an interactive web resource for the scientific community to explore the data.

Disclosures: K. Montgomery: None. **C. Soto:** None. **H. Divecha:** None. **D. Gonzalez-Padilla:** None. **E. Yalcinbas:** None. **E. Nelson:** None. **R. Zhang:** None. **A. Chandra:** None. **S.V. Bach:**

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Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.05/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Support: CONAHCYT No. 6390

Title: Heritability of the symptoms of alcohol abuse and anxiety

Authors: *U. CABALLERO SANCHEZ[1], T. V. ROMAN-LOPEZ[2], M. E. RENTERIA[3], X. DIAZ[4], S. ALCAUTER[5], A. MEDINA-RIVERA[6], A. E. RUIZ-CONTRERAS[7];
[1]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [2]Inst. de Neurobiologia, UNAM, Queretaro, Mexico; [3]Queensland Inst. of Med. Res., Herston, Australia; [4]UNAM, Querétaro, Mexico; [5]Neurobiología Conductual y Cognitiva, Inst. De Neurobiologia. Univ. Nacional A, Queretaro, Mexico; [6]Lab. Internacional de Investigación sobre el Genoma Humano, Univ. Nacional Autónoma de México, Juriquilla, Querétaro, Mexico; [7]Lab. Neurogenomica Cognitiva, Fac. Psicologia, Univ. Nacional Autonoma de Mexico, D.F., Mexico

Abstract: Comorbidity has been found between alcohol abuse and anxiety. One possibility is that there are genes that explain both phenotypes. This study aims to calculate the common heritability of alcohol consumption symptoms and anxiety symptoms. We analyzed 94 pairs of dizygotic twins and 162 pairs of monozygotic twins from the TwinsMx database. Alcohol abuse symptoms were assessed using the Alcohol Use Disorders Identification Test (AUDIT), and anxiety symptoms were assessed using the State-Trait Anxiety Inventory (STAI). A bivariate model was used to calculate the genetic contribution (heritability) of additive genes (A), common environmental contribution (C), and unique environmental contribution (E) for each phenotype, as well as the covariance between both phenotypes. According to the Akaike criterion, the AE model best fits our data. For symptoms associated with alcohol abuse, the value of A is 0.63 (40% of the variance explained) and the value of E is 0.77 (60%). In contrast, for trait anxiety symptoms, the value of A is 0.64 (42%) and 0.70 (49%) for E. The value of A that explains trait anxiety symptoms also explaining symptoms associated with alcohol abuse (i.e., shared heritability) is 0.30 (9%); the value of shared E for both phenotypes (i.e., common environment for both phenotypes) is 0.04 (0.001%). Our results suggest that both phenotypes are mainly explained by unique environments and moderately by additive genes. They also suggest that both phenotypes share few genetic factors. For future analyses, it is necessary to focus on individuals diagnosed with both conditions and not just on a continuum of symptoms for both phenotypes.

Disclosures: U. Caballero Sanchez: None. **T.V. Roman-Lopez:** None. **M.E. Renteria:** None. **X. Diaz:** None. **S. Alcauter:** None. **A. Medina-Rivera:** None. **A.E. Ruiz-Contreras:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.06/O8

Topic: G.09. Drugs of Abuse and Addiction

Support: P50DA037844
DP1DA054394

Title: Study of the subjective effects of prescription opioids in half a million people

Authors: *A. A. PALMER, S. SANCHEZ-ROIGE;
Psychiatry, UCSD, La Jolla, CA

Abstract: Opioid use disorder (OUD) is a devastating disease for which genetic predisposition is a known risk factor. A recent genome-wide association analysis (GWAS) by our group showed that using opioids “not as prescribed” is a heritable trait that is strongly genetically correlated with OUD, even when measured in populations that were not ascertained for high levels of substance use or misuse (Sanchez-Roige et al, Molecular Psychiatry, 2021). Based on this observation, as well as an extensive literature suggesting a relationship between the positive and negative subjective effects of drugs on the development of substance use disorder (SUDs), we have embarked on a project that will collect 500,000 responses to a 97-item survey from a population-based cohort that has already been genotyped. The survey includes questions about the subjective effects of opioids, the reasons for using opioids “not as prescribed”, and a variety of psychiatric and behavioral traits that are known or hypothesized to be comorbid with SUDs. While genetic results are not yet available, we have already obtained ~100,000 completed surveys, allowing us to examine the relationships among traits. The rate of self-reported OUD was almost 7%. This allowed us to calculate the odds ratio for developing OUD based on the (retrospectively, self-reported) initial sensitivity to the positive and negative subjective effects of prescription opioids. Sensitivity to the positive effects of opioids was an extremely strong risk factor for OUD, with odds ratios over 40 for individuals who endorsed the highest level of positive subjective response. The negative subjective effects of opioids (e.g. nausea) were significant but had much smaller odds ratios for OUD. Other psychiatric traits and abuse of other substances produced significant but modest odds ratios. These data suggest that our GWAS aimed at elucidating the genetic basis of differences in the subjective responses to opioids will contribute to ongoing efforts to understand the genetic basis of OUD. Moreover, our genetic results will be uniquely able to elucidate how specific alleles influence OUD risk, for example, by altering subjective drug responses. Finally, our data suggest that screening for the subjective

effects of opioids could provide a powerful and easily deployed tool for identifying people most at risk for transitioning from prescription opioid use to misuse.

Disclosures: A.A. Palmer: None. **S. Sanchez-Roige:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.07/O9

Topic: G.09. Drugs of Abuse and Addiction

Support: DC Center for AIDS Research (DC-CFAR)
Georgetown-Howard Universities Center for Clinical and Translations Science (GHUCCTS)
Artificial Intelligence/Machine Learning Consortium to Advance Health Equity and Researcher Diversity

Title: Understanding Addiction through AI: a predictive analysis powered by machine learning models

Authors: *D. ACHEAMPONG[1], S. D. WASHINGTON[2], M. C. GONDRE-LEWIS[3]; [2]McGovern Inst., [1]Howard Univ., Washington, DC; [3]Anat., Col. of Med., Washington, DC

Abstract: Better predictive models are needed to identify people who are morelikely to abuse substance or consume drugs such as opioidsand cocaine because of the drug's prevalence and the linkedsocioeconomic and health impact effects that are linked to it. Thisstudy investigates how well two machine learning algorithms—Lassoregression, and Random Forest—predict substance abuse in peoplebased on a variety of socioeconomic and demographic factors, suchas age, sex, income, and education level. We applied these models toa dataset of people classified as substance abusers and non-users tohelp identify the most important predictors of substance abuse.According to preliminary findings, the models' levels of accuracy vary.By providing insights into the potential of machine learning techniquesto identify high-risk individuals and enable more targeted interventions,this study advances the area of substance use prediction. The resultshighlight how crucial it is to include cutting-edge computationaltechniques into public health initiatives to address substance useproblems. Furthermore, our work creates opportunities for furtherresearch to improve predictive models and investigate how well theywork for various demographics.

Disclosures: D. Acheampong: None. **S.D. Washington:** None. **M.C. Gondre-Lewis:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.08/O10

Topic: G.09. Drugs of Abuse and Addiction

Support: VA RRD I01RX002916

Title: Integrating multi-modal neuroimaging data to identify a customized anatomical transcranial magnetic stimulation target for co-occurring alcohol use disorder and mild traumatic brain injury

Authors: *A. A. HERROLD[1,2], S. LIVENGOOD[3], D. K. BHAUMIK[4], P.-S. YEN[5], T. PARRISH[6], T. PAPE[7], N. JORDAN[8], P. RIORDAN[9], A. RAMIC[10], O. A. AJILORE[11], A. AARONSON[12], T. MALLINSON[13];
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Abstract: Alcohol use disorder (AUD) and mild traumatic brain injury (mTBI) impact functional abilities. AUD occurs in up to 35% of Veterans with mTBI. Evidence suggests that co-occurrence of AUD and mTBI (AUD+mTBI) leads to an exacerbation of brain dysfunction, symptom manifestation, and ultimately, functional disability. Alcohol-related characteristics are operationally defined per AUD symptoms and outcomes including, but not limited to, alcohol consumption, alcohol craving, and AUD severity. Transcranial magnetic stimulation (TMS) is a non-invasive neuromodulatory treatment with recent FDA clearance for the first substance use disorder (SUD) indication, smoking cessation. Preliminary TMS efficacy is demonstrated for AUD alone and mTBI alone using a variety of neural targets. TMS is, thus, a promising treatment for AUD+mTBI, and, yet there is no consensus on the optimal anatomical target. The objective is to identify a customized anatomical target (i.e. site of stimulation) for AUD+mTBI using a data-driven approach including multi-modal neuroimaging data. We have enrolled a total of 24 Veterans to date (n=15 with AUD+mTBI and 9 matched controls). We acquired resting state functional MRI (rsfMRI), Magnetization Prepared - Rapid Gradient Echo (MPRAGE), diffusion tensor imaging (DTI), and alcohol cue reactivity task fMRI data on a 3T Siemens PRISMA scanner. rsfMRI data were processed using the CONN toolbox. MPRAGE data were processed using the CAT12 toolbox to generate gray matter density (GMD) values. We used the Schaeffer 100 parcel atlas including default mode (DMN), salience, frontal parietal control networks. We added the following additional bilateral sub-cortical structures: nucleus accumbens, caudate, hippocampus and amygdala from the CONN atlas. GMD values were based on averaging 2 ROIs. We implemented a novel, recently published technique of integrating multimodal neuroimaging data using a Bayseian modeling approach which borrows strength of multiple modalities to adjust for local false discovery rates and to identify disrupted functional connectivity between groups (Wang et al 2024 PLoS ONE 19(4):e0289401). Preliminary results

incorporated rsfMRI and GMD values. We identified 1 ROI to ROI link of disrupted connectivity that met FDR cut-offs: left DMN retrosplenial cortex to left caudate ($\alpha = .001$). Next steps for this research will involve adding DTI and cue reactivity fMRI data. We hypothesize that the addition of each modality will improve disrupted connectivity prediction metrics. We will then correlate the disrupted connectivity values with alcohol related characteristics and functional disability to identify a TMS target.

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Poster

PSTR242: The Genetics of Addiction

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Program #/Poster #: PSTR242.09/O11

Topic: G.09. Drugs of Abuse and Addiction

Support: Baszucki Brain Research Foundation (to HP)
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Title: Increased expression of the L-type voltage-gated calcium channel CACNA1C in the hippocampus of subjects with alcohol use disorder and rhesus monkeys with chronic alcohol use

Authors: *T. PAREEK[1,2], D. M. PLATT[1,2], K. GRANT[3,4], S. M. O'DONOVAN[5], C. A. STOCKMEIER[1,2], H. PANTAZOPOULOS[1,2], B. GISABELLA[1,2];
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Abstract: Aim: Context-induced relapse is a major factor limiting recovery from alcohol use disorder (AUD). Identifying molecular targets involved in hippocampal contextual memories associated with chronic alcohol use may allow for the development of novel therapies for AUD. Our recent RNAseq profiling study of the hippocampus from rhesus monkeys with chronic alcohol use identified L-type calcium channels including CACNA1C as a top candidate gene. However, there is currently a lack of evidence regarding CACNA1C protein and cell type expression in the hippocampus following chronic alcohol use, as well as a lack of information regarding expression in the hippocampus of human subjects with AUD. We used a combination of human postmortem and rhesus monkey studies to evaluate CACNA1C hippocampal expression following chronic alcohol use. **Methods:** Hippocampal samples from adult human subjects with a history of either alcohol use disorder (AUD) only (n=20), major depressive disorder (MDD) only (n=20), comorbidity of both (n=24), or healthy controls (n=20) were used.

Gene expression of CACNA1C was quantified using qRT-PCR. CACNA1C protein expression was examined using immunohistochemistry and quantitative stereology-based microscopy in frozen hippocampal samples from adult, male rhesus monkeys with a history of chronic alcohol use (n=7) or no alcohol use (n=5). **Results:** CACNA1C mRNA expression was significantly increased in the hippocampus of subjects diagnosed with AUD ($p<0.03$) as well as subjects with comorbid AUD and MDD ($p<0.05$). In comparison, there was a significant downregulation of CACNA1C expression in subjects with MDD only ($p<0.04$). In monkeys with chronic alcohol use, we observed CACNA1C expression in neurons that were predominantly CamKIIa positive and glial cells that were GFAP positive. Further, densities of CACNA1C neurons ($p<0.01$) and glial cells ($p<0.02$) were increased, and these increases were consistent across monkey hippocampal sectors. **Conclusion:** Our results indicate increased CACNA1C expression in humans and rhesus monkeys with chronic alcohol use. Increased densities of CACNA1C cells encompassed excitatory neurons and astrocytes. Future studies examining the role of CACNA1C expression in context-induced relapse may allow for the development of CACNA1C-based therapeutic strategies for AUD, ultimately.

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Poster

PSTR242: The Genetics of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R00DA043573

Title: Effects of environmental enrichment and isolation housing on the striatal and hippocampal transcriptomes in genetically diverse mouse strains

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Abstract: Environmental factors influence a broad range of phenotypes including addiction related behaviors. Despite the impact of these phenomena on diseases such as addiction, the genetic mechanisms underlying these effects are poorly understood. Advanced mouse populations, when used in the context of a systems genetics approach, provide the ability to disentangle these complex relationships. To this end, we used environmental enrichment and isolation housing as models of enrichment and impoverishment to identify genetic mechanisms that interact with environmental factors to influence the striatal and hippocampal transcriptomes. As subjects, we used mouse strains from the genetically diverse Collaborative Cross recombinant inbred panel and their founder strains (C57BL/6J, A/J, NOD/ShiLtJ, PWK/PhJ, CC002/Unc,

CC005/TauUnc, CC019/TauUnc, CC051/TauUnc). At wean, male and female mice from these strains were housed in one of two conditions: Mice from the isolated group were singly housed in a shoebox cage and provided food, water, and bedding. Conversely, mice from the enriched group were housed in same-sex groups in large rat cages and provided enrichment items including a vertical running wheel, a horizontal running wheel above a small nesting box, Nestlets, a Shepherd Shack, and a tube. After ten weeks in these distinct housing conditions, striatum and hippocampus were collected and bulk RNA-Seq was used to quantify gene expression. The effects of strain, sex, housing condition, and interactions among these variables on striatal and hippocampal gene expression were assessed. These data reveal strain specific effects of housing condition on gene expression in brain regions that are critically involved in reward, compulsive drug seeking, and addiction. Identified mechanisms may underlie environmentally induced vulnerability and resistance to heritable diseases including addiction.

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Poster

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Support: NIDA P50DA037844
NIDA U01DA043799
NIDA 3U01DA051234-03S1

Title: Genome-wide association study of cocaine use in Heterogeneous Stock rats

Authors: *M. K. LARA[1], L. L. CARRETTE[1], T. MISSFELDT SANCHES[2], O. POLESSKAYA[3], B. SICHEL[4], S. BONNET-ZAHEDI[5], B. BOOMHOWER[6], M. BRENNAN[7], D. CHEN[8], A. S. CHITRE[9], D. E. CONLISK[10], M. FANNON[6], B. JOHNSON[5], E. KEUNG[6], A. J. KIMBROUGH[11], A. R. MARTINEZ[12], L. MATURIN[10], K.-M. NGUYEN[7], D. OTHMAN[2], J. RAMBORGER[6], P. SCHWEITZER[13], K. SHANKAR[14], S. SIMPSON[7], L. C. SMITH[6], E. A. SNEDDON[15], L. C. SOLBERG WOODS[16], G. DE GUGLIELMO[12], M. KALLUPI[17], O. GEORGE[18], A. A. PALMER[7];
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Abstract: Cocaine use disorder (CUD) is a major public health crisis with detrimental effects for both individuals and society. While previous genetic studies in both humans and animal models have already begun to establish the role of genetic factors influencing vulnerability to cocaine use, the identification of specific genes mediating that risk remains limited. Furthermore, the absence of FDA-approved medications to treat CUD highlights the urgent need to better understand the underlying genetic etiology. In order to identify genetic loci associated with cocaine use, we conducted a genome-wide association study (GWAS) using outbred N/NIH Heterogeneous Stock (HS) rats. HS rats have high genetic diversity comparable to the human population, and they exhibit a range of addiction-related behaviors falling on a spectrum from resilient to vulnerable, making HS rats an ideal model system to study CUD-related behaviors. To date, this large collaborative project has resulted in comprehensive phenotyping of approximately 900 HS rats for CUD-like behaviors using an extended access model of cocaine self-administration. GWAS for traits related to CUD, broadly including cocaine intake, escalation, and compulsion, identified multiple significant loci. These loci contain several genes of interest, including *Cesl1*, which is involved in cocaine metabolism, as well as *Slc6a2, Vsnl1, Kcns3,* and *Lsm6,* which all have been implicated in related behavioral or neuropsychiatric traits in humans or animal models. Additionally, several expression quantitative trait loci (eQTL) were identified, including an eQTL for *Lsm6,* which suggests that heritable differences in gene expression may be driving the association with cocaine intake. This study reveals novel genetic loci associated with CUD-related behaviors, representing an important step in characterizing the genetic architecture of CUD and identifying potential targets for future studies.

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Poster

PSTR242: The Genetics of Addiction

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Program #/Poster #: PSTR242.12/O14

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant U01DA044468

Title: Genome-wide association study of aversive influences on cocaine-seeking and food-seeking

Authors: M. EID[1], T. MISSFELDT SANCHES[2], A. S. CHITRE[3], A. A. PALMER[4], ***T. JHOU**[5];
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Abstract: Addictions to cocaine and other drugs of abuse have long been recognized to have heritable influences, but this heritability arises from multiple factors that are difficult to elucidate in human populations. We used several inbred rat strains (Buffalo, Brown Norway, Fischer, M520, Wistar Kyoto, ACI, and Lewis inbred strains), as well as heterogeneous stock (HS) rats, to examine heritable contributions to several tasks examining aversive influences on addiction-like behaviors. In particular, we used a runway operant cocaine-seeking task that is particularly sensitive to the aversive effects of cocaine, which protect against drug-seeking, along with a test of resistance to footshock punishment. Data from inbred rats suggest that aversive effects of cocaine and punishment resistance are both heritable, but via independent means. We next performed a genome-wide association screen (GWAS) in HS rats and identified several significant chromosomal loci associated with individual variations in cocaine-seeking and punishment resistance. These loci include one on the X chromosome that associates with cocaine-seeking in females, a chromosome 5 locus associating with resistance to footshock punishment, and a locus on chromosome 16 associating with resistance to delayed punishment. These and other loci identified are distinct for each task, further corroborating that distinct heritable influences on addiction vulnerability have independent genetic contributors. Further studies are underway to identify and characterize which genes at these loci are likely to be causal drivers of behavior.

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Poster

PSTR242: The Genetics of Addiction

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Program #/Poster #: PSTR242.13/O15

Topic: G.09. Drugs of Abuse and Addiction

Title: Single-nucleus transcriptomics reveals addiction-related, nicotine-induced transcriptional changes and mitochondrial dysfunction in the ventral tegmental area

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Abstract: Nicotine is widely recognized as the primary contributor to tobacco dependence. Previous studies have indicated that molecular and behavioral responses to nicotine are largely mediated by ventral tegmental area (VTA) neurons, but there is accumulating evidence that glia play prominent roles in nicotine addiction. However, VTA neurons and glia have yet to be characterized at the transcriptional level during the progression of nicotine self-administration. Here, a male mouse (C57BL/6J) model of nicotine self-administration was established and the timing of three important phases (pre-addiction, addicting, and post-addiction phase) was determined. Single-nucleus RNA sequencing (snRNA-seq) in the VTA at each phase was performed to comprehensively classify all unique cell subtypes. Adaptive changes occurred during the addicting and post-addiction phases, with the addicting phase displaying highly dynamic neuroplasticity that profoundly impacted transcription in each cell subtype. We illustrated the role of VTA serotonergic neurons in nicotine self-administration and revealed heterogeneity of VTA dopaminergic neurons during nicotine self-administration. Furthermore, we investigated alterations in mitochondrial homeostasis induced by nicotine and the impacts these changes have on the progression of self-administration. The results provide valuable insights into the cellular and molecular mechanisms underlying the progression of nicotine addiction, ultimately contributing to identification of molecular targets for nicotine cessation.

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Poster

PSTR242: The Genetics of Addiction

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA045795

Title: Elucidating Transcriptional Mechanisms of Addiction: Cocaine- and Stress-induced Immediate Early Gene interactions

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Abstract: Background: The global surge in neuropsychiatric disorders has been strongly associated with stress, depression, and substance abuse, which all contribute to mental health deterioration and increased risk of addiction and relapse. A deeper understanding of these factors is essential for developing targeted treatments for comorbid substance use disorders (SUDs) and stress-related disorders (e.g., depression, anxiety), with stress being a pivotal element in drug relapse post-withdrawal. Methods: We employed Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and next-generation sequencing to investigate protein-DNA interactions

in the nucleus accumbens (NAc) of mice subjected to chronic cocaine exposure or social defeat stress (CSDS). We examined transcription factors (TFs) including ΔFOSB, CREB, ZFP189, and NPAS4. Utilizing Targeted Recombination in Activated Neuronal Populations (TRAP), we assessed the potential of CSDS-responsive neurons to modulate cocaine-seeking behaviors. Results: CSDS significantly reduced social interaction ratios, while cocaine exposure heightened reward-seeking behaviors. CUT&RUN analyses revealed distinct and overlapping TF binding patterns in the NAc, illustrating the molecular divergence in response to stress and cocaine. Activation of CSDS-TRAPed neurons via the designer receptor hM3Dq markedly increased cocaine-seeking behavior, underscoring the role of specific neuronal ensembles in addiction. Discussion: Our findings illuminate specific changes in TF-DNA interactions due to cocaine and stress exposure, implicating critical brain regions in addiction and depression. This study enhances our understanding of the mechanisms underlying stress-induced anhedonia and addiction behaviors and opens avenues for further research into TF inhibitors or potentiators as potential treatments for neurological disorders. Supported by NIDA and NIMH

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Poster

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Title: Rna-sequencing analysis identifies sex-related differences in global gene expression in rats after withdrawal from methamphetamine self-administration.

Authors: *V. V. GUJAR, A. P. DAIWILE, J. L. CADET;
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Abstract: Significant behavioral differences in responses to methamphetamine (METH) exist between the two sexes in animal species. Some of these dissimilarities are probably related to sexual dimorphism in baseline molecular and biochemical mechanisms in reward pathways in the brain. As a first step towards identifying sex-based differences in methamphetamine-induced transcriptional signatures, we used RNA sequencing analysis to measure gene expression in the dorsal striatum of rats that had self-administered METH.
We trained rats to self-administer METH (0.1 mg/kg/infusion, i.v.) using two 3-hr daily sessions (with 30 minutes time out between session) for 20 days. Control rats self-administered saline under similar conditions. This was followed by drug seeking tests on withdrawal days 3 (WD3) and 30 (WD30). Behavioral results show that male rats took more METH than female rats. In both male and female rats, there were animals that escalated (high-takers) and others that did not escalate (low-takers) their METH intake during the behavioral experiment. Rats were euthanized

24 hours after the second drug seeking test and the dorsal striatum (dSTR) was isolated for RNA extraction followed by RNA sequencing analysis. RNA sequencing data identified marked differences between female and male control rats. In addition, female low-takers showed 435 mRNAs with higher and 467 mRNAs with lower expression in comparison to male low-takers. Moreover, female high-takers had 1217 mRNAs with higher and 1205 mRNAs with lower expression in comparison to male high-takers. Genes with differential expression in the female high METH takers belong to neuronal differentiation, cytoskeleton, immunity, and cell division whereas those in male high METH takers belong to glycoprotein, extracellular matrix, synaptic transmission, and classes of potassium channels. Ingenuity pathway analysis (IPA) revealed that some of these genes are involved in amphetamine-induced delusional disorder, methamphetamine dependence, addiction behavior, and cognitive impairments. Thus, our results provide more evidence for sexually dimorphic responses to METH exposure. These observations support the notion of sex-specific approaches to the treatment of patients who suffer from METH use disorder.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA038613
NIH Grant F32DA052966

Title: Regulation of metabolism-related gene expression by cocaine self-administration in mouse nucleus accumbens circuits

Authors: *C. A. CALARCO[1], I. A. WILLIAMSON[4], C. BARRETT[2], S. KEY[2], B. HERB[5], G. KUMAR[4], S. A. AMENT[3], M. LOBO[2];
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Abstract: Exposure to illicit drugs and subsequent chronic use profoundly impacts behavior, neuronal structure and firing, and gene expression in reward-related brain regions. Some of these changes are mediated by altered cellular energy homeostasis and mitochondrial function. Cocaine self-administration significantly reduces mitochondrial size in nucleus accumbens neurons, and disruption of this process is sufficient to blunt cocaine seeking. Taking a brain-wide perspective, we examined bulk and circuit-specific transcriptional changes relating to cellular metabolism and mitochondria, concentrating on reward-related brain regions and inputs to the nucleus accumbens after IV cocaine self-administration. We conducted gene ontology analysis

on bulk RNA-seq data generated from mice that had undergone either cocaine self-administration or received acute cocaine, with a focus on mitochondrial-related ontology terms. The sequencing data included tissue from the prefrontal cortex, nucleus accumbens, dorsal striatum, ventral pallidum, amygdala, hippocampus, and ventral tegmental area. We found significant representation of genes in metabolism and mitochondrial-related ontology terms, with regional and exposure-related variability. Further, predictive analysis of transcription factors regulating mitochondrial-related genes identified multiple transcription factors that may control cocaine-related changes in metabolic function. In a circuit-specific analysis, we have conducted ribotag-based labeling, isolation, and sequencing of mRNA from neurons in the prefrontal cortex, ventral hippocampus, and the ventral tegmental area that project into the nucleus accumbens after cocaine self-administration. Ongoing analysis is examining expression of metabolism-related genes, predicted transcription factors, and characterization of these projection-specific populations. Understanding circuit-specific transcriptional changes will inform how cellular metabolism supports responses to cocaine throughout reward circuits.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA038613

Title: The cell type selective impact of nicotine on nucleus accumbens medium spiny neurons

Authors: *S. L. KEY[1], C. A. CALARCO[2], I. A. WILLIAMSON[3], C. FLORES[4], M. LOBO[5]; [1]Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Neurobio., Univ. of Maryland Sch. of Med. Dept. of Neurobio., Baltimore, MD; [3]Univ. of Maryland Sch. of Med. Program In Neurosci., Parkville, MD; [4]Univ. of Maryland, Baltimore, Baltimore, MD; [5]Anat. and Neurobio., Univ. of Maryland SOM, Baltimore, MD

Abstract: Stimulant drug use disrupts behavior, neuronal structure and firing, and gene expression across multiple brain regions. Altered neuronal structure throughout brain reward regions, including the nucleus accumbens (NAc) can support persistent changes in drug craving and seeking that underlie continued drug use in the context of substance use disorders. Cocaine self-administration increases dendritic spine number in NAc D1- and D2-medium spiny neurons (MSNs), and alters mitochondrial size in a cell-type selective manner. These changes in mitochondrial morphology support drug-seeking behaviors and may support the increases in dendritic spine formation. Nicotine, a commonly used psychostimulant that drives chronic use despite knowledge of adverse health outcomes, also increases spine density of NAc MSNs and

increases MSN dendritic complexity, however, these morphological changes have not been examined at the cell-type selective level. Further, nicotine impacts cellular respiration and can directly interact with mitochondria within reward circuits, but NAc mitochondrial morphology has not been investigated in the context of repeated nicotine exposure. Changes in cellular mitochondrial function may support long term morphological plasticity underlying behavioral changes in response to drug exposure. To investigate this in a mouse model, we examined the effect of repeated intraperitoneal (IP) nicotine exposure (7 days, 0.5mg/kg) on NAc gene expression and cell-type selective MSN and mitochondrial morphology. Initial results show increased dendritic complexity, including the number of dendritic branch points, dendritic length, and intersection points in a sholl analysis specifically in D1-MSNs, with no significant changes in D2-MSNs. Analysis of gene expression in bulk NAc tissue by qPCR shows nicotine exposure changes mitochondrial related genes, with some differences by sex. Notable upregulated genes include Nrf1, Tfam, and Mfn1 and 2. Downregulated genes include Egr3, Polg, and Tfb1. Further analysis of plasticity-related genes are ongoing. Following the initial finding of increased dendrite morphology in D1-MSNs, current work is examining spine and mitochondrial morphology in D1-MSNs. Understanding how nicotine impacts mitochondria, cellular metabolism, and cell-type selective plasticity can clarify mechanisms of drug-induced plasticity as well as persistent drug seeking and taking.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA051551
NIDA Grant DA056592

Title: Effects of individual short chain fatty acids on cocaine related behaviors and gene expression

Authors: *M. KOPERSKA[1], J. P. SENS[2], D. D. KIRALY[3], R. S. HOFFORD[4];
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Abstract: Cocaine use disorder leads to extensive morbidity and mortality for which there remain no FDA approved treatments. Until recently, most approaches have focused on understanding the mechanisms of cocaine use disorder in the central nervous system, with little focus on peripheral systems that communicate with the brain. A growing body of research shows

that the resident microbes of the gastrointestinal tract, collectively the gut microbiome, alter brain and behavior in models of cocaine use. One potential avenue for this gut-brain signaling is via production of neuroactive metabolites such as the short-chain fatty acids (SCFA). These byproducts of fiber fermentation play key roles in numerous biological processes. Previous work from our group has shown a role for this class of molecules in response to cocaine, but specific molecular mechanisms remain unclear. Here, we evaluated the role of individual short-chain fatty acids in modulating the behavioral and neurobiological response to cocaine. Using established microbiome-depletion and metabolite repletion models, we examined the behavioral effects of individual SCFA on cocaine conditioned-place preference (CPP) and gene expression. Both male and female mice (C57BL6/J) were included in all studies. To interpret dose-dependent effects, animals underwent CPP at two doses of cocaine: 5mg/kg and 10mg/kg. In analyzing gene expression, q-PCR analyses surveyed changes in gene expression in the nucleus accumbens related to established transcriptomic and epigenetic mechanisms of SCFAs. Significant dose x microbiome status and metabolite x dose interactions for the formation of conditioned place preference were found. As previously seen in our group, treatment with antibiotics led to significant alterations in gene expression in the nucleus accumbens following cocaine, particularly in genes related to synaptic plasticity and epigenetic regulation. Treatment with individual SCFA led to reversal of cocaine + antibiotic changes with unique signatures of gene expression controlled by the individual SCFA. Our research indicates that SCFAs are critical mediators of behavioral changes in the context of cocaine use, yet the role of individual SCFA is understudied in this space. The studies conducted provide insight into this question and lay the foundation for more targeted studies into the molecular mechanisms underlying gut-brain signaling in cocaine use disorder. Future research will focus on detailed molecular mechanisms underlying these effects, using a translational model of self-administration.

Disclosures: M. Koperska: None. **J.P. Sens:** None. **D.D. Kiraly:** None. **R.S. Hofford:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.19/O21

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH NIDA R21DA055846
NIH NIDA R21DA059966
NIH NIDA R21DA057458
WSU Bridge II grant

Title: Methamphetamine self-administration alters cell type-specific gene expression in the rat nucleus accumbens

Authors: S. CHEHIMI[1], A. SHARMA[2], R. CRIST[1], B. C. REINER[1], ***A. MOSZCZYNSKA**[2]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Wayne State Univ., Detroit, MI

Abstract: The nucleus accumbens (NAc) is a crucial brain region implicated in reward processing and addiction. Methamphetamine (METH) abuse leads to profound neuroadaptive changes within the NAc, contributing to addictive behaviors. Here, we investigated how METH self-administration impacts cell type-specific gene expression profiles within the rat NAc. Using cutting-edge RNA sequencing techniques, we assessed gene expression changes in specific cell types, including medium spiny neurons (MSNs) and interneurons, following METH exposure. Our findings reveal significant alterations in the transcriptomic landscape, suggesting distinct molecular adaptations associated with METH addiction. Furthermore, we identified specific gene networks and pathways dysregulated by METH, shedding light on the underlying mechanisms driving addictive behaviors. Understanding these cell type-specific gene expression changes may provide novel insights into the neurobiology of METH addiction and offer potential targets for therapeutic intervention. This study underscores the importance of considering cell type-specific responses in elucidating the molecular basis of drug addiction and further highlights the potential of transcriptomic approaches in uncovering novel therapeutic avenues for treating substance use disorders.

Disclosures: S. Chehimi: None. **A. Sharma:** None. **R. Crist:** None. **B.C. Reiner:** 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.; Novo Nordisk, Boehringer Ingelheim. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Oxford Nanopore Technologies. **A. Moszczynska:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.20/O22

Topic: G.09. Drugs of Abuse and Addiction

Support: R00DA045795
R01DA058958

Title: A zinc finger transcription factor in the nucleus accumbens regulates cocaine-induced transcription and behaviors in a cell type specific manner

Authors: *G. SILVA[1], J. PICONE[2], R. K. KIM[3], N. L. TRUBY[4], R. L. NEVE[5], P. J. HAMILTON[6];
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Abstract: Previous work has demonstrated that *Zfp189* is a gene target through which the CREB transcription factor (TF) regulates the reinforcing effects of cocaine within the nucleus accumbens (NAc). However, the exact NAc cell-type specific mechanisms through which *Zfp189* expression is able to regulate cocaine-induced neuroadaptations remains unclear. The *Zfp189* gene product is a Krüppel associated box (KRAB) zinc finger TF of poorly understood function. To directly interrogate the transcriptional 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 ($ZFP189^{NFD}$). We demonstrate that these synthetic ZFP189 TFs exert divergent transcriptional regulation at a *luciferase* target gene, *in vitro*. Upon packaging these ZFP189 TF constructs in herpes viral vectors and surgically delivering to mouse NAc, we identify that synthetic $ZFP189^{VPR}$ affects cocaine-, but not saline-elicited behaviors and transcriptional regulation. To understand the NAc cell-type specific correlates of this drug-specific result, we performed single nuclei RNA sequencing on infected NAc tissues. We discovered that $ZFP189^{VPR}$ impeded expression of cocaine-induced differentially expressed genes across all NAc cell types compared to $ZFP189^{WT}$. Further, these synthetic ZFP189 TFs of opposing molecular function produce opposing gene expression profiles within *Drd1*- vs. *Drd2*-medium spiny neurons (MSNs)**.** We next investigated the MSN-specific contribution of our ZFP189 variants to cocaine-induced locomotor behavior and spine morphology. We utilized transgenic mice that express Cre recombinase under the *Drd1*- or *Drd2*-promoter in combination with Cre-dependent expression vectors to express our synthetic ZFP189 TFs selectively in *Drd1*+ or *Drd2*+ NAc MSNs. We saw a similar increase in cocaine-induced locomotor behavior when delivering $ZFP189^{VPR}$ to *Drd1*+ MSNs and $ZFP189^{WT}$ to *Drd2*+ MSNs. When investigating spine morphology on MSN subtypes, we saw the same pattern of $ZFP189^{VPR}$ within *Drd1*+ MSNs and $ZFP189^{WT}$ within *Drd2*+ MSNs both exerted an increase in mature spine morphology. Given the largely opposing roles of the two MSN subtypes in reward-related behaviors, and the observed opposite transcriptional control of our synthetic ZFP189 TFs, it is possible that we are dysregulating a ZFP189-governed opponent process between the MSN subtypes. Collectively, this work links MSN-specific function of a drug-induced TF in governing lasting drug-related transcriptional neuroadaptations and behaviors.

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Poster

PSTR242: The Genetics of Addiction

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Program #/Poster #: PSTR242.21/O23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P50AA022538

Title: A Cell-type Specific Investigation of Mettl3 mRNA Expression in AUD Brain

Authors: *J. TANDUKAR[1], E. MALOVIC[5], H. ZHANG[2], S. C. PANDEY[3], R. GAO[4]; [1]Biol. Sci., [2]Dept. of Psychiatry, Col. of Med., [3]Alcohol Res. Center, Dept Psychiatry, [4]Departments of Chem. and Biol. Sci., Univ. of Illinois Chicago, Chicago, IL; [5]Dept. of Psychiatry, Col. of Med., Univ. of Illinios Chicago, Chicago, IL

Abstract: Exposure to alcohol during adolescence increases the risk of developing alcohol use disorder (AUD) and can trigger long-lasting epigenetic effects in various brain regions. While increasing attention has been given to the changes in DNA and histones in AUD studies, a whole other layer of gene regulation mechanisms associated with RNA epigenetics has often been understudied. METTL3, an RNA methyltransferase, plays a crucial role in methylating internal adenosine residues of eukaryotic mRNAs, a process known as m6A methylation. To study how the level of METTL3 can be affected by alcohol, we established an adolescent AUD rat model by dividing the animals into two groups - adolescent intermittent ethanol (AIE) and adolescent intermittent saline (AIS). Our recent study using both male and female AIE and AIS rats revealed that *Mettl3* RNA was increased 0.6-fold in the central amygdala ($p < 0.01$) while the METTL3 protein level also increased (but was not significant, $p = 0.1572$) for the AIE rats. The higher level of *Mettl3* and METTL3 suggests an increased level of m6A methylation in the AUD central amygdala, which may be associated with specific differential gene regulation that are responsible for increased anxiety in the AIE rats. Despite this discovery that links AUD and RNA epigenetics, whether the observed increase of *Mettl3* originate from neuronal or non-neuronal pathophysiological processes remains unclear, as our quantification was not cell-type-specific. Additionally, whether such an increase can be observed in different brain regions (especially those susceptible to AUD) remains unstudied. Here we aim to elucidate these questions by quantifying *Mettl3* RNA levels across the AIE brain in a cell-type-specific manner. We developed an improved RESOLution enhanced Visualization using Expansion-coupled FISH (RESOLVE-FISH) pipeline to visualize and quantify individual RNA transcripts in a cell-type-specific manner while preserving the original spatial context of the brain tissue. By employing this method, we successfully identified fluorescent puncta representing single *Mettl3* mRNA or cluster in the central amygdala, basolateral amygdala (BLA), frontal cortex, and hippocampus. By assessing the *Mettl3* mRNA levels in distinct cell types, such as neurons, astrocytes, and microglia, we hope to discern any cell-type-specific differential epigenetic patterns associated with adolescent alcohol exposure compared to the control group within these brain regions. Our approach and results will provide cell-type and brain-region specific insights into the molecular mechanisms underlying the effects of alcohol exposure during adolescence on *Mettl3* expression.

Disclosures: J. Tandukar: None. **E. Malovic:** None. **H. Zhang:** None. **S.C. Pandey:** None. **R. Gao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A co-inventor on multiple patents related to expansion microscopy.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR242.22/O24

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA056602

Title: Mitigation of Addiction-Like Behaviors in Alcohol Dependent Rats by Nicotinic A3B4 Receptor Modulation

Authors: *C. P. WOOD[1,2], M. R. DOYLE[3], S. DIRIK[2], R. QIAO[2], A. R. MARTINEZ[2], P. CAMPO[2,4], N. T. ZAVERI[5], M. KALLUPI[2], G. DE GUGLIELMO[2];
[2]Psychiatry, [1]UCSD, San Diego, CA; [3]Psychiatry, UC San Diego/Scripps Res., San Diego, CA; [4]Psychiatry, Univ. of San Diego, California, San Diego, CA; [5]Astraea Therapeutics, LLC, Mountain View, CA

Abstract: Mitigation of Addiction-Like Behaviors in Alcohol Dependent Rats by Nicotinic A3β4 Receptor Modulation
Courtney P. Wood, PhD; Paola Campo; Michelle R. Doyle, PhD; Selen Dirik; Ran Qiao; Angelica R. Martinez; Nurulain T. Zaveri, PhD; Marsida Kallupi, PharmD, PhD; and Giordano de Guglielmo, PharmD, PhD Background: Alcohol and nicotine co-use is a prevalent issue, particularly among individuals with alcohol dependence; therefore, developing treatments that reduce both alcohol and nicotine intake is of great interest. Targeting nicotinic receptors with antagonists or partial agonists can successfully decrease nicotine use, and emerging evidence suggests this approach may also work for alcohol use. ATRX-52, an α3β4 nicotinic acetylcholine receptor (nAChR) functional antagonist, has shown promising results on attenuating drug + cue-primed reinstatement of nicotine-seeking behavior. Methods: In this study, we investigated the effectiveness of ATRX-52 in reducing alcohol consumption under fixed ratio and progressive ratio responding as well as a cue-seeking test in rats. Rats were characterized for their alcohol addiction-like behavior by pairing chronic intermittent access to alcohol vapor with measurements of self-administration and motivation for alcohol during withdrawal. Rats with high and low addiction indices (n=16/group) were treated with ATRX-52 (0.2, 0.4, 0.8 mg/kg) or vehicle injections, administered 30-min prior to self-administration sessions. ATRX-52 was also evaluated in a separate group of rats self-administering saccharin or nicotine. Results: ATRX-52 dose-dependently decreased ethanol intake in all rats in both the fixed and progressive ratio schedules of reinforcement. Notably, the treatment was more effective in rats with high addiction indices compared to those with low addiction indices. ATRX-52 (0.4 mg/kg) also reduced cue seeking and nicotine self-administration in high addiction rats. Conclusions: These findings suggest that ATRX-52 may have potential as a treatment strategy for alcohol use disorders, particularly in individuals with higher vulnerability to addiction.

Disclosures: C.P. Wood: None. **M.R. Doyle:** None. **S. Dirik:** None. **R. Qiao:** None. **A.R. Martinez:** None. **P. Campo:** None. **N.T. Zaveri:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Provided therapeutics. **M. Kallupi:** None. **G. de Guglielmo:** None.

Poster

PSTR242: The Genetics of Addiction

Location: MCP Hall A

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Program #/Poster #: PSTR242.23/O25

Topic: G.09. Drugs of Abuse and Addiction

Support: T32AA007456
R01AA030048
R01AA029688

Title: In depth characterization of alcohol addiction-like behaviors: from individual differences to medication development

Authors: *M. R. DOYLE[1,2], S. DIRIK[3], A. R. MARTINEZ[3], M. BALAGUER[3], C. P. WOOD[3], A. VAIYAPURI[3], A. MORGAN[3], X. LI[3], A. SHI[3], J. PIRI[3], A. SANCHEZ[3], A. A. PALMER[3], G. DE GUGLIELMO[3];
[1]UC San Diego/Scripps Res., La Jolla, CA; [2]The Scripps Research Institute, La Jolla, CA; [3]UC San Diego, La Jolla, CA

Abstract: Alcohol use disorder (AUD) affects approximately 15% of adults who consume alcohol, with only 10% of treated individuals responding to currently FDA-approved medications. Given the role of genetics in AUD development and the heterogeneous nature of the disorder, we investigated individual differences in alcohol addiction-like behaviors and responses to treatment medications in genetically diverse heterogeneous stock (HS) rats. We used 450 male and female HS rats that self-administered oral ethanol and measured multiple AUD-related behaviors. Dependence was induced using chronic intermittent ethanol vapor exposure, and behavioral experiments were conducted during acute withdrawal. We assessed withdrawal behaviors, sensitivity, tolerance, and the ability of pharmacotherapies to reduce alcohol drinking in dependent rats. We identified individual differences in response to alcohol during withdrawal, development of tolerance, and sex differences. Cluster analyses indicate variation in addiction phenotypes that can be helpful for identifying successful therapeutic targets for populations with varying symptomology. Significant individual differences in response to FDA-approved and new medications were observed. Our study highlights the importance of understanding individual differences in AUD-related behaviors and treatment responses. These data, combined with observed cellular and transcriptomic variations, provide valuable insights into potential novel therapeutic targets to improve treatment outcomes for AUD patients.

Disclosures: M.R. Doyle: A. Employment/Salary (full or part-time):; Scripps Research Institute. **S. Dirik:** A. Employment/Salary (full or part-time):; UC San Diego. **A.R. Martinez:** A. Employment/Salary (full or part-time):; UC San Diego. **M. Balaguer:** A. Employment/Salary (full or part-time):; UC San Diego. **C.P. Wood:** A. Employment/Salary (full or part-time):; UC San Diego. **A. Vaiyapuri:** None. **A. Morgan:** None. **X. Li:** None. **A. Shi:** None. **J. Piri:** None. **A. Sanchez:** None. **A.A. Palmer:** A. Employment/Salary (full or part-time):; UC San Diego. **G. de Guglielmo:** A. Employment/Salary (full or part-time):; UC San Diego.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.01/O26

Topic: G.09. Drugs of Abuse and Addiction

Title: Prolonged Voluntary Alcohol Consumption Attenuates Central and Peripheral Cytokine Responses to Lipopolysaccharide

Authors: *L. SINGLETON[1], J. CARROLL[2], K. THOMPSON[2], K. GEISINGER[2], R. A. KOHMAN[2];
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Abstract: Preclinical and clinical research demonstrates that alcohol misuse has long-term effects on immune function, as alcohol misuse alters the inflammatory profile basally and following an insult. Additionally, alcohol-induced inflammation may promote alcohol cravings and alcohol dependence (Leclercq et al., 2012). In support, rodent models show that administering lipopolysaccharide (LPS) enhances EtOH intake in the two-bottle choice paradigm (Blednov et al., 2011; Decker Ramirez et al., 2023). The present study extended this work by assessing the bidirectional association between alcohol use and inflammation in the Drinking in the Dark (DID) binge paradigm. A week after receiving LPS (1 mg/kg) or saline, female mice were evaluated for voluntary EtOH consumption in a prolonged DID paradigm. Next, we assessed whether prolonged voluntary EtOH exposure influenced the inflammatory response in the brain and liver following a secondary immune challenge. Our results demonstrate that voluntary binge drinking in the DID model was unaltered by a prior immune challenge. However, prolonged alcohol consumption attenuated the central and peripheral immune response to a secondary insult. Compared to mice who drank only water, EtOH-consuming mice showed diminished brain and liver levels of interleukin-1β and tumor necrosis factor-α four hours after LPS. Twelve days of DID was not sufficient to promote a basal increase in inflammatory cytokines. Collectively, the results demonstrate that prolonged voluntary EtOH exposure blunts the inflammatory response, which may indicate cross-tolerance. Our findings provide novel insight into the immunomodulatory effects of binge alcohol intake, which may inform our understanding of the health risks in alcohol-consuming individuals.

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Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01AA028488
NIH Grant R01AA025969

Title: Late positive responses to alcohol images in community alcohol use disorder sample

Authors: *A. BOLAND, C. FAIRBAIRN, K. D. FEDERMEIER;
Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: Alcohol use disorder (AUD) is a prevalent medical condition worldwide with an estimated lifetime prevalence rate of 29.1% (Grant et al., 2015). Past research has shown that individuals with AUD often demonstrate enhanced brain responses to alcohol-related stimuli when compared to healthy individuals, which may be indicative of enhanced emotional and motivational states related to alcohol cues (Heinze et al., 2007; Kim et al., 2006; Littel et al., 2012). The present study builds on previous literature by examining whether this alcohol cue salience extends to an individual's everyday environment. Moreover, this study examines event-related potential (ERP) responses linked to emotional salience (the Late Positive Potential; LPP) and explicit memory (the Late Positive Complex; LPC) in various types of images and explores the relationship between these brain responses and reported drinking episodes. Participants with AUD (n=34) underwent a 2-week ambulatory assessment period where they were asked to fill out surveys, take pictures, and report any drinking episodes throughout each day. Along with this, they wore a SCRAM ankle monitor to detect drinking episodes transdermally. At the conclusion of the assessment period, participants completed an EEG task where they were shown old (pictures they took) and new (pictures they didn't take) photographs; half of each of these kind of photographs included alcohol. Analyses using multi-level modeling found a main effect of memory, with larger LPC responses to old compared to new images (Mean difference=5.6 μV, $t(33)=-8.44$, $p<0.001$). Along with this, there was a main effect of image content, with participants showing an enhanced LPP response to images containing alcohol (Mean difference=1.89 μV, $t(33)=3.78$, $p<0.001$). The relationship between image content (alcohol vs not) and late positive response diverged significantly depending on participants' alcohol consumption frequency as detected via transdermal sensor during ambulatory assessment (Mean difference=-0.26 μV, $t(33)=-2.06$, $p=0.042$). The results support previous findings that individuals with AUD have enhanced brain responses to alcohol-related cues. They also suggest that the number of drinking episodes one has may influence their reactivity to these cues. Overall, the study builds on past research on alcohol cue salience and introduces a novel real-world component to this line of research.

Disclosures: A. Boland: None. **C. Fairbairn:** None. **K.D. Federmeier:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 AA029130-S1

Title: Effect of chemogenetic inactivation of the anterior insular cortex on ethanol-induced conditioned taste aversion

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Abstract: The aversive properties of ethanol (EtOH), which serve to constrain consumption, can manifest independently of its rewarding properties. While the neurobiology underlying ethanol reward is well-understood, the mechanisms underlying EtOH's aversive properties are unknown. The insular cortex (IC) plays a crucial role in the processing of interoceptive information including the subjective effects of addictive drugs. The anterior subregion of the IC (AIC) is particularly crucial as it serves as the final step in interoceptive processing by which internal conditions inform an organism and guide appropriate behavioral responses to environmental stimuli based on subjective experience. Previous work has demonstrated deficits in behavioral responding to aversive stimuli following loss of AIC function. In addition, lesion studies have established a link between the IC and acquisition of lithium chloride-induced conditioned taste aversion (CTA), a preclinical paradigm used to measure the aversive properties of drugs. Despite compelling data, whether AIC activity is also linked to the aversive properties of EtOH remains unexplored. To address this gap, we used a chemogenetic approach to inactivate the AIC during CTA. Adult male and female Long-Evans rats received bilateral injections of either a Gi-expressing DREADD or GFP into the AIC. After recovery, rats underwent a standard CTA paradigm during which a 0.1% saccharin solution was paired with intraperitoneal (i.p.) administration of saline, EtOH (1.5 g/kg), or LiCl (31.75 mg/kg) over three conditioning days. On test day, rats were given access to saccharin 30 minutes after receiving an i.p. injection of the DREADD ligand chlozapine-N-oxide (CNO; 1 mg/kg). Results demonstrated that both GFP- and Gi DREADD-expressing rats exhibited a significant decrease in saccharin intake when it was paired with either EtOH or LiCl by the third conditioning day compared to the saline group (*$p<0.05$). However, chemogenetic inactivation of the AIC had no effect on CTA expression on test day ($p>0.05$). These data suggest that AIC activity is not required for the expression of either EtOH- or LiCl-induced CTA. Whether the same manipulation disrupts acquisition of EtOH-induced CTA is unknown. Ongoing studies will explore this possibility as well as the effect of inactivation of distinct subcortical AIC projections on CTA expression.

Disclosures: J. Sanchez: None. **H. Yang:** None. **E.J. Glover:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Program #/Poster #: PSTR243.04/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Support: K01-AA027833

Title: Alcohol Consumption and Cognitive Decline in Older Adults: A Longitudinal Study

Authors: X. LU[1,2], ***N. MALEKI**[3,2,4];
[1]Harvard T.H. Chan Sch. of Publ. Hlth., Boston, MA; [2]Massachusetts General Hospital, Boston, MA; [3]Harvard Med. Sch., Charlestown, MA; [4]VA Boston Healthcare System, Boston, MA

Abstract: Motivation: To examine the associations between distinct levels of alcohol consumption and cognitive decline among individuals aged 60 and above in the United States.
Background: While extensive research exists on alcohol's impact on cognitive function and underlying key brain regions involved in those with alcohol use disorder, limited studies have explored the impact of alcohol consumption in individuals who use alcohol lightly or moderately. Particularly, the relationship between alcohol exposure and cognitive decline remains uncertain, lacking consensus across various studies.
Methods: Using the Health and Retirement Study (HRS), a nationally representative longitudinal study of American adults, we focused on a cohort of participants aged 60 and older from enrollment years spanning 1996 to 2016 biennially. Categorical drinking status was defined using two approaches that grouped drinking categories into three levels (no drinking, 21 drinks per week or less, and exceeding 21 drinks per week) or four levels (no drinking, low to moderate, intermediate, and heavy drinking) in accordance with NIAAA defined criteria for men and women separately. Cognitive decline was quantified by tracking the reduction across consecutive waves in word recall score and mental status score. Our analysis employed the Generalized Estimating Equations (GEE) approach with Gaussian distribution, an identity link function, and an exchangeable correlation structure to address the potential correlation within waves. Gender, age at current wave, education level, and race were considered as potential confounders.
Results: Within the cohort of 13,211 participants with a median age of 73, the average years of follow-up was 9.8 years. The adjusted models revealed no linear association, but a quadratic association between weekly drinking volume and cognitive decline. Consuming less than 21 drinks per week exhibited a significant association with less cognitive decline compared to no drinking, as indicated by coefficients of -0.04 and -0.05 for mental status and word recall (all p-values < 0.001). Furthermore, a consistent trend emerged where consumption of more than 21 drinks per week or heavy drinking correlated with amplified cognitive decline for mental status and word recall.
Conclusion: Low to moderate alcohol consumption may not have a detrimental effect on cognitive decline in older adults as they age and may even slow the decline. Heavy alcohol usage, on the other hand, is linked to more pronounced cognitive decline, emphasizing the potential risks and severity associated with excessive drinking.

Disclosures: X. Lu: None. **N. Maleki:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

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Topic: G.09. Drugs of Abuse and Addiction

Support: German Research Foundation (DFG, GRK 2350 project B2; TO 539/3-1)
German Federal Ministry of Education and Research (BMBF, grant 01GQ1102)

Title: Moderate drinking - exploring daily and neural mechanisms underlying AUD vulnerability

Authors: *O. BERHE[1], G. GAN[1], A. GHADAMI[1], M. REICHERT[2,1,3], A. S. MEYER-LINDENBERG[1], H. TOST[1];
[1]Dept. of Psychiatry and Psychotherapy, Med. Fac. Mannheim,, Central Inst. of Mental Hlth., Mannheim, Germany; [2]Ruhr-University Bochum, Bochum, Germany; [3]Dept. of Sport and Sport Sci., Karlsruhe Inst. of Technol., Karlsruhe, Germany

Abstract: Engaging in alcohol consumption in young age represents a significant risk factor for the later development of addiction or alcohol use disorder (AUD). Improved understanding of the complex interplay between occasional alcohol consumption and the daily-life affective and neural characteristics is important since it can help to identify salient risk markers in this vulnerable population. 378 young healthy social drinkers from a population-based cohort (13 to 28 years) took part in a 7-day Ecological momentary assessment protocol using GPS-triggered smartphone-based e-dairies with 9 to 23 prompts per day to rate momentary affective well-being. Only self-identified current drinkers (mild-to-moderate) were included in analyses . Participants also filled out a standard battery of questionnaires. Of those, 214 participants additionally completed a functional MRI with a well-established reward paradigm. We performed an activation and psychophysiological interaction (PPI) analysis with ventral striatum (VS) as region of interest. Our findings linked higher alcohol consumption to both, to changes in momentary affective well-being and alterations in neural reward processing and cognitive control mechanisms. Observed negative association between weekly alcohol intake and momentary valence ($p < 0.011$) may reflect the underlying self-medication motives - using alcohol as a coping mechanism. We further found the alcohol-related increase in VS reward sensitivity ($t = 2.87$, $pFWE = .044$) and increased functional connectivity between the VS and dorsolateral prefrontal cortex ($t = 3.97$, $pFWE = 0.006$), both are established neural risk markers predisposing individuals in this demographic to problematic alcohol use later in life. In this study we highlight daily life and neural mechanisms underlying AUD vulnerability in young healthy moderate drinkers. These findings may provide valuable insights into preventive strategies and intervention approaches tailored to this vulnerable population.

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Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.06/O30

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01DA052953
US Department of Veteran's Affairs (BX004440)
NIH NINDS 2T32NS007421-25
Roy J. Carver Charitable Trust

Title: Acid-sensing ion channels contribute to alcohol tolerance

Authors: *M. T. JAHNKE[1], G. I. S. HARMATA[2], A. C. CHAN[1], R. J. TAUGHER-HEBL[3], J. B. HARDIE[4], J. A. WEMMIE[1];
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Abstract: Alcohol tolerance is central to alcohol use disorder (AUD), but mechanisms underlying tolerance are understudied compared to other aspects of AUD. Recent work from our lab suggests that acid-sensing ion channel 1a (ASIC1A) may play a novel role in tolerance. ASIC1A is a key subunit of a cation channel permeable to Na^+ and Ca^{2+}, activated by extracellular H^+ ions. We found that alcohol directly potentiates this ASIC1A activation, and mice lacking ASIC1A are less intoxicated by an acute alcohol dose. Therefore, we hypothesized that loss of ASIC1A may further reduce alcohol intoxication with chronic exposure and promote development of alcohol tolerance. To test this, we exposed age- and sex-matched cohorts of *Asic1a*$^{-/-}$ and *Asic1a*$^{+/+}$ mice chronically to alcohol. This chronic intermittent alcohol exposure (CIE) was done in into alcohol vapor chambers perfused with a mixture of room air and vaporized alcohol for 16 hours followed by an 8-hour abstinence period. This was repeated for a total of 5 days of CIE. Blood alcohol levels post-CIE did not differ between *Asic1a*$^{-/-}$ and *Asic1a*$^{+/+}$ mice. Following CIE, we tested measures of alcohol tolerance. Mice were given a high alcohol dose and placed into an open field enclosure to quantify locomotor activity as a measure of intoxication. Alcohol suppressed locomotion in alcohol-naïve controls of both genotypes, indicating severe intoxication. Alcohol was less intoxicating following CIE, supporting CIE-induced tolerance. Moreover, this tolerance was greater in *Asic1a*$^{-/-}$ mice compared to their *Asic1a*$^{+/+}$ counterparts. We also measured effects of CIE on alcohol-induced hypothermia by rectal thermometer. CIE produced tolerance to alcohol-induced hypothermia, which may be greater in *Asic1a*$^{-/-}$ mice. As alcohol tolerance leads to withdrawal when consumption ceases, we investigated measures of alcohol withdrawal post-CIE. We tested withdrawal-related seizure activity with a handling-induced convulsion (HIC) scale, and in the open field we measured withdrawal-associated loss of spontaneous activity in the absence of alcohol. We found *Asic1a*$^{-/-}$ mice exhibited higher HIC scores and more severe hypoactivity post-CIE, suggesting that withdrawal was greater in *Asic1a*$^{-/-}$ mice. Taken together, these data support our hypothesis that ASIC1A disruption increases the development of alcohol tolerance, leading to greater alcohol withdrawal. These findings suggest that ASIC1A has a key role in alcohol intoxication and in opposing the development of tolerance and withdrawal. Because these are core features of AUD,

our data suggest that ASIC1A may be involved in the development of AUD and might therefore be an effective therapeutic target.

Disclosures: M.T. Jahnke: None. **G.I.S. Harmata:** None. **A.C. Chan:** None. **R.J. Taugher-Hebl:** None. **J.B. Hardie:** None. **J.A. Wemmie:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.07/

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant AA028680

Title: Differential effects of oxytocin on stress-enhanced alcohol drinking in sensitive and resilient mice

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Abstract: Alcohol use disorder (AUD) is a highly prevalent psychiatric condition that is frequently comorbid with post-traumatic stress disorder (PTSD). However, attempts to develop medications for patients with PTSD-AUD comorbidity have been largely unsuccessful. Preclinical and clinical evidence suggests that oxytocin (OXT) could be a potential pharmacotherapy for AUD. Despite this promise, it is not known whether OXT could be differentially effective in individuals prone or resistant to development of stress-enhanced enhanced alcohol drinking. To address this gap in knowledge, here we tested effects of OXT on alcohol consumption in a mouse model of stress sensitivity and resilience following repeated exposures to a naturalistic stressor. Male and female mice consumed alcohol in a 2-bottle choice (2BC) procedure with 10% ethanol and water for 40 days. Alcohol and water intake were assessed using an automated lickometer system. On experimental days 16, 19, 23 and 26 of 2BC mice were exposed to a predator stress (soiled rat bedding). Sixteen of the 64 tested mice robustly increased alcohol intake and preference after stress exposures and were classified as stress-sensitive. On the other hand, seventeen of the 64 tested mice, classified as stress-resilient, decreased alcohol intake after the stress exposures. On experimental days 37 and 39, mice were injected with OXT (1 mg/kg, intraperitoneal). OXT administration significantly decreased alcohol intake during the first four hours after OXT administration in male mice ($p<0.0001$). There was no difference in the sensitivity of sensitive and resilient males to the inhibitory effects of OXT on alcohol intake. OXT also significantly decreased alcohol intake in female mice during this time interval. Importantly, while the effects of OXT on alcohol intake were robust in stress-sensitive females ($p<0.0001$), there was no significant effect of OXT on alcohol intake in resilient females ($p=0.36$). These results not only confirm that OXT is a promising potential

pharmacotherapy for AUD, but also suggest that this treatment could be preferentially effective in subjects prone to stress-induced excessive alcohol use.

Disclosures: A.E. Ryabinin: None. **M.A. Nipper:** None. **M.L. Helms:** None. **D.A. Finn:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.08/O31

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P20GM103642
NIH Grant 2R25NS080687
NIH-RISE 5R25GM061151-19

Title: Effects of Tip60 expression modulation on alcohol-induced seizure susceptibility in Drosophila

Authors: *A. S. EMMANUELLI-MICHELI[1], N. JIMENEZ-VIZCARRONDO[2], A. MERCADO-ROSARIO[3], S. ITRIAGO FREITES[4], C. DEL VALLE-COLÓN[5], A. GHEZZI[6]; [1]Biol., Univ. of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico; [2]Univ. of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico; [3]Univ. of Puerto Rico, Río Piedras, San Juan, Puerto Rico; [4]Univ. de Puerto Rico, Rio Piedras, San Juan, Puerto Rico; [5]Biol., Univ. de Puerto Rico, Rio Piedras, Puerto Rico; [6]Biol., Univ. of Puerto Rico, Rio Piedras, San Juan, PR

Abstract: Alcohol use disorder, also known as AUD, is a disorder characterized by uncontrolled consumption of alcohol. This pattern of behavior can lead to the development of tolerance mechanisms towards alcohol ingestion. Moreover, excessive alcohol consumption is known to produce seizure-inducing adaptations as a symptom of withdrawal. Alcohol-induced neuroadaptations can also cause disruption of sleep patterns, which may trigger seizures as well. While some medications are available to alleviate seizure onset, alcohol consumption can affect the release of anti- epileptic drugs (AEDs) into the bloodstream. To comprehend the mechanisms underlying the effect alcohol consumption has on seizure susceptibility, we implemented a *Drosophila melanogaster* model given that approximately 60% of the fly's genes are homologous to those in humans, with 75% of these responsible for human diseases such as epilepsy. We aim to determine the role of Tip60, a histone acetyltransferase that has been previously linked to alcohol neuroadaptations, in the mechanisms previously mentioned after alcohol exposure. To achieve this, we tested Tip60 knockdown by using the UAS-Gal4 genetic manipulation system and RNAi against Tip60 (elav- Gal4/UAS-Tip60-RNAi), as well as Tip 60 over-expression. To delve into the effects of alcohol consumption, the number of days of alcohol exposure varied in the four groups that were used for both experimental groups, and the control flies. Nonetheless, each group of flies was exposed to 0.5mL of 95% EtOH for 40 minutes at a time. After 24 hours of the final exposure, the flies were subjected to mechanical stimulation to

induce convulsions**.** Our preliminary results obtained from recordings of the flies' seizure-like behavior show that the control group had an increase in seizure susceptibility following mechanical stimulation when exposed to alcohol. Furthermore, the experimental group whose Tip60 expression is knocked-down, presented an even higher susceptibility to seizures when exposed to alcohol. These results demonstrate a direct link between a knockdown in Tip60 expression and an increase in seizure susceptibility, providing the foundations for future interventions in the treatment of alcohol-induced seizure susceptibility.

Disclosures: A.S. Emmanuelli-Micheli: None. **N. Jimenez-Vizcarrondo:** None. **A. Mercado-Rosario:** None. **S. Itriago Freites:** None. **C. Del Valle-Colón:** None. **A. Ghezzi:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.09/O32

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA043461-05

Title: The effects of non-anesthetic doses of ketamine in a rat model of chronic alcohol consumption and abstinence

Authors: *S. JENNINGS;
Florida State Univ., Tallahassee, FL

Abstract: Title: The effects of non-anesthetic doses of ketamine in a rat model of chronic alcohol consumption and abstinence
Clinical and preclinical data support that therapeutic doses of ketamine, a noncompetitive NMDA receptor antagonist, may be effective for the treatment of Alcohol Use Disorder (AUD). However, studies have not established when the optimal therapeutic window of ketamine administration should occur to prevent relapse, sex differences in the effects of ketamine on drinking, or the neurocircuitry involved in ketamine's therapeutic effects in the alcoholic brain. The objective of the current study was to determine an effective therapeutic dose and treatment protocol of ketamine to reduce drinking. Male and female Long-Evans rats underwent a 24 h intermittent access drinking paradigm. After reaching a stable baseline of alcohol drinking, rats received 3 intravenous slow infusions of ketamine (0.0, 1.47, 2.35 mg/kg/infusion), or intraperitoneal injections of ketamine (0.0, 10, 20 mg/kg/injection) on withdrawal days. Our results show that under a 24 h intermittent access drinking paradigm, ketamine did not have acute or sustained effects on consumption or preference for ethanol regardless of sex or route of administration. Given that AUD is a chronic relapsing disorder, we next used a modified Drinking in the Dark (DID) paradigm followed by a 1-week abstinence period (2 weeks of DID + 1 week of abstinence), and repeated DID and abstinence cycles. At the start of the 6^{th} cycle, immediately after abstinence and 1 h before DID began, rats received ketamine (0.0, 10

mg/kg/injection, *i.p.*) for a total of 6 injections on Monday, Wednesday, and Friday of the 2 DID weeks. Rats continued into abstinence, followed by 2 weeks of DID (7^{th} cycle), to assess sustained effects of ketamine. Results suggest sex and individual differences in ketamine's effects on alcohol consumption under a chronic relapsing model of drinking. Ongoing work is underway to assess ketamine's effects in this model on negative affective-like states using the novelty suppressed feeding test (NSFT) during abstinence. Using this translational paradigm to model abstinence-induced drinking and negative affective-like states, key information about ketamine's therapeutic potential can be determined to inform optimal treatment approaches in AUD patients.

Disclosures: S. Jennings: None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.10/O33

Topic: G.09. Drugs of Abuse and Addiction

Support: NSF: HRD-2008186
NIH Grant 5P20GM103642
NIH-RISE 5R25GM061151-19

Title: Unveiling the Influence of Slowpoke on Alcohol-Induced Neuroadaptations in Drosophila's LNv Neurons

Authors: *I. A. MERCADO, C. DEL VALLE-COLÓN, A. GHEZZI;
Univ. of Puerto Rico, Rio Piedras, San Juan, Puerto Rico

Abstract: Alcohol has been identified as the most abused substance. Unrestrained alcohol consumption can have detrimental effect on neurological, psychological, and behavioral processes which can alter the homeostatic balance of the body and create psychological dependence of the substance. Furthermore, the conservation of the body's circadian rhythms, such as sleep, become affected as alcohol consumption rises. However, the molecular mechanisms controlling gene expression and neuroadaptations that regulate alcohol-induced behaviors and tolerance are not fully understood. This study focuses on determining the role the voltage gated potassium channel Slowpoke (*slo*) gene has in alcohol-induced tolerance and the effect on the circadian rhythm of sleep. Utilizing a *Drosophila* model, the aim of the study focuses on the lateral ventral neurons (LNv) that function in the production and release of the neurotransmitter pigment-dispersing factor (PDF) which stabilizes and regulates the sleep-wake cycles. Employing the UAS-Gal4 gene manipulation system and an RNAi targeting sequence to knockdown slo-gene expression in the LNv neurons affecting the regulation of PDF (pdf-Gal4/UAS-slo-RNAi). Age-equivalent adult female flies were used to measure the outcome of this genetic manipulation by utilizing activity monitors in quantitative assessment of the effect it

has on sleep and alcohol-induced tolerance. *Slo* gene knockdown *Drosophila* present considerable sleep distortion with an increase in alcohol sensitivity caused by a reduced alcohol tolerance. Also, our data show that flies expressing slo-RNAi into LNv neurons have decreased fraction of time sleeping, suggesting *Slo* knockdown into LNv plays a role modulating sleep behavior in flies. Results suggest that the Slo gene is involved alcohol-induced behavior regulation and the development of alcohol tolerance. Results shown in this study can advance and innovate beneficial targets for therapeutic treatment in alcohol-induced behaviors.

Disclosures: I.A. Mercado: None. **C. Del Valle-Colón:** None. **A. Ghezzi:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.11/O34

Topic: G.09. Drugs of Abuse and Addiction

Support: Bradley University Department of Psychology Elias Fund

Title: A novel neurobehavioral index shows attentional biases to alcohol-related cues emerge early in habitual use

Authors: *S. METCALFE[1], S. SEIFIANJOO[1], J. SWEET[1], A. BACON[2], J. A. HARRIS[2]; [2]Psychology, [1]Bradley Univ., Peoria, IL

Abstract: Attentional biases to substance related cues are a central feature of addiction and predictive of problematic use. We employ a novel neurobehavioral index rooted in biased competition models of attention to characterize alcohol-related attentional biases among college-age drinkers and non-drinkers. First, a behavioral measure of attentional biases to different beverage types was obtained during an adaptive staircasing task, wherein the relative dominance of superimposed transparent face and beverage images was adjusted according to participant responses. Specifically, the opacity of the image element indicated as perceptually dominant by the participant was reduced on subsequent trials of the same type, such that a higher percent face opacity would result from a stronger attentional bias toward the competing beverage image type. Here, the maximum percent face opacity achieved for each independently-staircased composite image type (containing alcoholic or nonalcoholic beverage elements) was submitted to a repeated-meaures analysis of variance with the within-subjects factor of nonface image element, and the between-subjects factor of drinking status. This analysis revealed a drinking status by nonface image element interaction, suggesting that alcoholic beverage cues were significantly more relevant to college-age drinkers than to age-matched nondrinkers. In addition, event-related potential measures of object-category-specific processing reflected in the N170 and later face-specific negativity, were extracted for composite images containing faces and nonface beverage images, sorted by type (alcoholic and nonalcoholic). Mean amplitude responses during the N170 and later negativity time windows, submitted to a repeated-measures analysis of variance with

the within-subjects factor of nonface image type and the between-subjects factor of drinking status, revealed a similarly distinct attentional status for composite images containing an alcoholic beverage element among the drinkers, a pattern not seen in among the nondrinkers. These findings indicate that attentional biases to alcohol-related cues emerge at early stages of habitual use and are evident in both behavioral and neural responses to composite images depicting directly competing elements.

Disclosures: S. Metcalfe: None. **S. Seifianjoo:** None. **J. Sweet:** None. **A. Bacon:** None. **J.A. Harris:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.12/O35

Topic: G.09. Drugs of Abuse and Addiction

Support: Butler University Holcomb Awards
Indiana Academy of Sciences

Title: Sex differences in combined alcohol and caffeine consumption in adolescent mice

Authors: A. KAUR, ***J. BERRY**;
Psychology, Butler Univ., Indianapolis, IN

Abstract: The notion that stimulating qualities of energy drinks reduce overall depressant effects of alcohol has resulted in an increase in the popularity of combining energy drinks with alcohol amongst young adults and teenagers. Alcohol misuse often emerges during or shortly after vulnerable adolescent years. Even though energy drinks are frequently advertised to and enjoyed by adolescents, there are limited investigations on the neurobehavioral effects of combining caffeine with alcohol during adolescence. This study sought to examine the impact of exposing C57BL/6J adolescent mice to concurrent alcohol and caffeine. In the current study, adolescent (n= 74, 38 males and 36 females; arrived at 4 weeks of age) C57BL/6J mice were given access to two bottles using an intermittent access (IA) two bottle choice drinking paradigm for a total of 4 weeks. The experimental bottle contained either caffeine (0.015% w/v), alcohol (10% v/v EtOH), or a combination of alcohol+caffeine at the aforementioned concentrations. Adolescent male and female mice were given free access to the substance(s) for 24 hours, after which the experimental bottle was taken away for another 24 hours. Male adolescent mice consumed significantly more alcohol when combined with caffeine compared to male adolescent mice that had access to alcohol alone. Further, this trend was shown only in male adolescent mice and not in female adolescent mice as females did not show any differences in alcohol consumption regardless of whether there was caffeine in the mixture. This data suggests that adolescent males may be more vulnerable to consume higher amounts of alcohol when combined with caffeinated beverages.

Disclosures: A. Kaur: None. **J. Berry:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.13/Web Only

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of social isolation on memory, anxiety, anhedonia, alcohol consumption and seeking behavior, and the expression of cannabinoid receptor 1 during the aging process of Wistar rats

Authors: *J. GOMEZ VILLATORO[1,2], I. RODRÍGUEZ[3], A. MOZO[3], A. HERRERA-SOLÍS[4], A. E. RUIZ-CONTERARAS[5], A. OSTOS VALVERDE[6], O. E. PROSPERO-GARCIA[6], M. MENDEZ DIAZ[3];
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Abstract: By 2050, it is estimated that 22% of the global population will be aged over 60, with approximately 1 in 3 older individuals experiencing loneliness, which may lead to adverse effects such as depression, anxiety, cognitive decline, and substance misuse and addiction. The expression of the cannabinoid receptor 1 (CB1R) is vulnerable to social isolation during postnatal and adolescent development, prompting inquiry into its expression during old age. This investigation aims to elucidate the impact of social isolation on CB1R expression, as well as its effects on anxiety, anhedonia, memory function, and alcohol consumption in aging rats. Male and female Wistar rats aged postnatal day (PND) 360 were randomly assigned into either a socialized or an isolation group commencing from PND90 onwards. To address this inquiry, we assessed anxiety (utilizing Elevated Plus Maze), anhedonia (via Sucrose Preference Test), memory function (using the Object Recognition Test), alcohol-seeking behavior (via Conditioned Place Preference), and CB1R expression (via Immunofluorescence) in various brain regions including the prefrontal cortex, the cingulate cortex, the hippocampus, the amygdala, and the bed nucleus of the stria terminalis. The results indicate that isolated male rats (n=8) exhibit a heightened preference for alcohol consumption compared with their social counterparts (n=8, 5.75 ± 0.58 vs 8.19 ± 0.74 (SEM) g of ethanol over the total training days). Likewise, the depressive-like behaviors are more pronounced in isolated male rats. Additionally, we observed that male and female isolated rats have diminished survival rates at year (7 out of 10), suggesting that social isolation compromise well-being and survival. This study furnishes essential insights

into the repercussions of social isolation on mood, cognitive abilities, and substance misuse and addiction among older individuals, and suggests underlying CB1R expression changes occurring in isolated aging rats.

Disclosures: J. Gomez Villatoro: None. **I. Rodríguez:** None. **A. Mozo:** None. **A. Herrera-Solís:** None. **A.E. Ruiz-Conteraras:** None. **A. Ostos Valverde:** None. **O.E. Prospero-Garcia:** None. **M. Mendez Diaz:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.14/O36

Topic: G.09. Drugs of Abuse and Addiction

Title: Sleep/wake patterns contribute to initiation, maintenance, and relapse of adolescent alcohol consumption

Authors: A. TEJADA[1], A. MORA[2], C. CUETO[3], K. GUERRERO LEON[4], M. R. GONZALES[5], S. GOMEZ[6], L. GONZALEZ[7], ***L. R. AMODEO**[8];
[1]Psychology, CSUSB, Apple Valley, CA; [2]California State Univ. San Bernardino, San Bernardino, CA; [3]California State Univ., San Bernardino, San Bernardino, CA; [4]California State Univ., San Bernardino, Highland, CA; [5]Cal State Univ. San Bernardino, Moreno Valley, CA; [6]California State Univ., San Bernardino, Victorville, CA; [7]CSU San Bernardino, Ontario, CA; [8]Psychology, CSU San Bernardino, San Bernardino, CA

Abstract: Adolescence is a time of neurophysiological changes that manifest in hallmark developmental behaviors. What is less discussed are the changes in sleep patterns that are concurrently exhibited during this period. This is of concern since early initiation of alcohol use can lead to a higher risk of alcohol-related problems, sleep disturbances, and relapse in adulthood. While there are many factors that may play a role in the initiation and maintenance of alcohol consumption it is not clearly understood how sleep/wake activity patterns can precipitate this maladaptive cycle. The purpose of the proposed study is to determine which developmental factors predict future high or low alcohol consumption in a rodent model. Specifically, this study seeks to determine whether rest/wake activity rhythms, circadian measures, and specific cognitive/social behaviors may contribute to the initiation, maintenance, and relapse of alcohol consumption during adolescence. To this end, adolescent male and female Long-Evans rats were given 10 sessions of intermittent 2-bottle 20% ethanol access during the dark phase beginning on postnatal day (P) 39. Rats were then divided into high and low ethanol consumers via median split. Prior to ethanol access, adolescent rats (P34-38) were assessed for 24h activity rhythms using a non-invasive activity monitor in the home cage and a battery of behavioral assays including social play, open field, spontaneous alternation, and 2% sucrose preference. Activity monitoring was also conducted at various timepoints through the initiation, maintenance, and relapse of alcohol consumption to track the development of alcohol use. Preliminary results

suggest that compared to low consumers, high consumers had greater overall activity and more fragmented inactivity episodes during the light phase prior to ethanol access. Additionally, activity patterns seemed to shift across consumption days during the light phase when the animals spend more time sleeping, but not during the dark phase. High consumers also demonstrated an increase in baseline sucrose preference. These results suggest that initial sleep/wake patterns might precipitate the maintenance of voluntary alcohol consumption in adolescent rats.

Disclosures: A. Tejada: None. **A. Mora:** None. **C. Cueto:** None. **K. Guerrero Leon:** None. **M.R. Gonzales:** None. **S. Gomez:** None. **L. Gonzalez:** None. **L.R. Amodeo:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.15/O37

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA027213
R01AA026306

Title: Assessment of Motivational and Sex-Specific Influences on Punishment Resistance to Alcohol

Authors: *S. M. SOTO[1], C. OJI[1], C. A. VARGAS-HUBNER[1], P. H. JANAK[1,2,3];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Kavli NDI, Baltimore, MD; [3]Neuroscience, Johns Hopkins University, Baltimore, MD

Abstract: A defining characteristic of problematic alcohol consumption is the continued use of alcohol despite negative consequences. Preclinical models of punished alcohol seeking are used to examine the choice between abstinence or drug use at the cost of a negative consequence. It is, therefore, critical to identify sex and motivational differences that could account for individual variation in resistance to punished alcohol use. In this experiment, we aimed to evaluate the effects of alcohol motivation and sex-specific effects of estrous cycle on punished alcohol seeking. To assess these aims, we provided Long Evans rats (n=22; 9F/13M) homecage access to alcohol (EtOH, 15% w/v), followed by alcohol self-administration training. After training, rats were tested on a progressive ratio task wherein the number of lever presses required for an alcohol reward increased exponentially after every trial. Progressive ratio tests were followed by punishment sessions wherein task conditions were identical to training, with the addition of an aversive footshock stimulus (0.25 mA; 0.5 s) contingent with the last lever press of the ratio (FR2). Consistent with prior literature, we observed prominent individual differences in punished alcohol seeking that were confirmed using unsupervised k-means clustering of each rat's resistance score, a measure that compares punished to baseline rewards. Through clustering, we identified low alcohol seeking rats as punishment sensitive and high alcohol seeking rats as

punishment resistant (~32%) and confirmed that these two groups differed in punished alcohol seeking. We next assessed motivational breakpoints between punishment resistant and sensitive rats on the preceding progressive ratio task. We found that progressive ratio breakpoints did not correlate with resistance scores in early nor late punishment. We next aimed to evaluate sex differences between male and female rats during punished alcohol seeking. Female rats appear more sensitive to punishment than male rats (2/9 resistant vs. 5/13 resistant). To better understand possible sex-specific effects of the estrous cycle on punishment, we collected estrous samples from female rats immediately after testing sessions. We did not observe significant changes in rewards earned during punishment between estrous cycle stages or between high and low fertile stages. Taken together, while our results did confirm individual differences in punished alcohol seeking consistent with prior work, these differences do not appear to be due to variations in the motivation to seek alcohol nor to estrous cycle influences on sensitivity to punishment.

Disclosures: S.M. Soto: None. **C. Oji:** None. **C.A. Vargas-Hubner:** None. **P.H. Janak:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.16/P1

Topic: G.09. Drugs of Abuse and Addiction

Support: BGSU Building Strength Grant
BGSU Psychology Department Fund

Title: Relative reward processing of ethanol following sucrose exposure in non-food-restricted rats

Authors: *E. SHULTZ, K. THOMPSON, H. C. CROMWELL;
Psychology, Bowling Green State Univ., Bowling Green, OH

Abstract: Alcohol Use Disorder (AUD) is a disease that has a devastating effect on millions of individuals around the world. While around 50% of the risk for developing AUD has been determined to be genetic, examining predictors of addictive-like behavior and individual variance in models without a family history of addiction is also important. Factors such as impaired reward sensitivity and response to natural reward, such as sucrose, may play a role in an individual's propensity to develop alcohol addiction. Additionally, little research has been done on predictors of alcohol addiction without the use of food or water deprivation to coax animals into addiction. The study of motivation and reward-sensitivity related functions in non-food restricted animals could provide us with novel possible predictors of addiction in a manner more reflective of the reality in which humans develop addiction. This study examined the appetitive and consummatory behavior of female Wistar rats in self-administration tasks of sucrose and ethanol solutions. The rats had ad-libitum access to food and water throughout the

study. Unique time-based manipulation of reward value was utilized to evaluate relative reward effects and reward sensitivity was assessed on both an inter-session and intra-session basis. This allowed for evaluation of motivational changes across different time periods and how those changes contribute to addictive behavior. Sucrose consumption during the shortest time access predicted both 15% and 30% ethanol consumption in the home cage during 30-minute exposure. The current study allows a closer examination of the motivational processes shared by alcohol and sugar that could result in addiction. Using natural reward sensitivity to predict future addiction could aid significantly in preventing and treating substance use disorders.

Disclosures: E. Shultz: None. **K. Thompson:** None. **H.C. Cromwell:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.17/P2

Topic: G.09. Drugs of Abuse and Addiction

Support: PASA Grant VPR-75-12742

Title: Reduction in ethanol consumption in mice following (2R,6R)-Hydroxynorketamine

Authors: *M. CAMPANILE[1], J. PAMPALONE[1], K. CASTELL[1], C. A. BROWNE[2], I. LUCKI[2];
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Abstract: Alcohol use disorder (AUD) remains a growing concern among military members, including veterans, where 40.8% of all US veterans met the criteria for lifetime alcohol use disorder in 2022. There is an urgent need for novel treatments to help reduce alcohol intake and effectively treat AUD. Literature suggests that ketamine can increase abstinence length and reduce consumption on heavy drinking days for individuals diagnosed with AUD. The ketamine metabolite, (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) also exhibits ketamine's' antidepressant and analgesic activity but without producing gait impairment, rewarding effects, or the sedation associated with NMDA antagonism. In this study, the effect of (2R,6R)-HNK) on an intermittent access (IA) model of binge drinking was examined in male (n=45) and female (n=45) C57Bl/6J mice were individually housed under a 12-hour light/dark cycle, with food and water available *ad libitum*. Mice were given three 16 h duration IA sessions per week, commencing with the onset of the dark cycle, with access to both 16% ethanol and water. Volumes of fluid consumed were measured at 2 and 16 h. Both the volume and % ethanol consumption were normalized to bodyweight. Blood ethanol concentration (BEC) was tracked weekly, with samples taken 2 h during the first session of the week. On week four of the experimental timeline, mice were given three intraperitoneal injections of (2R,6R)-HNK (0, 3, 10, 30, and 56 mg/kg dissolved in sterile 0.9% saline) administered at 48 h intervals. As AUD

produces a distinct electrocardiogram (ECG) profile of humans, prolongation of the QT wave and QRS complex abnormalities, mice were assessed weekly for ECG profile using the ECGenie (Mouse Specifics, Inc). Ethanol consumption led to alterations in the ECG waveforms, in particular in QT, QTC, PQ, PR, and ST segments, with distinct Sex X Week interactions. Female mice showed a significant increase in duration of the PR and PQ segment compared to the male mice. Starting at week 1, female mice show a significant increase in duration of QT, QTC, and ST segments. There was an overall treatment effect of (2R,6R)-HNK, reducing ethanol consumption by approximately 10% in both male and female mice, during the treatment week and the week following treatment cessation, indicative of sustained action of the drug. In conclusion, (2R,6R)-HNK may be a novel therapeutic for AUD.

Disclosures: M. Campanile: None. **J. Pampalone:** None. **K. Castell:** None. **C.A. Browne:** None. **I. Lucki:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.18/P3

Topic: G.09. Drugs of Abuse and Addiction

Title: The interaction of sex and chronic pain displacement and intensity in alcohol and cannabis co-users

Authors: *M. SINCLAIR, J. BOISSONEAULT;
Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Approximately 20% of adults in the United States experience chronic pain, and self-medication using cannabis and/or alcohol is common. The Catastrophizing, Anxiety, Negative Urgency, and Expectancy (CANUE) model describes modifiable (e.g., pain attitudes, impulsivity, negative affect) and non-modifiable (sex, pain characteristics) biopsychosocial factors associated with risk for pain self-medication. However, in addition to intensity, pain varies in widespreadness, which is thought to represent the degree to which pain is centralized. In this study, we examined how these modifiable and non-modifiable factors may interact to predict recent pain self-medication behavior in co-users of alcohol and cannabis.
Method: Using Amazon Mechanical Turk, participants (N=163, 55.3% female) reported recent use of both alcohol and cannabis. The risk of self-medication of pain with substance use was assessed via the CANUE-14 screening questionnaire. Recent self-medication behavior and the expectation that alcohol and cannabis will relieve pain were assessed with visual analog scales. Pain intensity and widespreadness were assessed using the Brief Pain Inventory. Using separate three-way ANOVAs, we examined the effect of sex, pain intensity, CANUE-14 score, relief expectancies, number of pain sites, and their interactions on self-medication behavior.
Results: For self-medication of pain using alcohol, we identified significant positive associations with pain intensity ($F(1,151)=23.37$, $p<.001$, $\eta^2_p=.13$), expectancy that alcohol relieves pain

(F(1,151)=48.70, p<.001, η^2_p=.24), and CANUE-14 score (F(1, 151)=7.27, η^2_p=.05). Similarly, pain intensity (F(1,153)=21.65, p<.001, η^2_p=.07), expectancy that cannabis relieves pain (F(1,153)=14.59, p<.001, η^2_p=.09), and CANUE-14 score (F(1,153)=12.88, p<.001, η^2_p=.08) each predicted more frequent self-medication of pain using cannabis.
Conclusion: Taken together, results indicate that pain intensity and CANUE-related psychosocial factors, including expectancies that alcohol or cannabis will relieve pain, are each important predictors of self-medication among co-users. However, number of pain sites was not related to self-medication behavior, suggesting pain intensity is a more relevant predictor than the degree to which pain is centralized. Assessments of risk for self-medication of pain with alcohol and cannabis should include measures of pain severity in addition to putative psychosocial risk factors.
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Disclosures: M. Sinclair: None. **J. Boissoneault:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.19/P4

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA025337

Title: Effects of Acute Alcohol on Insular Cortex Connectivity During and Pain Processing

Authors: *A. CUSHNIE[1], J. BOISSONEAULT[2];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Anesthesiol., Univ. of Minnesota, Minneapolis, MN

Abstract: The insular cortex (IC) is involved in pain processing and exhibits widespread connectivity and functional dissociation, with distinct connectivity patterns across its anterior (AIC) and posterior (PIC) subdivisions. PIC encodes pain intensity and discrimination, mediated by connections with the primary and secondary somatosensory cortex, thalamus, and cingulate. AIC underpins the affective-motivational component of pain processing via connections with the primary and secondary somatosensory cortex, prefrontal cortex, amygdala, and striatum. Aberrant IC connectivity has been implicated in alcohol use disorder (AUD) and chronic pain. Moreover, AUD and chronic pain frequently co-occur and are reciprocally related. Furthermore, alcohol intake is acutely analgesic, but chronic use can increase pain susceptibility. However, the effects of acute alcohol consumption on pain-related IC functional connectivity (FC) with the rest of the brain are incompletely understood.
Healthy adults (N=99, M=25.87 age (SD = 4.30)) completed two task-based fMRI sessions after consuming a placebo or alcohol-containing beverage targeting 0.08 g/dl BrAC. An MR probe attached to an fMRI-compatible stimulator delivered noxious thermal stimuli to the glabrous skin of the foot at temperatures individually calibrated to produce pain ratings of 5cm on a 10cm

VAS.
We investigated the impact of acute alcohol on pain-related changes in FC of IC using generalized psychophysical interaction analysis. We hypothesized that alcohol would significantly alter the FC of the IC following pain stimulation compared to placebo (pFDR<.05), with differential impacts on AIC and PIC.
Application of noxious heat induced widespread FC changes in IC FC that were significantly affected by alcohol intake. Alcohol increased pain-related changes in FC of the IC with several regions under pain stimulation compared to placebo: lateral occipital cortex and middle frontal gyrus. Decreased pain-related FC of the IC with frontal pole and precentral gyrus was also observed. Alcohol increased pain-related FC of the AIC to a greater extent than PIC with 3postcentral gyrus, precentral gyrus, and center-operculum. The opposite pattern was observed for superior frontal gyrus, fronto-operculum, left insular cortex, posterior supramarginal gyrus, frontal pole, supramarginal gyrus, and middle frontal gyrus. Taken together, results indicate acute alcohol intake induces distributed changes in IC connectivity during pain processing, particularly affecting regions involved in motor, visual, and language functions, providing mechanistic insight into alcohol's effects on pain.

Disclosures: A. Cushnie: None. **J. Boissoneault:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.20/P5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA026598

Title: Female mice exhibit different alcohol withdrawal behaviors and withdrawal-induced neuronal activation compared with male mice

Authors: N. PAYNE, M. SCALF, R. DOBBELMANN, S. ENGEL, S. MULLOY, *A. LEE; Univ. of Minnesota, Minneapolis, MN

Abstract: Investigating the neurobiological changes produced by alcohol withdrawal provides insight into the mechanisms that contribute to alcohol use disorder and identifies targets for pharmacological treatments for alcohol withdrawal. However, the vast majority of pre-clinical data on the behavioral manifestations and mechanisms of alcohol withdrawal have been collected from male animals. The objective of this study is to determine the effect of alcohol administration and withdrawal in female mice. Adult male and female C57BL/6J mice were given daily injections of alcohol (2.5 g/kg, ip) or saline, both paired with alcohol dehydrogenase inhibitor 4-methylpyrazole (9 mg/kg, ip) for nine days. Alcohol withdrawal behaviors such as somatic withdrawal signs and the marble burying test, which measures compulsive- and anxiety-like behavior, were assessed at 24h into withdrawal. Male mice, but not female mice, showed a

statistical increase in somatic withdrawal signs and increased alcohol-withdrawal induced marble burying behavior. Instead, female mice showed significant alcohol withdrawal-induced deficits in social interaction via examining their behaviors in the social interaction test. We observed a trend for increased c-Fos in non-dopaminergic cells of the ventral tegmental area (VTA) but a decrease in c-Fos positive dopamine neurons of the VTA after 24h withdrawal in alcohol-treated female mice compared with saline-treated mice. Chronic alcohol injections did not increase c-Fos expression in mesopontine tegmentum (MPT) cholinergic neurons in female mice, whereas male mice showed an increase in c-Fos when measured with immunohistochemistry when assessed 2 hours after the last alcohol injection. A separate group of male and female C57BL/6J mice were injected acutely with 4 g/kg alcohol and a loss-of-righting-reflex test was performed to assess alcohol-induced sedation. Both male and female mice lost their righting reflex for 18-21 minutes, with no significant difference between the sexes in sedation time. Our data shows sex differences in the behavioral manifestation of alcohol withdrawal in mice, in baseline cholinergic neuron activation, and in alcohol withdrawal-induced changes in neuronal activity. These data further emphasize the need to investigate both sexes to fully understand the effects of alcohol.

Disclosures: N. Payne: None. **M. Scalf:** None. **R. Dobbelmann:** None. **S. Engel:** None. **S. Mulloy:** None. **A. Lee:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.21/P6

Topic: G.09. Drugs of Abuse and Addiction

Title: The glucocorticoid receptor antagonist miricorilant decreases alcohol self-administration in alcohol-dependent female rats

Authors: *V. P. ACOSTA[1,2,3], M. MCGINN[1,2,3], J. VENDRUSCOLO[1,3], O. GEORGE[4], B. J. MASON[5], L. F. VENDRUSCOLO[1,2];
[1]Integrative Neurosci. Res. Br., Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Stress and Addiction Neuroscience Unit, National Institute on Drug Abuse, Baltimore, MD; [3]Neurobiology of Addiction Section, National Institute on Drug Abuse, Baltimore, MD; [4]Dept. of Psychiatry, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA; [5]Scripps Res., La Jolla, CA

Abstract: Alcohol use disorder (AUD) is associated with the dysregulation of brain stress and reward systems. The nonselective glucocorticoid receptor (GR) mifepristone has previously been shown to reduce alcohol consumption in alcohol-dependent male rats and in humans with AUD. However, mifepristone’s action on progesterone receptors may have undesired effects; thus, testing the efficacy of selective GR antagonists in alcohol drinking is critical. CORT118335 (miricorilant) has shown efficacy in decreasing alcohol drinking in alcohol-dependent and

nondependent male rats and is currently under investigation for the treatment of AUD in humans. Whether CORT118335 modulates alcohol drinking in alcohol-dependent and nondependent female rats remains to be determined. Here, we tested the effects of CORT118335 on alcohol-related behaviors in alcohol-dependent and nondependent female Wistar rats. Female rats were trained to self-administer alcohol in standard operant chambers. Next, half of the cohort was made dependent on alcohol via chronic, intermittent alcohol vapor exposure, while the other half was exposed to room air only (nondependent). Alcohol-dependent rats escalated alcohol intake, whereas nondependent rats exhibited stable drinking. Intraperitoneally injected CORT118335 (2 mg/kg, 6 mg/kg, and 20 mg/kg) dose-dependently reduced alcohol self-administration, with an increased effect in dependent compared with nondependent rats. CORT118335 decreased saccharin self-administration in alcohol-dependent and nondependent female rats. Locomotion was selectively reduced in nondependent rats, but motor coordination was not affected in alcohol dependent or nondependent rats. CORT118335 selectively reduced irritability in alcohol-dependent female rats. These findings indicate the potential of CORT118335 as a novel treatment for AUD.

Disclosures: V.P. Acosta: None. **M. McGinn:** None. **J. Vendruscolo:** None. **O. George:** None. **B.J. Mason:** None. **L.F. Vendruscolo:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.22/P7

Topic: G.09. Drugs of Abuse and Addiction

Title: Inhibition of the Cortical Amygdala reduces alcohol-dependent drinking behavior in female mice

Authors: *T. XIAO[1], Y. CHEN[2], A. BOISVERT[3], X. CHENG[1], J. ZHANG[4], X. CHEN[4], Z. QUE[5], Y. YANG[4], A. J. KIMBROUGH[3];
[1]purdue Univ., West Lafayette, IN; [2]Basic Med. Sci., Purdue Univ., Lafayette, IN; [3]Basic Med. Sci., Purdue Univ., West Lafayette, IN; [4]MCMP, Purdue Univ., West Lafayette, IN; [5]Eli Lilly and Co., Cambridge, MA

Abstract: Alcohol Use Disorder (AUD) is a significant public health concern, marked by chronic and excessive drinking leading to cycles of intoxication, withdrawal, and craving. While extensive research has characterized the importance of key brain regions in AUD, the cortical amygdala (CoA) has recently been identified as a critical brain region involved in AUD. Prior data has shown that inhibiting activity in the CoA reduced alcohol consumption in alcohol-dependent male mice, but its impact on withdrawal behavior and excessive drinking in females remains unexplored. Thus, we aimed to determine the impact of inhibiting CoA signaling on alcohol dependent drinking and withdrawal behavior in alcohol-dependent female mice. Using a chemogenomic approach, we injected the CoA of mice with either pAAV8-hSyn-mCherry

(sham) or pAAV8-hSyn-hM4D(Gi)-mCherry (Gi). To establish alcohol dependence, C57BL/6J female mice underwent two-bottle choice (2BC) / chronic intermittent ethanol vapor exposure (CIE) for 6 cycles or remained Air exposed during CIE weeks for nondependent controls. During the final week of 2BC mice were given injections of saline or CNO during drinking. Mice were then given a final CIE session and then tested during withdrawal the following week for behavioral signs of withdrawal with saline or CNO injections. Behavioral tests included the open field test, von Frey test, and tail suspension test. The average alcohol intake, prior to CNO testing, during the final week of drinking was 0.97 ±0.20 g/kg in non-dependent and 2.36 ±0.25 g/kg in alcohol-dependent mice. Following CNO injection to inhibit CoA signaling, the alcohol-dependent::Gi groups exhibited alcohol consumption of 2.07 ±0.36 g/kg with saline injection and 1.08 ±0.23 g/kg with CNO injection, demonstrating a significant reduction in alcohol intake, an effect that was not shown in alcohol-dependent mice with sham virus. Intriguingly, although alcohol-dependent mice showed significant signs of withdrawal behavior, inhibition of the CoA did not influence these behaviors, suggesting that the CoA may be key for alcohol dependent drinking independent of effect on withdrawal symptoms. Brain tissue was collected during withdrawal to examine the impact of inhibiting the CoA in alcohol-dependent mice on neural network structure and function and this data is currently being processed/analyzed.

Disclosures: T. Xiao: None. **Y. Chen:** None. **A. Boisvert:** None. **X. Cheng:** None. **J. Zhang:** None. **X. Chen:** None. **Z. Que:** None. **Y. Yang:** None. **A.J. Kimbrough:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.23/P8

Topic: G.09. Drugs of Abuse and Addiction

Support: P20GM103423

Title: Wishful Drinking: Semi-naturalistic, versus standard laboratory, housing yields markedly lower rates of drinking in female Long Evans rats

Authors: J. LAGOMARSINO[1], S. VILLAGOMEZ[2], M. MACOMBER[1], *M. J. GLENN[1]; [1]Psychology, Colby Col., Waterville, ME; [2]Colby Col., Waterville, ME

Abstract: According to the most recent National Survey on Drug Use and Health (NSDUH, 2022) rates of alcohol use in the United States are substantial with nearly 80% of Americans aged 12 or older reporting drinking alcohol at some point. Though not as high, a troublesome trend is the rate of reported binge drinking in the same population - just over 20% or 61.2 million people. While the reports of alcohol use and misuse are not markedly different for men and women, the rates of drinking in women is on the rise. Further compounding that increase are findings that alcohol use in women can result in more adverse outcomes for health and well-being. These alcohol-associated problems in women appear to be exacerbated by the recent

pandemic, suggesting that social factors, such as isolation, home and/or work stress, and increased social media use, may be potent contributors. The present study examined rates of drinking in female rats living continuously under different housing and social conditions. Female Long-Evans rats were approximately 60 days of age at the start of the experiment. They were divided into three housing/social conditions: single-housed rats lived one per standard laboratory cage (n=6); pair-housed rats lived two per standard laboratory cage (n=6; 3 pairs); and semi-natural-housed rats lived six in a complex, multi-level housing systems (n=6; 1 social group). High ethanol consumption in rats was sought using an intermittent access to ethanol protocol (Simms et al. 2008). In brief, rats were given continuous access to tap water and 20% ethanol 3 times weekly over a period of 6 weeks. On other days, rats had access to 2 bottles of tap water. On drinking days, ethanol and water intake (g) was measured for the first 30 minutes and the full 24 hours. After 3 weeks on the drinking protocol, rats' responses to a noxious stimulus was evaluated using the shock-probe test. Based on previous research showing that environmental enrichment reduced self-administration of alcohol and decreased anxious behavior in rodents, we hypothesized that rats living under semi-natural conditions with a rich social group would drink less than the other two housing/social groups. Further, consistent with increases in drinking in women that were worsened by pandemic isolation and/or stress, we hypothesized that that single housed rats would drink the most. The findings supported our hypotheses and we are poised to integrate them with the shock-probe test results and other neural and behavioral indices of sociality and activity. These results will help us better understand how and to what extent contextual factors, specifically social ones, contribute to motivated behaviors.

Disclosures: J. Lagomarsino: None. **S. Villagomez:** None. **M. Macomber:** None. **M.J. Glenn:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

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Program #/Poster #: PSTR243.24/P9

Topic: G.09. Drugs of Abuse and Addiction

Support: K99AA029168
R00AA029168
P50AA022538

Title: Withdrawal from chronic alcohol increases CO_2 inhalation sensitivity in mice and rats: a novel model relevant to panic and PTSD comorbidity following AUD

Authors: *K. M. J. MCMURRAY[1], H. KARTHIKEYAN[2], M. ALLRED[1], H. KUSUMO[1], S. C. PANDEY[3];
[1]Psychiatry, Univ. of Illinois Chicago, Chicago, IL; [2]Univ. of Illinois Chicago, Chicago, IL; [3]Alcohol Res. Ctr., Dept Psychiatry, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Alcohol use disorder (AUD) increases risk to develop comorbid psychiatric disorders like panic disorder and PTSD. AUD treatments are limited and comorbid panic or PTSD results in worse treatment outcomes, pointing to a need for novel treatments. AUD, panic and PTSD have overlapping genetics and symptomology suggesting they share underlying molecular mechanisms and neurocircuitry. Emerging evidence supports dysregulated acid-base homeostasis as a shared mechanism in AUD, panic and PTSD. Maintaining physiological homeostasis (e.g. neutral pH) is critical for survival, and threats to homeostasis elicit behavioral, emotional and physiological responses directed toward this goal. Alcohol use induces acidosis which positively correlates with withdrawal severity. Strong evidence supports dysregulated acid-sensing in panic, but its role in PTSD and AUD is not well understood. Homeostatic stressor CO_2 inhalation (non-hypoxic) produces acid-base imbalance and elicits greater fear/anxiety, hyperventilation, and cardiovascular effects (even panic attacks) in panic disorder and PTSD patients, in previously alcohol-dependent individuals, and during alcohol withdrawal. Thus, dysregulated acid-sensing mechanisms likely contribute to AUD, panic and PTSD pathophysiology and may facilitate the increased risk to develop panic or PTSD in previously dependent individuals. Investigating the effect of alcohol use on CO_2 sensitivity may yield unique and impactful findings, increase our understanding of comorbidity and help prevent or treat these disorders. Therefore, we recently developed a model to investigate the role of chronic alcohol use and withdrawal on CO_2 sensitivity in rats and mice. Adult Sprague-Dawley male rats received control or ethanol (9% v/v) Lieber-DeCarli liquid diets for 9 days while adult C57/Bl6J male mice received control (4-methylpyrazol (4-MP) 9mg/kg) or ethanol (2g/kg) + 4-MP injections for 9 days. Following chronic ethanol, both species were withdrawn for 24h then exposed to 10% (rats) or 5% (mice) CO_2 inhalation for 10m. We found withdrawal from chronic ethanol exposure increased CO_2-evoked defensive behaviors. Together, these data suggest chronic ethanol use and withdrawal increases panic and PTSD-relevant behaviors in mice and rats. Overall, these data support the use of this highly translational chronic alcohol use/withdrawal and CO_2 inhalation model to improve our understanding of shared mechanisms underlying comorbid AUD, panic and PTSD.

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Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant 5R01DA047994
NIH grant 2P60AA007611-36

Title: Virtual alcohol drinking agent demonstrates patterns of alcohol use disorder

Authors: *G. BOBASHEV[1], A. S. KUZNETSOV[2];
[1]RTI Intl., Research Triangle Park, NC; [2]Mathematical Sci. and Ctr. for Mathematical Biosci., Indiana Univ. Indianapolis, Indianapolis, IN

Abstract: Few attempts have been made to simulate the complex natural history of alcohol use disorders. We developed a model to simulate an agent's alcohol use over time intervals from days to months in order to replicate a gradual transition among disease states. We followed the principles of control theory and opponent process theory, formalizing representations of several processes (toxicity, tolerance, effect, craving) as weighted integrals of alcohol concentration, which was modeled with a pharmacokinetic equation. We calibrated our model to reproduce several qualitative alcohol use trajectories commonly observed in the literature such as weekend drinker, daily use, occasional/social drinker. The model shows transitions among these patterns as escalation occurs with accumulating history of alcohol use. We demonstrate how a relatively simple control theory approach can reproduce many of the key characteristics of real-world alcohol use.

Disclosures: G. Bobashev: None. **A.S. Kuznetsov:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Program #/Poster #: PSTR243.26/P11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-PREP-#5R25GM075149-18

Title: Investigating Anticipation for Alcohol Elicited by Cues Predicting its Availability and Associated Neural Circuitry in Mice.

Authors: *S. DAVID[1], D. FURLANO[1], M. OROZCO[2], M. MORNINGSTAR[1], D. N. LINSENBARDT[2];
[2]Neurosciences, [1]Univ. of New Mexico, Albuquerque, NM

Abstract: Excessive alcohol consumption leads to $249 billion in economic burden and a mortality rate of140,000 annually, partially driven by intense cravings triggered by alcohol paired cues. Asignificant gap exists in our understanding of the neurological underpinnings of alcohol-cuelearning and associated behaviors, which if filled may produce avenues for the development ofnovel therapeutic targets to mitigate cue-induced cravings. In the present study, 48 C57BL/6Jmice (split evenly by sex) were provided access to either alcohol (EtOH) or water for 2 hoursdaily for 19 days using the drinking in the dark paradigm and specialized sippers that providetemporally accurate drinking volumes. An additional control group received no specializedsippers (Naïve). Immediately prior to the experimenter entering the room and providing fluidaccess, 1 minute of discrete auditory clicks were presented in the testing room using a remotecomputer interface. Home cage behavior was continuously monitored using

overhead IRcameras. We were surprised to observe progressive increases in home cage activity during cuepresentation in all groups. These increases reached statistical significance in both male andfemale alcohol consuming groups by the final session (male mean=26 SEM=4.7; femalemean=46.15, SEM=5.5), in addition to water drinking males (mean= 26.9, SEM=3.5) and naïvefemales (mean=32.72, SEM=4.48). Time spent oriented toward sipper and distance from thesipper during the cue period were not predictive of alcohol or water consumption, but females inall groups spent significantly less time oriented toward the sipper than chance during cuepresentation. Additionally, a separate cohort of mice received virus injections to retrogradelylabel and map neurons projecting to the nucleus accumbens - neurons known to respond toreward-predictive cues. Virus expression was quantified using an unbiased QUINT workflow toevaluate 1300 brain regions and revealed many dense projection origins that displayed uniquecortical layer specificity. Of note, 13.07% (SEM=2.1) of the amygdala, 24.08% (SEM=6.04) ofthe infralimbic cortex, and 10.73% (SEM=2.7) of the prelimbic cortex were found to project tothe nucleus accumbens. In conclusion, mice displayed behavioral evidence of sensitization tocues regardless of subsequent reward, but this effect was most robust in mice receiving EtOHand for water and naïve groups was sex specific. Additionally, we quantified the density andspecificity of circuits thought to regulate these cue-associated behavioral responses, setting thestage for testing their causal role in excessive alcohol consumption.

Disclosures: S. David: None. **D. Furlano:** None. **M. Orozco:** None. **M. Morningstar:** None. **D.N. Linsenbardt:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.27/P12

Topic: G.09. Drugs of Abuse and Addiction

Title: Identification of neurological mechanisms for impulsivity in voluntary and involuntary administration models of alcohol addiction

Authors: *S. JUNG[1,2], H.-I. IM[3,2];
[1]Korea Inst. of Sci. and Technology(KIST), Seoul, Korea, Republic of; [2]Div. of Bio-Medical Sci. &Technology, KIST Sch., Univ. of Sci. and Technol. (UST), Seoul, Korea, Republic of; [3]Ctr. For Neurosci., Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of

Abstract: According to the World Health Organization (WHO), alcohol use disorder (AUD) is considered the second most common mental illness, accounting for 5-6% of deaths worldwide. Among the many causes of alcohol consumption, 'impulsivity' is considered a factor in the inability to control excessive drinking, and such drinking can also cause impulsive behavior. The orbitofrontal cortex (OFC) is known to be a key region regulating excessive alcohol seeking. γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in synaptic transmission, and its role of the regulation of impulsivity in the OFC is not clearly defined. Here, we investigated

the effect of changes in GABA-related signaling in the OFC on impulsivity control by repeated alcohol exposure. We established two types of alcohol addiction models: intermittent two-bottle choice of alcohol (IA2BC) and intraperitoneal injection (I.P injection). For the IA2BC procedure, mice (CJ57/B6J) were individually housed and exposed to two bottles containing 20% ethanol or water for 24 h on an intermittent schedule for 6 weeks. Drinking time started at 11 a.m. and ended in 24 hours. For the I.P injection procedure, mice (CJ57/B6J) were individually housed and injected with 2 g/kg 20% ethanol in saline or saline twice daily, 6 hours apart, for 2 weeks. Injection times were 11 a.m. and 5 p.m., respectively. We performed the Cliff Avoidance Task (CAT), which assesses maladaptive impulsive rodent behavior, to determine whether alcohol induces impulsive behavior. CAT was measured 3 days and 2 weeks after alcohol exposure ended. In the IA2BC model, the alcohol group (ALC) showed a trend toward increased jump number, but not first jump latency, after 2 weeks of alcohol exposure. In the I.P model, alcohol exposure increased the number of jumps and shortened the latency to the first jump. We confirmed that alcohol exposure increased OFC's GABA-related mRNAs, gad1 (glutamic acid decarboxylase), gabra2 (alpha subunit of GABA A receptor), and gat3 (subtype of GABA transporters) in the IA2BC model. In the future, we will determine whether alcohol-induced impulsive behavior is alleviated by regulating GABAergic signaling in the OFC by muscimol (GABA agonist). In addition, we plan to check whether these results are the same in I.P injection model.

Disclosures: S. Jung: None. **H. Im:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.28/Q1

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA031028
R01AA027766

Title: Sexually dimorphic responses of parvalbumin^{+}interneurons (PV-INs) to alcohol exposure and withdrawal in the dorso-ventral axis of the hippocampus

Authors: *S. DAS, S. BASU, H. SUH;
Neurosciences, Cleveland Clin. Fndn., Cleveland, OH

Abstract: With alcoholism being one of the most debilitating addictions in the United States, efforts to characterize its cyclic nature between dependence and withdrawal have revealed a myriad of areas to study the neurobiology of addiction. One area is alcohol withdrawal syndromes, in which efforts to abstain after continuous consumption can result in severely negative symptoms. A striking example of this is alcohol withdrawal-associated seizures (AWS), where an individual withdrawing from chronic alcohol consumption can start to experience

convulsions, due to a sudden deprivation of alcohol's inhibiting effects and dysregulation of the excitation-inhibition (E/I) balance in the brain. We previously demonstrated that chronic alcohol exposure disrupted the structural development of hippocampal newborn neurons. Furthermore, specific activation and inhibition of hippocampal newborn neurons by chemogenetic manipulations increased and decreased the expression of AWS, respectively. These observations led to the hypothesis that alcohol exposure and withdrawal may induce the aberrant integration of hippocampal newborn neurons and the formation of pathological neural circuits, and as a result, may be responsible for the altered E/I balance leading to AWS. In the current study, we aim to determine the connectivity of hippocampal newborn neurons with parvalbumin-expressing inhibitory neurons (PV-INs), an abundant and potent subtype of GABAergic neurons in the hippocampus. We fed 9-week-old C57BL/6 mice with a liquid diet containing 5% alcohol or calorie-matched diet for 4 weeks and investigated the number and morphology of PV-INs at 3 weeks during alcohol exposure (AE), as well as 24 hours and 4 weeks after alcohol withdrawal (AW). At 24 hours after AW, the number of PV-INs are not significantly different in the hippocampus between control and the AW mice. However, after separating the count by sex and dorso-ventral axis, a sexual dimorphic pattern emerges—AW causes a marked decrease in PV-INs in the male ventral hippocampus, but an increase in PV-INs in the female ventral hippocampus. In addition, we observed the potential differential expressions of the perineuronal net (PNN) in a sex- and position-dependent manner. We will present the sexual dimorphic and positional effects of alcohol on the number and structure of PV-INs during AE and AW, providing an insight into the role of PV-INs in the sexually dimorphic effects of alcohol.

Disclosures: S. Das: None. **S. Basu:** None. **H. Suh:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.29/Q2

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA031028
R01AA027766

Title: Alcohol-induced Aberrant Neural Circuitry Integration of Hippocampal Newborn Neurons

Authors: *S. BASU, S. DAS, H. SUH;
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Abstract: Chronic alcohol exposure (AE) causes marked neuroadaptation in the brain and a consequence of this is manifested as alcohol withdrawal (AW). Generalized tonic-clonic seizures and cognitive decline represent the most severe and prevalent conditions among AW-associated syndromes. However, the precise neural substrates that undergo neuroadaptation during AE and are responsible for AW syndromes are yet to be understood. Hippocampal dentate granule cells

are principal excitatory neurons that are continuously generated and integrated into neural circuits. While this process of neurogenesis provides plasticity to the brain, disrupted neurogenesis has been implicated in addiction, deficits in cognition, and emotional stability. Moreover, hippocampal newborn neurons play a critical role in the maintenance of excitability of the hippocampus. We previously showed that a 4-week chronic AE in mice led to an expression of seizures during a protracted period of AW. AW globally increased the dendritic spine densities in hippocampal newborn neurons, indicating increased neuronal connectivity with excitatory neurons. Functional studies with chemogenetics revealed that hippocampal newborn neurons that mature during chronic AE are necessary and sufficient for the expression of alcohol withdrawal-associated seizures (AWS) in mice. These results suggest that AE induced aberrant integration of hippocampal newborn neurons and the consequences of their abnormal circuit formations are manifested in the seizure expression during AW. Using a rabies virus, we mapped altered neuronal connectivity of the hippocampal newborn neurons in alcohol fed C57BL/6J mice compared to a calorie matched control group. We found a significant increase in the excitatory inputs from the entorhinal cortex, medial septum, CA3, CA1, and dentate gyrus to the hippocampal newborn neurons after 1 week of AW. These results collectively suggest that chronic AE altered the morphology and connectivity of hippocampal newborn neurons, which contributes to the expression of seizures during AW. Moreover, we identified a significant increase in the connectivity of hippocampal newborn neurons with astrocytes at 1 week into AW, suggesting a potential involvement of astrocytes in the control of excitability of hippocampal newborn neurons. Our results shed light on hippocampal newborn neuron as a neural substrate in regulating the excitation-inhibition balance of the brain during AE and AW and provide hippocampal neurogenesis as a potential therapeutic target to treat alcohol addiction.

Disclosures: S. Basu: None. **S. Das:** None. **H. Suh:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR243.30/Q3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA026577 – JPS
NIH Grant AA027516 – DRS

Title: Sex-dependent effects of environmental enrichment on solitary binge ethanol consumption in adult c57bl/6 mice

Authors: *M. VALCHINOVA[1], J. P. SEVIGNY[2], S. SCHRANK[3], N. YUNUS[4], K. VETTER[4], V. ILY[4], O. D. AGUILAR[4], D. R. SPARTA[4];
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Abstract: SEX-DEPENDENT EFFECTS OF ENVIRONMENTAL ENRICHMENT ON SOLITARY BINGE ETHANOL CONSUMPTION IN ADULT C57BL/6 MICE
M. Valchinova[1], J. P. Sevigny[1,2], S. Schrank[1], N. I. Yunus[1], K. R. Vetter[1], V. Ily[1], O. D. Aguilar[1], D. R. Sparta[1,21]Department of Psychology, University of Illinois Chicago, Chicago, IL 60607, USA[2]Graduate Program in Neuroscience, University of Illinois Chicago, Chicago IL 60607, USA
Alcohol Use Disorders (AUD) is one of the most ubiquitous substances of abuse, globally contributing to 5.3% of deaths worldwide. Recent studies show alcohol consumption has increased as a result of social isolation and stress from COVID pandemic regulations. Previous research, demonstrate environmental enrichment (EE) transiently modulates ethanol consumption and anxiety-like behaviors in adult mice. We hypothesized that prior enrichment conditions may modulate future isolated binge ethanol consumption, anxiety-like behavior and novelty-seeking behavior. To examine the effect of an enriched environment we housed adult mice (>P60) in either enriched (EE) or impoverished (IE) conditions for twelve days. Post-treatment, animals were isolated and given access to ethanol using the drinking in the dark (DID) paradigm, for a period of three weeks. Animals were behaviorally assessed periodically using the elevated zero maze, two bottle choice anhedonia, and a novel-object open field behavioral paradigm. We found no difference in level of ethanol consumption nor anhedonia response between enriched and impoverished mice. Both male and female impoverished mice exhibited increased anxiety-like behavior in the open-field paradigm. Enriched male mice exhibited increased neophilia compared to any other group. In summary, we found that enrichment does not modulate isolated binge ethanol consumption in mice but does modulate anxiety-like behavior and novelty seeking behavior in a sex-dependent manor. Further research will explore anxiety and ethanol consumption effects of social defeat in the context of environmental enrichment and impoverishment.

Disclosures: M. Valchinova: None. **J.P. Sevigny:** None. **S. Schrank:** None. **N. Yunus:** None. **K. Vetter:** None. **V. Ily:** None. **O.D. Aguilar:** None. **D.R. Sparta:** None.

Poster

PSTR243: Alcohol: Drinking and Behavioral Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027516 – DRS
NIH Grant AA026577 – JPS

Title: Diverging effects of environmental enrichment on binge ethanol consumption in male and female adolescent c57bl/6 mice

Authors: *J. SEVIGNY[1], S. SCHRANK[2], N. YUNUS[2], K. VETTER[2], M. VALCHINOVA[2], V. ILY[2], O. D. AGUILAR[2], D. R. SPARTA[2];
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Abstract: Alcohol use disorder (AUD) is a widespread problem in the United States affecting 12% of adults and 3% of adolescents. Drinking in solitude as a teen is highly predictive of future AUD. Solitary alcohol consumption spiked during the COVID-19 pandemic demonstrating how environmental conditions modulate consumption behavior. In mammalian models, environmental enrichment has been shown to impact stress and anxiety-like behaviors. For example, enrichment transiently modulates alcohol consumption and anxiety-like behavior in adolescent mice. It is unknown as to the long-lasting developmental effects of environmental enrichment on solitary drinking behavior. Our investigation shows that environmental enrichment in early adolescent mice increases ethanol consumption in males but not females. Paradoxically we see that enrichment decreases anxiety-like behavior in a zero-maze task in male but not female mice. To probe the physiological basis of these changes we used single cell patch clamp electrophysiology to record excitability measurements in corticotropin-releasing factor (CRF) labeled neurons within the ventral bed nucleus of the stria terminalis (BNST) and the central amygdala (CeA). We found that adolescent enrichment may suppress the excitability of CRF neurons in the both the CeA and the vBNST by depolarizing resting membrane potential. In summary, our results indicate that environmental enrichment increases solitary binge-ethanol consumption and decreases anxiety-like behavior in adolescent male mice due in part to suppressed excitability of CRF neurons in the vBNST and the CeA.

Disclosures: J. Sevigny: None. **S. Schrank:** None. **N. Yunus:** None. **K. Vetter:** None. **M. Valchinova:** None. **V. Ily:** None. **O.D. Aguilar:** None. **D.R. Sparta:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: National Plan on Drug abuse, Ministerio de Sanidad of Spain (PNSD2019I015 and PNSD2023I018)
ISCIII Redes de Investigación Cooperativa Orientadas a Resultados en Salud (RICORS), Red de Investigación en Atención Primaria de Adicciones (RIAPAd; grant RD21/0009/0013)

Title: Pleiotrophin and phosphacan regulate ethanol consumption and ethanol effects on hippocampal neurogenesis and perineuronal nets in a sex-dependent manner

Authors: M. GALÁN-LLARIO[1], M. RODRÍGUEZ-ZAPATA[2], T. FONTÁN-BASELGA[3], E. GRAMAGE[4], A. GARCÍA GUERRA[5], ***G. HERRADON**[6];

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Abstract: Pleiotrophin (PTN) is a cytokine that modulates ethanol reward. PTN is an endogenous inhibitor of Phosphacan (a.k.a. RPTPβ/ζ). We hypothesize that PTN and Phosphacan also play important roles in chronic ethanol consumption and in the neural alterations associated with ethanol during adolescence. Male and female adolescent mice with transgenic PTN overexpression in the brain (PTN-Tg) and their wild type counterparts were used in an intermittent access to ethanol (IAE) model. Before each drinking session, wild type mice received an administration of MY10 (60 mg/kg, i.g.), a small-molecule inhibitor of Phosphacan, or vehicle as control. Male mice treated with MY10 drank less ethanol than controls. In contrast, MY10 did not seem to have relevant effects on ethanol intake in female mice. Interestingly, PTN overexpressing male and female mice drank less ethanol than controls. IAE induced a significant decline of doublecortin (DCX)+ cells in the dentate gyrus (DG) of male wild type mice, but not in females. Treatment with MY10 fully prevented ethanol-induced loss of neural progenitors in the male DG. Surprisingly, chronic ethanol induced a greater decline of DCX+ cells in the DG of PTN-Tg male and female mice compared to wild type mice. IAE induced a decrease in the intensity of PNNs in different cortical and hippocampal areas of male and female mice. We found a significant correlation between ethanol consumption and ethanol-induced alterations in the intensity of PNNs in the DG. Treatment with MY10 prevented this correlation in the DG of males and even reverted it in CA1 from both sexes, suggesting a connection between the capacity of MY10 to modulate the intensity of PNNs in the hippocampus and its ability to reduce ethanol consumption. In the insular cortex, we found that inhibition of Phosphacan with MY10 potentiated the ethanol-induced increase of Parvalbumin (PV)+ cells in male mice, whereas in females did the opposite. The data demonstrate that PTN and Phosphacan differentially modulate ethanol consumption and ethanol effects on hippocampal neurogenesis in male and female mice, which may be related to a sex-dependent regulation of ethanol-induced changes in the intensity of PNNs and number of PV+ cells in the hippocampus and the insular cortex.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR244.02/Q6

Topic: G.09. Drugs of Abuse and Addiction

Support: CONACyT 252348
INPRF 18101

Title: Acute alcohol administration induces endoplasmic reticulum stress and caspase-12 activation in the rat brain

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Abstract: Alcohol is the most commonly abused drug worldwide, and its excessive consumption is associated with numerous health, social, and economic consequences, including the development of alcohol use disorders (AUD). Many people with AUD exhibit a heavy drinking pattern known as binge drinking, which is the consumption of at least five drinks for men and four drinks for women, reaching a blood alcohol concentration (BAC) of 0.08 mg/dL or more within about two hours. However, many studies in humans have documented even more extreme binge drinking, leading to blood alcohol concentrations of around 400 mg/dL. Excessive alcohol consumption has several harmful effects on the brain. There is evidence that endoplasmic reticulum (ER) stress (accumulation of misfolded proteins) is involved in the neuronal damage caused by alcohol. Therefore, we aimed to investigate whether acute administration of higher doses of alcohol as an acute binge-drinking model triggers ER stress and activates an apoptosis mechanism in the hippocampus of adult rats. Male Wistar rats were treated intraperitoneally with a single acute dose of ethanol (3.0 g/kg) or water and the expression of proteins and the activity of some proteins involved in ER stress and the apoptosis mechanism were determined by Western blot and immunohistochemistry. Acute administration of higher doses of ethanol leads to an increase in the expression of ER stress marker proteins, Grp78 and CHOP, as well as proteins involved in the unfolded protein response (UPR): PERK, IRE1, ATF4, and IRE1- and eIF2α-phosphorylated proteins; some proteins maintain their expression longer or increase 12 or 24 hours after the start of ethanol treatment. Furthermore, acute treatment with higher doses of ethanol increases the enzymatic activity and protein expression of caspase-12 and the anti-apoptotic protein Bcl-2. Acute ethanol exposure induces ER stress in adult rat hippocampal neuronal cells by activating the UPR signaling pathway, which triggers activation of the caspase-12-dependent apoptosis mechanism and cell death. These findings are among the few studies that have documented the effects of extremely heavy alcohol consumption on the central nervous system and provide new insights into the acute effects of alcohol on the brain.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA026642

Title: Sex-dependent modulation of PKA subunit expression in multiple brain regions following withdrawal from chronic ethanol

Authors: R. MOOREHEAD[1], J. HARRIS[1], B. HUGHES[3], N. BELCHER[1], A. M. MALDONADO-DEVINCCI[2], ***R. CANNADY**[1];
[1]Dept. of Biol., [2]Dept. of Psychology, North Carolina A&T State Univ., Greensboro, NC;
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Abstract: Alcohol dependence and withdrawal are associated with neuroadaptations that are thought to underlie continued misuse and relapse episodes in individuals with alcohol use disorder (AUD). However, the cell and molecular pathways contributing to heavy alcohol (ethanol) use and withdrawal are not fully understood. Protein Kinase A (PKA) mediates behavioral effects and neuronal function following acute ethanol and sub-chronic exposure, but limited studies have examined PKA expression patterns during withdrawal periods in preclinical models of heavy use. Furthermore, evidence indicates that sex may influence drinking patterns and perception of withdrawal symptoms; prompting the need to examine sex-dependent effects on PKA expression during withdrawal episodes. The current study examined the effects of chronic intermittent ethanol exposure (CIE) on PKA catalytic subunit expression in various brain regions of Long Evans (LE) rats. Brains were extracted following 10 days of CIE with an additional 72 hours of withdrawal and then processed for western blot analysis. After CIE and 72 hours of withdrawal, PKA catalytic subunit expression was increased in the prelimbic prefrontal cortex of male, but not female, LE rats relative to air-exposed controls. Infralimbic prefrontal cortex PKA expression was increased irrespective of sex. Interestingly, there was a bidirectional effect on PKA catalytic subunit expression within the CA3 subregion of the hippocampus between males and females. These preliminary findings suggest that withdrawal from chronic ethanol exposure regulates PKA catalytic subunit expression in a sex-dependent manner. Future studies examining other PKA subunits, and behavioral, and neuronal functions will further uncover the role of PKA in mediating the effects of ethanol withdrawal and will seek to provide evidence of which PKA substrates are modified by ethanol dependence.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: USA Pennsylvania State Health Department grant Project 10: 420491-04400-02 to Dr. Nune Darbinian
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National Institute On Alcohol Abuse And Alcoholism (NIAAA) of NIH grant R01AA031319 to Dr. Michael Selzer
Gates Foundation grant OPP1119489 to Dr. Laura Goetzl

Title: Prenatal Opioid and Alcohol Exposures: Association with Altered Placental Serotonin Transporter Structure and/or Expression

Authors: *N. DARBINIAN[1,2], N. MERABOVA[3,2], G. TATEVOSIAN[1], S. ADELE[4], A. DARBINYAN[5], M. F. MORRISON[6], C. L. DEVANE[7], S. RAMAMOORTHY[8], L. GOETZL[9], M. E. SELZER[10,2];
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Abstract: Introduction. Fetal exposures to many drugs of abuse, e.g., opioids and alcohol (EtOH), are associated with adverse neurodevelopmental problems in early childhood, including abnormalities in activity of the serotonin (5-HT) transporter (SERT), which transports 5-HT across the placenta. Little is known about the effects of these drugs on SERT expression. **Methods.** Pregnant women who used EtOH or opioids were compared to gestational age-matched controls using a structured questionnaire to determine prenatal substance exposure. Following elective pregnancy termination, placental membrane fractions and exosomes were prepared from 1st and 2nd trimester human placentas. Changes in EtOH- or opioid-exposed placental SERT expression and modifications were assessed by quantitative western blot. Novel SERT isoforms were sequenced and analyzed. **Results.** Opioid-exposed, but not EtOH-exposed maternal placentas showed SERT cleavage and formation of new SERT (fragments) isoforms. Alcohol-exposed cases showed reduced SERT levels. Antibodies to the N-terminal SERT region did not recognize either of the two cleavage products, while antibodies to the central and C-terminal regions recognized both bands. The secondary band seen in the opioid group may represent a hypo-phosphorylated SERT fragment. **Conclusions.** These changes in SERT modifications and expression may result in altered fetal brain serotonergic neurotransmission, which could have neurodevelopmental implications.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5P20GM103642
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Title: Circuit specific ethanol-induced transcriptional adaptations in Drosophila melanogaster

Authors: *D. FIGUEROA VARGAS[1], C. DEL VALLE-COLÓN[1], A. MONTES-MERCADO[1], S. I. MORALES-CANCIO[1], A. SEEDS[2], A. GHEZZI[1];
[1]Biol., Univ. of Puerto Rico, Río Piedras, Puerto Rico; [2]Inst. of Neurobio., Univ. of Puerto Rico, Med. Sci. Campus, San Juan, Puerto Rico

Abstract: Alcohol Use Disorder (AUD) disrupts neurological, physiological, and behavioral processes, posing significant challenges to both young adults and adults alike. This disruption often leads to the development of adaptations in the brain, resulting in alcohol tolerance and physiological dependence. Such adaptations, particularly within neural circuits regulating sleep and wake cycles, intensify the complexities of alcoholism recovery. To understand the behaviors affected by AUD at a genetic level, it is crucial to investigate how the dysregulation triggered by alcohol consumption influences the gene expression profiles within neuronal populations such as the ventrolateral neurons (LNv) in the *Drosophila* model. LNv neurons, which express the neuropeptide pigment dispersing factor (PDF), regulate behaviors such as circadian rhythms and sleep/wake cycles and have recently been involved in the development of alcohol tolerance in flies. PDF synchronizes the circadian clock and regulates locomotor activity. This study focuses on the role of LNv in alcohol-induced disorders. Using the INTACT method, we extract nuclear RNA from specific Drosophila cell types in this case, LNv. Nuclei from LNv neurons were epitope tagged with a GFP targeting the nuclear membrane and then purified by immunoprecipitation using an anti-GFP antibody. Twenty-four hours after exposure to alcohol, the mRNA was extracted from the isolated nuclei and submitted for RNA-sequencing. Whole genome differential expression analysis reveals that ethanol exposure induces significant changes in the expression of key regulators of neuronal activity within LNv. We found 253 genes differentially expressed genes between control and alcohol exposed flies. These genes were highly and enriched for the biological process mitochondrial translation. Through this approach, we identified new candidate genes involved in alcohol-induced neuroadaptation, providing insights into the molecular mechanisms underlying alcohol-related behavioral changes. This

study not only enhances our understanding of alcohol-induced neuroadaptations but also offers potential targeted interventions in alcohol use disorders.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR244.06/R4

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in alcohol consumption and differential expression of mu opioid receptors after naltrexone treatment

Authors: *I. ZAPEDOWSKA[1], L. MOLINA-MARTÍNEZ[1,2], J. JUAREZ[1];
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Abstract: Naltrexone is an opioid antagonist, used in clinic for alcohol use disorder. However, experimental studies have provided evidence of an increase in alcohol consumption after naltrexone treatment is interrupted. There is evidence that this phenomenon is due to an up-regulation of mu opioid receptors as a compensatory effect of the opioid antagonist treatment. As a consequence, the rewarding effect of alcohol is heightened. Furthermore, sex differences in alcohol consumption indicate that female rats drink more alcohol compared to males and there are also sex differences in mu receptor expression in areas related to motivation and reward. Nevertheless, it remains unclear, if there are sex differences in the mu opioid receptor expression after naltrexone treatment. On this basis, 16 male and 16 female *Wistar* rats were exposed to 10% alcohol consumption for 8 days after an alcohol induction period. Afterward, rats were treated with naltrexone (1 mg/kg) or vehicle (saline solution) intragastrically twice a day for 6 days. Subsequently rats were sacrificed to analyze mu opioid receptor density for the ventral tegmental area, nucleus accumbens and ventral pallidum, using the western blot method. In the ventral tegmental area, sex differences were observed in the vehicle groups, males had significantly higher receptor density compared to females. In nucleus accumbens we didn´t find any differences between sex or treatment. However, in ventral pallidum, we observed that there was a down-regulation of mu opioid receptor in females treated with naltrexone compared with males. These results suggest intrinsic sex differences in the expression of the mu opioid receptors in some reward areas, and a differential expression of these receptors after naltrexone treatment that could be related to sexual differences in alcohol consumption.

Disclosures: I. Zapedowska: None. **L. Molina-Martínez:** None. **J. Juarez:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

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Program #/Poster #: PSTR244.07/R5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA AA028549
NIAAA AA026999
NIAAA 006420
NIAAA T32 AA007456

Title: Ly2444296, a k-opioid receptor antagonist, selectively reduces alcohol drinking in male and female wistar rats with a history of alcohol dependence.

Authors: *F. FLORES RAMIREZ[1], J. M. ILLENBERGER[2], R. MARTIN-FARDON[1];
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Abstract: Alcohol use disorder (AUD) remains a major public health concern. The dynorphin (DYN)/κ-opioid receptor (KOP) system is involved in actions of alcohol, particularly its withdrawal-associated negative affective states. This study tested the ability of LY2444296, a selective, short-acting, KOP antagonist, to decrease alcohol self-administration in dependent male and female Wistar rats at 8 h abstinence. Animals were trained to orally self-administer 10% alcohol (30 min/day for 21 sessions) and were made dependent via chronic intermittent alcohol vapor exposure for 6 weeks or exposed to air (nondependent). After 6 weeks, the effect of LY2444296 (0, 3, and 10 mg/kg, p.o.) was tested on alcohol self-administration at 8 h of abstinence. A separate cohort of rats was prepared in parallel, and their somatic withdrawal signs and alcohol self-administration were measured after LY2444296 administration at 8 h, 2 weeks, and 4 weeks abstinence. LY2444296 at 3 and 10 mg/kg significantly reduced physical signs of withdrawal in dependent rats at 8 h abstinence, only. Furthermore, 3 and 10 mg/kg selectively decreased alcohol self-administration in dependent rats at only 8 h abstinence. These results highlight the DYN/KOP system in actions of alcohol during acute abstinence, suggesting KOP antagonism could be beneficial for mitigating acute withdrawal signs and, in turn, significantly reduce excessive alcohol consumption associated with AUD.

Disclosures: F. Flores Ramirez: None. **J.M. Illenberger:** None. **R. Martin-Fardon:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

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5R25GM061838-22

Title: Ethanol-induced activation of Wnt/B-catenin signaling is miR-9 dependent

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Abstract: Ethanol-induced activation of Wnt/β-catenin signaling is MiR-9 dependent Yaren L. Peña[2], *Cristina Velázquez-Marrero[1]
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MicroRNAs (miRNAs) play a crucial role in addiction by regulating gene expression in various brain regions involved in reward processing, learning, and memory. Studies have shown that alcohol exposure increases the expression of miR-9. In separate studies alcohol also leads to activation of the Wnt/β-catenin signaling pathway. Activation of this pathway is associated with neuroadaptations that contribute to alcohol tolerance and dependence. Our current study proposes a causal relationship between elevation of miR-9, in response to ethanol (EtOH), and activation of the Wnt/β-catenin signaling pathway. To investigate, we employ Rt-qPCR, immunocytochemistry, and mRNA-Seq analysis in both a heterologous expression system and within murine nucleus accumbens (NAc). Our results delineate the temporal changes in both miR-9-3p and miR-9-5p expression levels in response to EtOH and LiCl linked to significant alterations in the activation of the Wnt/ß-catenin pathway. This further shows activation solely in response to miR-9 treatment. Moreover, we find that the activation of Wnt/ß-catenin by EtOH is hindered in the presence of a miR-9 inhibitor, supporting our hypothesis that miR-9 is essential for EtOH-induced activation of the Wnt/ß-catenin pathway and thus plays a role in both components of alcohol molecular tolerance. Thus, miR-9 plays a multifaceted role in alcohol long-term adaptations, influencing various aspects of molecular and functional responses to alcohol exposure. Further research into the specific mechanisms underlying miR-9 dysregulation in alcohol addiction will lead to the development of novel therapeutic interventions for this complex disorder.
Conflict of Interest: There is no conflict of interest for any co-authors. **Acknowledgements:** This research project was funded by the U-RISE Program of the University of Puerto Rico in Cayey (NIH Grant: 1T34GM145404-01A1). It was mentored by Dr. Cristina Velázquez-Marrero, Associate Professor at the University of Puerto Rico in the Medical Sciences Campus and funded by NIH project grant 5R01AA027808-02. The experiments were conducted using the RCMI Research Education Core at the University of Puerto Rico in the Medical Sciences Campus, funded by NIH grant 5R25GM061838-22.

Disclosures: Y.L. Peña: None. **C. Velazquez-Marrero:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR244.09/S1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027660

Title: Activating calcium-activated chloride channels decreases alcohol consumption in rats.

Authors: G. R. CARDOSO[1], ***P. N'GOUEMO**[2];
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Abstract: Excessive and uncontrolled alcohol consumption can lead to the development of an alcohol use disorder. Ca^{2+} influx plays a role in regulating neuronal excitability and behavioral responses toward alcohol. However, the role of Ca^{2+} signaling in alcohol use disorder is not yet fully understood. The role of L-type Ca^{2+} channels in alcohol consumption is complex, with both activation and inhibition of these channels reducing alcohol intake. Recently, we reported that inhibiting the reverse mode of the sodium-calcium exchanger decreased alcohol intake in both male and female rats. The present study investigated the potential role of Ca^{2+}-activated chloride channels (CaCCs) in voluntary alcohol consumption in adult male and female Sprague-Dawley rats. We used EACT and T16Ainh-A01 to activate or inhibit transmembrane protein 16A (TMEM16A) CaCC, respectively. Over four weeks, animals were first trained to drink ethanol (7.5% vol/vol in water) using the two-bottle choice paradigm, one with ethanol and the other with water. Animals were then randomly divided into eight groups (n=8), each consisting of male or female rats treated with either EACT (2.5, 5, or 10 mg/kg, p.o.) or T16Ainh-A01 (5 or 10 mg/kg, p.o.). We measured ethanol intake, ethanol preference, water intake, and total fluid intake per kilogram of body weight after 2 or 24 hours of access. We also monitored the estrous cycle to find that all females were in the estrus cycle. The results showed that activating TMEM16A channels reduced alcohol consumption in both male and female rats. EACT also reduced alcohol preference and increased water intake in both males and females. Blocking TMEME16A channels does not alter alcohol intake and preference or water intake. Our findings suggest that TMEM16A may represent a potential molecular target for controlling alcohol consumption. Supported by NIH grant R01AA027660 (PN).

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Poster

PSTR244: Alcohol: Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR244.10/S2

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01NS111122
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Title: Anti-homeostatic excessive alcohol consumption exacerbated by genetic vitamin B6 deficiency

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Abstract: Alcohol is a leading contributor to preventable deaths in the United States and excessive alcohol use has been linked to severe neurological conditions including seizures. In the central nervous system, one of the main targets of alcohol are $GABA_A$ receptors, where it acts mostly as a positive modulator. However, activation of GABAergic signaling via pharmacological manipulation in turn causes increased alcohol consumption in rodent models, suggesting a bi-directional relationship. Moreover, chronic alcohol consumption has been shown to cause deleterious effects on vitamin content in animal models and humans. Among these affected vitamins, the active form of vitamin B6, pyridoxal-5'-phosphate (PLP), is critical for the synthesis of GABA. However, the intricate inter-relationship among alcohol use, PLP alteration, and GABAergic transmission has not been fully explored.
Dietary vitamin B6 (pyridoxine or pyridoxamine) is inactive and is metabolized into PLP by the highly conserved pyridox(am)ine-5'-phosphate oxidase (PNPO) enzyme. We previously generated and characterized knock-in fly models in which we replaced the fly *PNPO* gene with mutant human *PNPO* from epilepsy patients across a range of severity (PNAS 119:e2115524119; Hum Mol Genet. 28:31). Combining these genetic models of PNPO defects with dietary PLP supplementation offer a unique approach to investigate how PLP and GABAergic signaling may contribute and respond to alcohol use. The present study demonstrates that PNPO defects play a highly significant role in both acute and chronic alcohol use. We show that 1) alcohol consumption leads to PLP reduction; 2) PNPO defects increase alcohol consumption; 3) PNPO defective flies exhibit higher residual alcohol content following alcohol exposures; and 4) PNPO defects have potentially lethal consequences which are worsened by alcohol consumption and rescued with PLP supplementation. We also discovered altered neurotransmitter levels and altered behavioral responses to alcohol in *PNPO* mutant flies. Biochemically, alcohol treatment also induced changes in several metabolic pathways, including the pathway involving converting glutamate to GABA. In summary, PNPO-defective flies exhibit an increase in alcohol consumption and increased residual alcohol content following alcohol exposure, both of which lead to increased body alcohol content and further exacerbation of endogenous PLP deficiencies. These findings suggest 2 separate vicious cycles, both of which are fed by *PNPO* mutations and/or excessive alcohol consumption, leading to continual increase of anti-homeostatic alcohol consumption.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 to Cristina Velazquez 5R01AA027808-02
NIMHD to Abiel Roche U54 007600

Title: Comparative mRNA Profile Analysis from NAc Core of Adolescent Male Mice after "Binge-Like" Alcohol Exposure Eliciting Deficits in Context Fear Extinction Learning.

Authors: *M. E. LLORET[1], C. VELAZQUEZ-MARRERO[2], Y. PEÑA[3];
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Abstract: Post-traumatic stress disorder (PTSD) and alcohol abuse disorder (AUD) are known to have high comorbidity. In humans, increased alcohol consumption is believed to be a means by which PTSD patients cope with their symptoms triggering a comorbidity. However, little is known about the physiological and molecular factors that may facilitate this co-occurrence. Binge drinking is the most common method of consuming alcohol amongst adolescent males and represents a significant risk in the development of anxiety disorders and AUD. Here we have developed an animal model of binge drinking administered during fear conditioning (FC) aimed to model the effects of single episodic binge alcohol exposure on anxiety like behaviors. Previously we have shown fear extinction (FE) deficits in animals exposed to binge-like alcohol 24hrs. prior to extinction trials. Subsequent voluntary drinking assays showed no significant effect on alcohol consumption nor preference as measured by the every-other-day two-bottle choice (EOD) drinking assay. Currently we report the results from mRNA-Seq analysis, profiling the differential expression of mRNA's within the nucleus accumbens (NAcc) core 20 days post binge-like alcohol exposure and immediately after EOD, linked to observed contextual extinction deficits in adolescent mice. Lasting changes in genes expression were measured associated to the development of alcohol tolerance such as cdh1, as well as, calcium signaling related genes, structural collagens and genes associated to other addictive substances like OPRM1, suggesting crosstalk between maladaptive reward behaviors, amongst others. In summary, this study demonstrates that a single binge-like exposure to alcohol causes both extinction learning deficits associated to the development of anxiety-like disorders and long-lasting changes in gene expression which may account for an increased risk of subsequent maladaptive addictive behaviors.

Disclosures: M.E. Lloret: None. **C. Velazquez-Marrero:** None. **Y. Peña:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

Location: MCP Hall A

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Program #/Poster #: PSTR244.12/S4

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant 5R01AA028770-04
NIH Grant 1F31AA031597-01

Title: Chemogenetic manipulation of ventral pallidal projections to the lateral habenula alters aversion-resistant alcohol consumption

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Abstract: One of the most notorious elements of alcohol use disorder (AUD) is compulsive use despite adverse physical or psychological consequences, often referred to as 'aversion resistance'. Animals with a history of long-term intermittent alcohol access (IAA) continue to drink alcohol irrespective of its adulteration with the bitter-tasting additive quinine, offering a useful preclinical model of aversion-resistant ethanol use. Here, we examined whether chemogenetic manipulation of ventral pallidum (VP) projections to the lateral habenula (LHb), a neural pathway known to contribute to aversion-related processes, can alter aversion-resistant ethanol consumption in male and female Long-Evans rats. In this study, long-term ethanol access (LTA) IAA rats received 14 weeks of intermittent home-cage access to a bottle of 15% ethanol alongside water, while short-term ethanol access (STA) rats received 14 weeks of intermittent home-cage access only to a second bottle of water. For projection-specific targeting, all rats received infusions of a Cre-expressing retrograde AAV into the LHb. To assess whether activation of this pathway could ameliorate aversion-resistance, LTA rats received Cre-dependent viruses expressing either Gq designer receptors or mCherry control in the VP. To evaluate whether we could effectively initiate aversion-resistance via inhibition of this pathway, STA rats received Cre-dependent viruses expressing either Gi designer receptors or mCherry control in the VP. Subsequent to IAA, each animal underwent 30-minute drinking tests with unadulterated 15% ethanol as well as 15% ethanol adulterated with 45, 90, and 180 mg/L quinine following injections of the chemogenetic ligand deschloroclozapine (DCZ). As expected, LTA mCherry controls were aversion-resistant relative to STA mCherry controls at 45 mg/L quinine. Interestingly, we found that chemogenetic excitation of VP-to-LHb neurons in LTA rats significantly suppressed consumption of ethanol adulterated with 45 mg/L quinine. In contrast, we found that chemogenetic inhibition of VP-to-LHb neurons in STA rats evoked a trend towards aversion-resistant ethanol consumption at 45 mg/L quinine. All rats reduced drinking at higher concentrations of 90 mg/L and 180 mg/L quinine. These data support a pivotal role for VP afferent inputs to the LHb in aversion-resistant ethanol consumption. We speculate that dysregulation of the VP-to-LHb circuit, marked by progressive hypoactivity of VP projection neurons, may be influential in the development of aversion-resistant drinking, an outcome that chemogenetic excitation can effectively 'rescue' and chemogenetic inhibition may induce.

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Poster

PSTR244: Alcohol: Molecular Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-NIAAA Grant R01AA028770
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Title: Effects of ventral tegmental area dopamine neuron $GABA_{B1}$receptor deletion on cue-evoked relapse

Authors: *J. I. AGUIRRE[1], T. TRAN[2], P. REMDE[2], A. PAULSON[2], M. LORTIE[2], J. M. RICHARD[2];
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Abstract: Alcohol use disorder (AUD) is characterized by the inability to control or stop the use of alcohol, even in the face of negative consequences or after long-term abstinence. Thus, it is important to understand the contributing factors to AUD such as environmental cues that can contribute to an increase in the probability of relapse. Ventral tegmental area (VTA) dopamine (DA) neurons have been shown to modify the learned value of reward-associated cues by driving an increase in cue reactivity, cue-reward seeking, and cue-induced relapse to alcohol seeking while the systemic activation of $GABA_B$ receptors is shown to counteract these behaviors. Yet, which receptors control the activity of VTA DA during cue-related behaviors have yet to be determined. Therefore, our goal was to test the impact of $GABA_{B1}$ receptor deletion from VTA DA neurons on alcohol cue reactivity and cue-evoked relapse using a CRISPR-Cas9 approach in TH-Cre transgenic Long Evans rats. We bilaterally microinjected Cre-dependent AAV viral vectors harboring guide RNAs targeting $GABA_{B1}$ receptors (n=14, 7 females) or control (Rosa; n=13, 6 females). $GABA_{B1}$ deletion was carried out at two different time points: 1) before any access to EtOH and 2) after the self-administration, prior to extinction and reinstatement testing. In addition, some rats (n= 8, 3 females) that received post-training deletion also received bilateral guide cannula implants to deliver baclofen intracranially into the VTA. We found that rats with $GABA_{B1}$ receptor deletion before any access to EtOH showed a decrease in free-access consumption and self-administration of alcohol as well as a decrease in active lever presses during extinction and reinstatement testing. However, when the deletion took place after self-administration training, rats had an increased number of active lever presses when compared to the control group. While VTA DA neuron $GABA_{B1}$ receptor deletion did not affect the ability of systemic baclofen to reduce alcohol seeking, it appears to disrupt the ability of intra-VTA baclofen to alter alcohol seeking during extinction and cue-induced reinstatement. Overall, our data suggests that dopamine neuron $GABA_B$ receptors may play distinct roles during different phases of reward learning and reinstatement. Future work is needed to better understand the impact of $GABA_{B1}$ deletion at each timepoint on dopamine neuron activity, whether these effects

differ based on sex, and the role of these receptors in the effects of intra-VTA and systemic baclofen.

Disclosures: J.I. Aguirre: None. **T. Tran:** None. **P. Remde:** None. **A. Paulson:** None. **M. Lortie:** None. **J.M. Richard:** None.

Poster

PSTR244: Alcohol: Molecular Mechanisms

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR244.14/S6

Topic: H.03. Decision Making

Support: NIAAA Grant P60 AA011605

Title: Impact of Adolescent Alcohol Exposure on Functional Connectivity and Choline Expression in the Basal Forebrain

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Abstract: In 2022, the National Institute on Alcohol Abuse and Alcoholism (NIAAA) reported that 8% of adolescent males and 9% of adolescent females in the United States engaged in binge alcohol use, totaling approximately 3.2 million individuals aged 12-20. Binge alcohol consumption is widely recognized to induce persistent cognitive impairments, including diminished behavioral flexibility. We previously reported that adolescent intermittent ethanol (AIE)-induced deficits in behavioral flexibility were statistically mediated by changes in cortical-subcortical functional connectivity by using MRI in rats. Other studies found that AIE exposure led to ~15-25% decrease in the number of choline acetyltransferase positive (ChAT+) neurons within basal forebrain projection neurons, coinciding with deficits observed in reversal learning and other tasks. These findings suggest a strong potential involvement of cholinergic neurons in the modulation of behavioral flexibility. However, no studies have assessed AIE-induced functional connectivity and choline expression changes in the same animals. To fill this gap, we extended our prior study by focusing on ChAT+ neurons in those rats that underwent MRI. Methods: From postnatal day (P)25-54, adolescent rats received ethanol or water (5 g/kg) via gavage, on a 2-day-ON/2-day-OFF regimen. In adulthood (>P100), rats underwent a 2-week attentional set shift task (ASST). Post behavioral training, the rats underwent resting-state functional magnetic resonance imaging (fMRI) using a 9.4T magnet for a whole-brain analysis. Immunohistochemical analysis was performed in basal forebrain slices focusing on the medial septal nuclei (MS) to visualize ChAT+ density. Results: In the ASST, AIE did not significantly alter the total trials required for initial acquisition. Though for reversal 1, AIE rats showed significant increases in prepotent errors ($p<0.01$). For reversal 2, AIE rats showed significant

increases in active errors ($p<0.05$). These findings were consistent with our previous work showing that AIE exposure reduces behavioral flexibility. Ongoing immunohistological analyses will address how AIE impacted ChAT+ neurons and whether that measure correlates with behavioral flexibility and/or functional connectivity.

Disclosures: S.E. Giang: None. **G.E. Kirkpatrick:** None. **D.L. Robinson:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.01/T1

Topic: H.02. Perception and Imagery

Title: Non-local interactions between voltage-gated sodium channels form the physical basis of correlations between neuronal activities underlying perception and voluntary motor control

Authors: *D. GUPTA;
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Abstract: During the brain's interaction with external surroundings during a task, sensory stimuli and movements transmit and generate action potentials (spikes). Given the task constraints, the degree of correlation in a spiking pattern reduces measures of surprise in those patterns, forming a basis for voluntary movements and awareness. Since most neurons that are activated during a task do not have significant or direct anatomical connections, the correlation between the neuronal activities is likely to be due to non-local causes. Voltage-gated sodium (NaV) channels play a central role in the generation and propagation of spikes. Furthermore, NaV channels open and close like a switch in response to very tiny voltage changes of a few mVs. The switch-like function of NaV channel proteins depends on conserved basic amino acids and aromatic amino acids. The author argues that delocalized pi electrons of aromatic amino acids and the guanidinium group of the arginine side chain may exist in superposition states. The author further argues that brain oscillations, common during these tasks, allow interactions between the quantum states associated with NaV channel proteins, leading to their entanglement. This entanglement, which can be between quantum states that do not coexist, is likely to be the non-local basis of the correlation in the spiking patterns given a task involving physical surroundings.

Disclosures: D. Gupta: None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.02/T2

Topic: H.02. Perception and Imagery

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Title: Enhanced frontotemporal theta and alpha connectivity during the mental manipulation of musical sounds

Authors: *D. R. QUIROGA-MARTINEZ[1], A. O. BLENKMANN[2], T. ENDESTAD[2], A.-K. SOLBAKK[3], J. T. WILLIE[4], O. KIM-MCMANUS[5], P. BRUNNER[6], M. DASTJERDI[7], J. LIN[8], R. T. KNIGHT[9];
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Abstract: The ability to mentally hold and manipulate a sequence of sounds enables essential human capacities such as language and music. However, the neural networks and dynamics supporting these processes remain largely unknown. Here, we leveraged the high spatiotemporal resolution of human intracranial recordings (N=15) to investigate how different brain regions communicate when we mentally manipulate a sequence of musical sounds. On each trial in the task (96 in total), participants listened and then vividly imagined a short, three-note melody. In the recall condition, they imagined the melody as presented, while in the manipulation condition they imagined it backwards. At the end of the trial, a test melody was presented, and participants answered whether the melody was the same as the first one (recall block; 50% same, 25% inverted, 25% different) or it's inverted version (manipulation block; 50% inverted, 25% original, 25% different). Using the imaginary part of coherence, we found that, compared to recall, manipulation enhanced synchrony in low-frequency oscillations across distributed brain areas during the imagination period. This enhancement was band specific such that alpha (7.5-12

Hz) oscillations connected auditory (Heschl's gyrus, superior temporal gyrus) and cognitive control (prefrontal, medial temporal) areas, while theta (3-7.5 Hz) oscillations specifically connected prefrontal and medial temporal regions. Furthermore, Granger causal analyses provided evidence of a central role of the dorsolateral prefrontal cortex in the coordination of both networks. Our findings shed light on the brain networks and dynamics that support auditory working memory and imagination.

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Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.03/T3

Topic: H.03. Decision Making

Support: NIA R21 AG067108
HHMI

Title: Sequential sampling from memory during perceptual decisions about stimuli that are difficult to categorize

Authors: P. SHARMA[1,2], M. N. SHADLEN[2], *S. SHUSHRUTH[3,4];
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Abstract: Perceptual decision-making refers to the class of decisions in which sensory evidence is used to categorize percepts and guide actions. For instance, a soccer goalkeeper might decide a ball is moving to their right (categorize motion as "right") and choose to dive in that direction (decide on action). Conventionally, categorical decisions are assumed to precede motor actions. But studies in animals and humans indicate that the accumulation of sensory evidence towards a decision transpires in the same neurons involved in action planning. This suggests that perceptual decisions are embodied in terms of the motor actions that a percept affords.
Behavioral tasks commonly used to study perceptual decision-making involve choosing between known actions based on sensory information. Recently, we showed that when perceptual decisions are uncoupled from the actions they bear upon, monkeys postpone evidence accumulation until the relevant actions are revealed (Shushruth et al., 2022). This postponement could arise from an inability of the monkeys to effectively categorize the stimulus, possibly due to their limited conceptual understanding of direction. We hypothesized that when confronted with stimuli that are difficult to categorize, humans also resort to a strategy similar to that used by monkeys.
Six subjects, all naïve to our hypothesis, estimated the net direction of a random dot motion

(RDM) stimulus which varied between 0° to 350° across trials. After observing an RDM, they waited for a fixed delay period before two choice targets were made available. They indicated their decision by choosing the target closest to their perceived direction of motion.
The decision accuracy varied across the six subjects, reflecting differences in their ability to integrate motion evidence. Notably, subjects with higher decision accuracy showed prolonged deliberation after choice-target presentation, and the time they took to report their decisions depended on the strength of the motion evidence. We found that this pattern of accuracy and decision reporting time can be explained by a model in which subjects sequentially sample stored information from memory while choosing the targets. When the stimulus was hard to categorize, the appearance of the targets provided a framework to interrogate the stored evidence and render a decision. Our results reveal a strategic feature of working memory that can take into account the future utility of its content. This observation opens new avenues for investigating how memory and decision-making interact.

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Poster

PSTR245: Perception, Imagery, and Imagination II

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Topic: H.02. Perception and Imagery

Support: NIH Grant R00MH117226
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Title: Hippocampal ripple rate increases during mental imagery in humans

Authors: *L. SHI[1], C. CYR[1], N. L. ANDERSON[1], A. M. HOLUBECKI[1], V. KOKKINOS[1], J. ROSENOW[2,1,3], S. SCHUELE[1,3], E. L. JOHNSON[4,5,6], C. ZELANO[1], J. SMALLWOOD[7], R. M. BRAGA[1,6];
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Abstract: Sharp-wave ripples (SWRs) are rapid bursts of synchronized oscillatory activity in the hippocampus within the range of 70-250 Hz. Extensively studied in rodents for their role in navigation, memory formation, and offline consolidation, SWRs have also been linked to declarative memory in humans (Axmacher et al., 2008; Chen et al., 2021; Dickey et al., 2022; Iwata et al., 2023; Kunz et al., 2024; Norman et al., 2019, 2021, 2023; Sakon & Kahana, 2022). However, the exact role of SWRs in mnemonic processes remains to be understood. The aim of this study is to examine how hippocampal ripples relate to mental imagery, including different

forms of imagined content and levels of vividness. Participants were tasked with visualizing various scenarios involving scenes (e.g., “Imagine a movie theater”), faces (e.g., “Imagine a bearded face”), or inner speech (e.g., “Imagine reciting the alphabet”). Prompts targeted either specific past contexts (e.g., “Remember your bedroom”) or time-independent imagination (e.g., “Imagine a city skyline”). Each prompt appeared for 2 seconds, followed by a 6-second period for imagining, during which participants focused on a crosshair. After each trial, participants rated the vividness of their visual and auditory imagery. Participants completed between one and four runs, each including 18 trials, with the order of ratings balanced across runs. Participants also provided data from a 5-minute resting-state run involving passive fixation to a crosshair. We collected intracranial electroencephalography (iEEG) data from the hippocampal formation, including CA1, CA2/3, CA4, and subiculum, from 12 epilepsy patients. After exclusion of periods with interictal epileptiform discharges, hippocampal ripples were identified using the procedures outlined by Norman et al. (2023). Preliminary analyses of four participants revealed that within the medial temporal lobe, at or near CA1, contacts were identified where the average ripple rate was significantly higher during the 6-second imagination period compared to an equivalent number of 6-second epochs from the resting state task ($p < 0.05$). This suggests that hippocampal ripples play a role in mental imagery. Ongoing analyses are investigating how ripple rates vary with different types of imagined content and their relation to visual and auditory vividness.

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Poster

PSTR245: Perception, Imagery, and Imagination II

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Topic: H.02. Perception and Imagery

Support: NSERC

Title: Color, contrast, and low spatial frequencies do not explain the extraction of average sound level from scene ensembles

Authors: *V. THARMARATNAM[1], D. B. WALTHER[2], J. S. CANT[1];
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Abstract: In order to circumvent capacity limitations in working memory and attention, the visual system can make rapid and accurate ‘gist’ estimates of groups of visual items (i.e., visual ensembles) to compress redundant information. For example, in a fraction of a second and without reliance on visual working memory (VWM), humans can accurately extract visual

statistics from ensembles of objects and faces, such as average orientation and emotion, respectively.
Jung and Walther (2021) showed that non-visual multisensory attributes (i.e., apparent sound level: how quiet or loud a scene would feel) of single scenes are represented in the prefrontal cortex and are accurately rated by observers. Extending this finding to research on ensemble perception, Tharmaratnam and colleagues (SfN 2023) found that average sound level could be rapidly extracted from groups of scenes without reliance on VWM or color. We here explore to what extent average sound level from scene ensembles can be explained by certain lower-level visual features, such as contrast or low spatial frequencies.
Participants rated the average sound level of scene ensembles, from either grayscale stimuli with a 75% contrast reduction (Exp. 1), or grayscale high spatial frequency filtered stimuli (> 6 cycles/degree, Exp. 2). In both experiments, we varied set size by randomly presenting 1, 2, 4, or 6 scenes to participants on each trial, and measured VWM capacity using a 2-AFC task. Participants were able to accurately extract average sound level in both experiments, with all 6 scenes being integrated into their summary percepts. This occurred without relying on VWM, as fewer than 1.2 scenes were remembered on average. These results reveal that with minimal low-level visual information available, multiple pieces of multisensory information can be rapidly retrieved from long term memory and combined to form summary statistical representations. Overall, these results help explain a universal mechanism by which the brain mitigates sensory overload by showing that high spatial frequency information can be used to extract multisensory summary statistics from scene ensembles.

Disclosures: V. Tharmaratnam: None. **D.B. Walther:** None. **J.S. Cant:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.06/T5

Topic: H.02. Perception and Imagery

Support: Else Kroener-Fresenius-Foundation

Title: Perceptual suppression is reduced by cycling exercise

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Abstract: Conscious perception is not only shaped by external stimuli but is also influenced by fluctuations in factors such as intrinsic neural dynamics, ongoing cognitive processes, general arousal level, as well as by visceral signals such as those generated by respiration and cardiac activity. Yet, the physiological mechanism by which bodily signals affect neural activity and

ensuing perception remains poorly understood. Here, we aimed to investigate the relationship between visual perception, cardiac and neural activity by combining a perceptual suppression task with a mild cycling exercise. We tested 30 subjects (16 female, age = 23.93 ± 2.41) who reported subjective target visibility in a bistable flash suppression task while at rest or while cycling using a desk ergometer at two different resistance levels. ECG and EEG data were collected and correlated with perceptual suppression rates. Cycling resulted in an average heart rate increase of 16% during low-resistance cycling and 41% during high-resistance cycling (rANOVA, $p < 0.0001$). Compared to rest, alpha (8-12 Hz) amplitude was reduced during cycling (Wilcoxon signed-rank test, $p < 0.01$). Importantly, we found a significant decrease in perceptual suppression rates during cycling compared to rest, with a 15% decrease during low-resistance cycling and 19% decrease during high-resistance cycling (Wilcoxon signed-rank test, $p < 0.01$). However, there was no statistically significant correlation between the individual change in heart rate and the changes in associated perceptual suppression rates. Our findings indicate an effect of exercise on conscious visual perception, while changes in heart rate alone cannot account for this effect. Since cycling did not change perceptual performance in catch trials with physical target removal, this result cannot be attributed to reduced task performance. Replicating our previous study (Poland et al., 2021), within a given rest or exercise condition, pre-stimulus alpha power was lower in target suppression trials. Given the large decrease in alpha power during cycling, we had thus expected individual suppression rates to increase during cycling compared to rest. In contrast, we observed an exercise-induced decrease of suppression rates, i.e. prolonged target visibility. Whether these exercise-induced perceptual effects are due to dual task conditions and associated small eye movements or can be attributed to the neural processing of cardiac signals remains to be seen.

Disclosures: A. Bhonsle: None. **C. Schmidt-Samoa:** None. **M. Wilke:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.07/T6

Topic: H.02. Perception and Imagery

Title: Modeling biases in human intuitive physical judgments

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Abstract: People can rapidly infer the properties of different objects and the relationships between them when reasoning about the physical world. However, this becomes more challenging as the relationships between objects grow more complex or ambiguous. People are prone to making false inferences about physical scenarios and are influenced by perceptual biases. In the current work, we test the hypothesis that people's intuitive physical judgments about stability are influenced not only by the visual information they attend to but also by their

motivations. In an eye-tracking study, we presented participants (N = 57) with images of block towers varying in stability and ambiguity and rewarded them for correctly judging whether each tower would remain standing or fall. Subjects' fixation patterns were significantly correlated with the average fixation patterns of other subjects who made the same choice, suggesting that how people sample information might contribute to their judgments about physical scenes. Moreover, we incentivized participants (N = 29) to judge towers as stable or unstable using monetary bonuses, hypothesizing that people would be more likely to judge a given tower as stable versus unstable when financially incentivized to make a particular stability judgment. Although these bonuses did not depend on participants' responses, participants were, on average, more likely to judge a tower as stable when motivated to judge it as stable than when motivated to judge it as unstable, and vice versa. Additionally, participants' fixation patterns were significantly correlated with the average fixation patterns of other subjects with the same motivation, indicating that motivation may influence how people sample information when making intuitive physical judgments. To enhance our understanding of how biases affect these judgments, we employed a convolutional neural network (Inception-v4) to model and predict human stability judgments. Using a subset of 100 block tower images, we analyzed how the model's predictions compared to human judgments. Preliminary results suggest that the model accurately captures some human predictions but not others, particularly in ambiguous cases. Taken together, this research may illuminate potential limitations in human physical scene understanding and guide further refinement of AI models in simulating human intuitive physical judgments.

Disclosures: R. Calabro: None. **K. Bhattacharyya:** None. **Y. Leong:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.08/T8

Topic: H.02. Perception and Imagery

Support: SNSF Grant P500PB_211117

Title: Induction of local neuronal fatigue in a perception related brain network in humans

Authors: *G. Y. T. LEGENDRE[1], G. AVVENUTI[1], D. GROLLERO[1], R. BASANISI[1], L. CECCHETTI[1], T. ANDRILLON[2], G. BERNARDI[1];
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Abstract: After sleep loss, we often feel irritable, impulsive or less inclined for social contacts. According to the scientific literature, this behavior is caused by deficits in emotional regulation and cognitive functions. Brief local sleep-like episodes may underlie such impairments. Indeed, recent evidence suggests that slow waves similar to those of sleep can manifest in restricted cortical areas even during a global state of wakefulness. When such sleep-like episodes occur in

a neural network, they disrupt the cognitive function the neural network operates. The prevailing scientific model posits that such local sleep-like episodes occur in a brain network following its prolonged activity but, so far, studies on sleep-like episodes focused on motor or executive functions and induced slow waves in fronto-parietal networks. It is yet to be determined whether sleep-like episodes can be restricted to brain areas operating perceptual functions. In other words, can the brain grow tired of seeing, say, smiling faces?In this work, we introduce a novel paradigm designed to induce sleep-like episodes in a perceptual network and to measure their consequences. To root the experiment in an ecological setting, we aim to impair the recognition of emotional facial expressions by inducing sleep-like episodes in the associated brain network. The paradigm is divided into three phases, an initial test phase to assess participants' performance on a set of tasks related to facial expression recognition, an intervention phase to induce sleep-like episodes and, lastly, a test phase, similar to the first phase, to measure participants' change in performance. The paradigm includes EEG and source reconstruction to track slow wave density in perceptual brain networks. We use different tests for assessing explicit and implicit facial expression recognition such as two-alternative forced choice and binocular rivalry. Pilot data showed that, after being exposed to many happy faces, participants are biased toward seeing anger in ambiguous facial expressions and, conversely, toward happiness when repeatedly exposed to angry faces. These observations are consistent with the induction of sleep-like episodes in brain networks involved in the recognition of specific emotional facial expressions. With this work, we aim to provide valuable insights on how cognitive fatigue can impact our perception, particularly in social contexts.

Disclosures: G.Y.T. Legendre: None. **G. Avvenuti:** None. **D. Grollero:** None. **R. Basanisi:** None. **L. Cecchetti:** None. **T. Andrillon:** None. **G. Bernardi:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.09/T9

Topic: H.02. Perception and Imagery

Support: JSPS Kakenhi 21H03787
JSPS Kakenhi 23K21851

Title: Perceptual decision confidence modulates event-related responses to 3D structure-from-motion stimuli

Authors: *S. IWAKI[1,2], A. OTSUKA[3], T. MIMURA[3], H. NISHIMOTO[3], Y. UESUGI[4,5], S. TORIGE[4,5];
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Abstract: Two-dimensional optical flow in the retinal images is an important cue to perceive structure of three-dimensional objects (3-D structure-from-motion: 3DSFM). Brain mechanisms underlying 3DSFM had been studied by using fMRI and MEG (Orban 1999, Iwaki 2013), in which neural interaction between the dorsal and ventral visual systems appear to be critical (Iwaki 2013). In this study, we investigated how perceptual decision confidence during 3DSFM modulates the event-related brain activities while perceiving 3DSFM stimui by MEG measurement.Eighteen participants observed visual stimuli consisted of 1,000 randomly placed dots with motion coherence ranging from 0 to 100 %, which were perceived as rotating 3-D sphere. During the interstimulus interval, the subjects were required to report the tilt angle of the rotation axis and to rate subjective confidence of the answer by a 0 to 10 scale. Stimulus related MEG epochs were recorded by using a 360-ch MEG system (MEGIN, Helsinki) with a sampling rate of 1 kHz.The minimum L2-norm algorithm (Gramfort 2014) was used to estimate brain activities at 450 regions-of-interest (ROIs) defined by APARC-sub parcellation (Khan 2018). Correlation analysis between estimated brain activities and subjective decision confidence revealed significant positive correlations in regions including the posterior cingulate cortex, lateral occipital cortex, precuneus, and supramarginal gyrus, particularly within the latency range of 700 to 900 ms.The previous study implicates involvement of feedback neural network between dorsal and ventral visual subsystems in the robust perception of 3DSFM (Iwaki 2013). The current findings suggest the possible role of specific brain regions that facilitates perceptual decision confidence during 3DSFM.
References Orban GA et al, Neuron 24: 929-940, 1999. Iwaki S et al., J. Integr. Neurosci. 12: 355-367, 2013. Gramfort A et al, Neuroimage 86 :446-460, 2014. Khan S et al, Neuroimage 174 :57-68, 2018.

Disclosures: S. Iwaki: None. **A. Otsuka:** None. **T. Mimura:** None. **H. Nishimoto:** None. **Y. Uesugi:** None. **S. Torige:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.10/T10

Topic: H.02. Perception and Imagery

Title: Individual differences in psychological distance and mental imagery associations.

Authors: *H. SATO[1,2], T. TAKANO[2], K. MOGI[3,2];
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Abstract: Construal level theory (CLT) is a comprehensive theory that explains the relationship between psychological distance and mental construal, stating that distant objects are represented

more abstractly, while close objects are represented more concretely (Trope and Liberman, 2010). Response category width, the difference between minimal and maximal estimates of a stimulus attribute, has been proposed as a psychophysical manifestation of construal level and distance (Krüger et al., 2014). Mental imagery refers to sensory representations without direct external stimuli (Pearson et al., 2015). There are large individual differences in vividness of mental imagery, ranging from aphantasia, which has no mental imagery, to hyperphantasia, which has photo-like mental imagery (Zeman, 2024). There are a lot of studies reporting that mental imagery plays an important role in various cognitive functions, including perception (Ishai and Sagi, 1995), working memory (Keogh and Pearson, 2011), and moral judgement (Amit and Greene, 2012), together with the neural processes involved (Kosslyn 2005, Breedlove et al. 2020). A study investigating the association between pictorial and verbal representations and psychological distance (Amit et al., 2009) suggests that vivid visual mental imagery promotes more concrete mental construal. We hypothesize that individual differences in mental imagery use may affect the relationship between psychological distance and mental construal. We predict that individuals who rely more on mental imagery will show a stronger association between psychological distance and response category width. Their vivid mental imagery may lead to more concrete construal of close objects and more abstract construal of distant objects. Conversely, individuals who rely less on mental imagery may show a weaker or no association between psychological distance and response category width, as their mental representations may be less affected by psychological distance. Here we study individual differences in the correlation between distance perception and construal using stimuli designed to represent a spectrum of psychological distances and mental imagery categories. The variability of the subject's trait in imagery was assessed by the Vividness of Visual Imagery Questionnaire (VVIQ) (Marks 1973). We discuss insights into the cognitive mechanisms underlying CLT and the role of individual differences in the use of mental imagery in shaping mental construal and decision-making processes, and the neural mechanisms involved.

Disclosures: H. Sato: None. **T. Takano:** None. **K. Mogi:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.11/T11

Topic: H.02. Perception and Imagery

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National Nature Science Foundation of China Grant Number: 31871136]
Science and Technology Ministry of China 「Grant Number: 2021ZD0204200]
Sino-German Center for Research Promotion 「Grant Number: M-0705]

Title: Understanding face representation in mental imagery: is it categorical or featural?

Authors: *Y. HUANG[1], S. CHANG[2], M. MENG[1];
[1]Sch. of Psychology, South China Normal Univ., Guangzhou, China; [2]Zhongshan Ophthalmic Ctr., Sun Yat-sen Univ., Guangzhou, China

Abstract: There is a specialized brain area for face perception in the fusiform gyrus (i.e., the fusiform face area, FFA). Previous neuroimaging studies focusing on hemispheric lateralization in human perception suggested that the left FFA activity patterns correlated with encoding facial features whereas the right FFA activity patterns correlated with differentiating face/non-face categories. Without direct perceptual stimulation, humans can also form visual representation of face through mental imagery. However, neural mechanisms underlying face imagery remain unclear. For example, is there hemispheric lateralization in forming face representation through imagery? To address this issue, we conducted a human fMRI study. The study recruited 9 participants with self-reported normal imagery ability (6 females, aged 18-30, the vividness of visual imagery questionnaire's score over 40). We used a retro-imagery paradigm: participants were asked to visually imagine one (imagery condition) of two sequentially presented images (perception condition) during fMRI scanning, and the blood-oxygen-level-dependent (BOLD) signal was recorded (TR = 500ms, voxel size = $3\times3\times3mm^3$). A pre-study questionnaire (n = 269) rated 16 pictures on a 10-point scale for how much these pictures resemble faces, creating four stimulus conditions (4 pictures each): faces, high-similarity faces, low-similarity faces, and non-faces (houses). Separate functional localization experiment was used to delineate the left and right FFAs as the regions of interest (ROIs). The average BOLD signal of each ROI was extracted for a univariate activation amplitude analysis to compare the effects of face similarity during perception and imagery. The results of perception conditions showed that the BOLD signal reached its peak at 5-5.5s after stimulus onset, thus subsequent analysis was based on these 2 TRs. The mean BOLD signal of the 2 TRs after stimulus onset was used as baseline to calculate the average percent signal changes, which were then used for a two-factor (left/right ROI × face similarity) ANOVA. In contrast to the significant main effect and interaction in perception conditions, only significant main effect was found in imagery conditions, with no interaction between the left/right ROI and face similarity level. Specifically, we only found greater activation for faces than non-faces in the right FFA during mental imagery. The preliminary results failed to show a hemispheric lateralization in face imagery, perhaps due to weaker averaged activation during imagery than during perception. Further conclusions will require more refined experimental designs and multi-voxel decoding analyses.

Disclosures: Y. Huang: None. **S. Chang:** None. **M. Meng:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.12/Web Only

Topic: H.02. Perception and Imagery

Title: Dissociating the neural bases of predictive and postdictive metacognitive evaluations

Authors: *T. MATSUHASHI[1], K. HOSOKAWA[1], C. HOSODA[2];
[1]Tohoku Univ., Sendai, Japan; [2]Tohoku Univ., Tokyo, Japan

Abstract: Metacognitive judgments can be prospective, predicting one's future performance, or retrospective, assessing confidence in a past choice. Several studies provide evidence that these two aspects of metacognition are dissociable, suggesting they rely on distinct cues or cognitive resources. However, because prospective and retrospective judgments are often elicited and studied in separate experimental paradigms, their similarities and differences remain unclear. Here we characterize prospective judgments of performance in a geometry estimation task.Thirty-two participants (13 females; mean age 21.9 years) gave written informed consent. All had normal/corrected vision and no neurological/psychiatric history. The study was approved by ethics committees at the University of Tokyo and Tohoku University. Task stimuli were adapted from the RAVEN task, a non-verbal test measuring abstract reasoning involving visual geometric designs with a missing piece and 6-8 choice options to fill it in. The participants had 4000ms to indicate whether the stimulus was a prospective time estimation, which predicted the performance to solve the geometric task (within 30, 60, 90 and over 120 seconds). They also had to retrospectively estimate the number of differing spots. Brain images were acquired using 3T PRISMA Siemens scanner. BOLD functional images used gradient-echo EPI sequences (TR:1500ms, TE:30ms, 2mm slices, FA=60°). The main experiment had 3 runs of 268 volumes. T1-weighted anatomical scans were also collected. Functional/anatomical images were processed using FSL. We then examined brain activation related to retrospective and prospective judgments by contrasting prospective estimation trials with retrospective estimation trials (Prospective>Retrospective and Retrospective>Prospective). The resulting activation threshold was Z>3.1, p=0.05. We found influences on prospective judgment that bilateral DLPFC were strongly influenced by predicting performance preceding decision, whereas retrospective estimation of counting differing spot numbers did not influence the DLPFC. Our results suggest that prospective judgments about future task performance relied more on bilateral DLPFC, whereas retrospective judgments about the past did not significantly engage frontal cortex. These findings indicate that metacognitive monitoring of past and future performance may involve partially distinct neural mechanisms.

Disclosures: T. Matsuhashi: None. **K. Hosokawa:** None. **C. Hosoda:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.13/T12

Topic: H.02. Perception and Imagery

Support: National Institute of Mental Health Intramural Research Program (ZIAMH002783)

Title: Visual evoked pupil, blink, and eye movements in cortical blindness

Authors: V. E. GOBO[1], T. T. LIU[2], S. JAPEE[1], E. MERRIAM[1], B. OSBORNE[3], P. BANDETTINI[1], *S. I. KRONEMER[4];
[1]Lab. of Brain and Cognition, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Dept. of Neurol., Georgetown Univ. Med. Ctr., Georgetown, DC; [3]MedStar Georgetown Univ. Hosp., Washington, DC, DC; [4]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Damage to the visual cortex can result in homonymous hemianopia - a condition where individuals experience blindness in half of their visual field. While homonymous hemianopia has been studied for understanding pathways supporting conscious visual perception, there is limited use of eye metrics (i.e., pupil size, blinks, and microsaccades) as a possible covert measure of visual conscious and unconscious processing in this patient group. Previous research using the glare illusion - an image with illusory brightness - found that pupils constrict more for illusory glare than non-glare stimuli of equal luminance. We hypothesized that pupillary responses to the glare illusion in the blind field of homonymous hemianopia may indicate preservation of higher-order visual processing linked to conscious perception. To test this, we studied the pupillary, blink, and microsaccade dynamics evoked by the glare illusion in homonymous hemianopia. Adult participants with homonymous hemianopia (N = 7; mean age = 51 years; age standard deviation = 16.9 years; 3 females) completed a computerized, visual perception task with simultaneous eye tracking (EyeLink 1000 Plus, SR Research Ltd). Participants centrally fixated on a black plus sign while plus-shaped images (3.25 degrees radius; 3 seconds) appeared in the periphery either deep within their blind field or in the mirror-symmetric location in their intact visual field. Participants were instructed to press a button whenever this peripheral stimulus was a red cross (target stimulus, 1/3 probability) and withhold responses for monochrome crosses: glare and non-glare stimulus (1/3 probability each). Analysis of button responses and participant subjective reports confirmed that most participants had no conscious perception of visual stimuli presented in their blind field. In the sighted field, all participants showed stimulus-evoked pupil size, blink and microsaccade rate responses. In the blind field, however, one group of participants showed pupil, blink, and microsaccade responses to the task stimuli, whereas the other group did not. Interestingly, those participants with blind field pupillary responses did not show differences in pupil size between the glare and non-glare stimuli. These findings suggest that while some individuals with homonymous hemianopia may maintain visual processing without conscious perception, conscious perception may be necessary to drive the pupillary responses linked with the glare illusion. Ongoing research aims to assess the functional implications of these findings for conscious visual perception in cortically blind individuals and their rehabilitation potential.

Disclosures: V.E. Gobo: None. **T.T. Liu:** None. **S. Japee:** None. **E. Merriam:** None. **B. Osborne:** None. **P. Bandettini:** None. **S.I. Kronemer:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.14/U1

Topic: H.02. Perception and Imagery

Support: STI2030-Major Projects+2021ZD0204300
National Natural Science Foundation of China (32030045)
National Natural Science Foundation of China (32061143004)

Title: Human intralaminar and medial thalamic nuclei transiently gate conscious perception through the thalamocortical loop

Authors: *Z. FANG[1], Y. DANG[2], A. PING[1], W. CHENYU[1], X. LI[1], M. ZHANG[1];
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Abstract: Human high-order thalamic nuclei have been known to closely correlate with conscious states. However, given the great difference of conscious states and contents (conscious perception), it is nearly unknown how those thalamic nuclei and thalamocortical interactions directly contribute to the transient process of conscious perception. To address this question, we simultaneously recorded local field potentials (LFP) in the human intralaminar, medial and ventral thalamic nuclei as well as in the prefrontal cortex (PFC), while patients (n=5) with implanted electrodes performing a visual consciousness task. Overall, compared to the ventral nuclei, intralaminar and medial nuclei showed earlier and stronger consciousness-related activity. Moreover, the transient thalamocortical neural synchrony and cross-frequency coupling were both driven by the theta phase of the intralaminar and medial nuclei during conscious perception. These results suggest that contrary to common beliefs, the intralaminar and medial thalamic nuclei, rather than PFC, play a decisive ‘gate’ role in conscious perception.

Disclosures: Z. Fang: None. **Y. Dang:** None. **A. Ping:** None. **W. Chenyu:** None. **X. li:** None. **M. Zhang:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.15/U2

Topic: H.02. Perception and Imagery

Support: KHIDI Grant HI19C1095

Title: Investigating beta burst rate dynamics in motor Imagery: A comparative study with motor execution in a stop-signal task

Authors: *E. OH[1,2], P. G. BISSETT[2], S.-P. KIM[1];
[1]Biomed. Engin., Ulsan Natl. Inst. of Sci. and Technol. (UNIST), Ulsan, Korea, Republic of;
[2]Psychology, Stanford Univ., Stanford, CA

Abstract: Beta frequency oscillations (15-29 Hz) are a well-known neural signature related to human movement. The power of beta oscillations changes not only during movement execution

(ME) but also during motor imagery (MI), often referred to as event-related desynchronization/synchronization (ERD/S) (Pfurtscheller and Silva, 1999). This neural characteristic has been widely used as a feature in brain-computer interfaces (BCIs), which can help patients with motor injuries by linking their neural activity to movement-aid devices. In recent years, other characteristics of beta oscillations such as the rate and timing of beta bursts, transient increases in the power of beta oscillations, have been shown to be a predictor of single-trial behavior in inhibitory control, with more frequent beta bursts (Wessel, 2020) before successful response inhibition. However, most research has focused on beta bursts related to response inhibition during ME, with little exploration into beta burst during MI, despite the significance of MI research for developing BCIs that could assist patients who have lost the ability to move. Therefore, this study aims to evaluate whether beta bursts share similar characteristics between MI and ME, in line with previous findings that ERDS characteristics are evident in both contexts. To address this, we recruited 47 subjects and recorded EEG signals during a stop-signal task (SST), a common paradigm for studying response inhibition. The task consisted of frequent go trials (75%) and infrequent stop trials (25%), with a total of 800 trials per session. Each participant completed 2 conditions: a ME condition involving physical movements and a MI condition involving imagined movements without physical execution. The analysis of the temporal dynamics of beta burst rates revealed significantly higher beta burst rates near stop signal reaction time (SSRT) in stop trials compared to go trials (paired t-test, $p<0.05$), including at the frontal and contralateral central areas during the MI session. This spatiotemporal pattern of beta burst rates was similar in both the MI and ME sessions. Our findings suggest that beta bursts can be modulated by the inhibition of imagined movements as well as executed movements, consistent with shared neural mechanisms for imagined and executed movements. Furthermore, our results suggest that response inhibition can be detected using EEG when making imagined motor responses, which holds promise to improve motor control in BCIs for individuals with motor injuries and limitations.

Disclosures: E. Oh: None. **P.G. Bissett:** None. **S. Kim:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.16/U3

Topic: H.02. Perception and Imagery

Support: Bradley University Department of Psychology Elias Fund

Title: Individual differences in visual imagery vividness as they relate to component processes of attention and susceptibility to object-substitution masking

Authors: J. SWEET, *J. HARRIS;
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Abstract: Object-substitution masking (OSM), also known as common-onset masking, has, for the past twenty-five years, provided valuable insight into the neural mechanisms underlying the emergence of visual perceptual awareness. While an early reentrant mechanism has been identified as a key determinant of awareness during OSM (Enns and Di Lollo, 2000; Boehler et. al, 2008), the effective deployment of attention to the target, reflected in the N2pc event-related potential component, as well as visual working memory processes reflected in the sustained posterior contralateral negativity (SPCN), also track with the emergence of awareness during masked trials (Prime et al., 2011; Harris et al., 2013). In addition, there is significant variability in suceptibility to OSM effects, with masked trials yielding decrements in target detection anywhere between 5 and more than 60 percent, depending on the viewer. The present study seeks to characterize the contribution of component processes of attentional deployment otherwise conflated in the N2pc (i.e., the target-related negativity (Nt) and the distracter positivity(Pd)), as well as visual working memory processes reflected in the sustained posterior contralateral negativity (SPCN), to the emergence of awareness during OSM. For this, a repeated-measures multivariate ANOVA, comparing mean amplitude evoked responses for each of these components across target visibility (seen and unseen) in the masked condition, probes the relative predictive value of the Nt, Pd and SPCN in the emergence of awareness during OSM. In addition, individual differences in visual imagery vividness measured using the Plymouth Sensory Imagery Questionnaire (PSI-Q; Andrade et al., 2014) are correlated with mean-amplitude Nt, Pd, and SPCN components, as well as susceptibility to OSM reflected in target detection rate decrements from unmasked to masked trials.

Disclosures: J. Sweet: None. **J. Harris:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.17/U4

Topic: H.02. Perception and Imagery

Support: NSERC CGS-D 2024
NSERC: RGPIN-2023-04231
CRC-2019-00107
CFI/ORF-2014-34479

Title: Network cofluctuation dynamics are related to both behavioural and neural event boundary timing

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Abstract: People segment ongoing experience into a nested series of discrete events, separated by boundaries (Zacks et al., 2007). This hierarchical event structure is reflected in patterns of

fMRI activity observed during movie and auditory narratives, with patterns maintained during events and sharp transitions observed at moments that are commonly judged to be event boundaries (e.g., Baldassano et al., 2017; Geerligs et al., 2021). While recent findings suggest that these neural boundaries are coordinated within large scale networks (Geerligs et al., 2022), little is known about how network dynamics relate to event segmentation, in part because the temporal resolutions of most measures of dynamic fMRI functional connectivity are too coarse. In the current study, we overcame this hurdle using Edge Timeseries (Zamani Esfahlani et al., 2020) to measure network cofluctuation dynamics at a TR-by-TR level in a publicly available fMRI movie narrative dataset (Chen et al., 2015). This allowed us to ask if network coordination shifts (1) around moments that were independently rated as event boundaries and (2) around within-network neural event boundaries that were modeled within individual participants using the Greedy State Boundary Search. Our preliminary results show that shifts in both within-network and between-network cofluctuations were correlated with normative event boundary judgements in time, especially within the Dorsal Attention Network (DAN; $p < .01$). We also found that changes in within-network cofluctuation, including in the DAN, were correlated in time with neural event boundaries ($p < .05$). These results suggest that shifts in network coordination dynamics may also support our segmentation of experience into meaningful events, as we in turn coordinate with our environments.

Disclosures: R.E. Wilford: None. **K.D. Duncan:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.18/U5

Topic: H.02. Perception and Imagery

Title: Evaluating the computational efficiency of a biologically plausible action potential backpropagation mechanism for memory retrieval and generative cognition

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Abstract: Reinstating the details of a previously seen stimulus, given a recall cue, is a common cognitive function, yet the neural mechanisms enabling such generative retrieval are still largely unknown. While studies suggest that recollection involves a top-down reactivation of the same neural ensembles active during encoding, the precise mechanisms of such reactivation remain unclear.We formulate a biologically plausible computational hypothesis in which transient cholinergic modulation, known empirically to favor backpropagated action potentials and trigger cue-based recall, initiates a cascade of backward signals from the sparse neurons representing the cue to the cortical areas encoding the trace. These signals flow through gap junctions, increasingly known to be prevalent in memory-related brain regions, retracing the original forward pass in reverse to reactivate the same neurons. We simulate the computational efficiency

of this mechanism, with code available, in two cue-based recall tasks: image-to-category classification and reconstruction of an image from sparse activations representing the cue.In the first, we compare our method's accuracy to an SVM classifier on learning from many examples and one-shot learning, using the Caltech face/motorbike dataset, ensuring statistical robustness through multiple train/test splits and Gardner-Altman estimation plots.In the second, we use the weights of AlexNet, a pre-trained neural network, to extract feedforward visual processing features. By activating a small subset of neurons in a deep layer of AlexNet and propagating their activity backwards using synaptic weights, we attempt to reconstruct the original input, mimicking the hypothesized role of backpropagating action potentials in generating mental imagery from sparse representations.In image-to-category mapping, our mechanism achieves 96.8% average accuracy across 49 splits, only 0.8% behind the SVM [95% CI: 0.5%, 1.1%]. In the more challenging one-shot learning, our model reaches 96% accuracy for certain pairs, surpassing the SVM which peaks at 84.5% across 1500 experiments. For image reconstruction, our model generates less vivid but high-quality images from sparse activations in higher layers, even using less than 1% of top neurons. We observe a relationship between reconstruction quality and network performance.The mechanism's computational efficiency, together with growing empirical evidence, offer new avenues to interpret generative retrieval, with implications spanning cued attention, mental imagery, and future episodic thinking. More broadly, it builds a bridge between sparse and distributed neural coding.

Disclosures: Z. Ben houidi: None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.19/U6

Topic: H.02. Perception and Imagery

Support: Hertie Foundation

Title: Decoding brain activity underlying mental imagery from ultra-high field 7T fMRI

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Abstract: Mental imagery enables the exploration of virtual scenarios, objects and events involving complex neural networks in the ventromedial prefrontal cortex (vmpfc), hippocampus, and visual areas. Current scientific evidence fails to map these neural correlates within a hierarchical structure. We hypothesize that the vmpfc is intiating and coordinating hippocampal scene construction in the generation of extended mental events. Therefore, we investigated neural activity in a task-based fMRI experiment, where participants were instructed to visualize either objects, scenes or events in comparison to a control condition (counting non-word

characters). Data were acquired using a MAGNETOM 7T Plus ultra-high field scanner. Preprocessing was performed using SPM12. Multivariate methods were used to compare covariance brain activation patterns between the experimental and control conditions. Data were collected from a sample of 19 young healthy right-handed participants (28±4 years, 11 females). We were able to replicate previous findings that visual imagery involves a network of brain areas from the frontal cortex to sensory areas, overlapping with the default mode network. For the first time, we found that the vmpfc showed stronger activation for events than scenes (p<.05) and objects (p<001). Our results support the notion that visual mental imagery and its neural underpinnings follow a hierarchical model. This lays the groundwork for functional and effective connectivity analysis.

Disclosures: J. Taube: None. **P. Leelaarporn:** None. **M. Bilzer:** None. **C. McCormick:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.20/U7

Topic: H.02. Perception and Imagery

Support: Tiny Blue Dot Foundation
Templeton World Charity Foundation

Title: Intracranial EEG correlates of dreaming sleep

Authors: *M. SOLVIK[1], U. GORSKA[2], M. GROBBELAAR[1], C. KOZMA[3], C. BRACE[1], J. AZAM[1], H. HOLMES[1], M. KALKACH APARICIO[1,4], R. CILIENTO[1], E. MONAI[1], C. PAPANTONATOS[5], D. SCOTT[1], B. SEVAK[6], J. VOGEL[1], G. TONONI[2], M. BOLY[1,2]; [1]Dept. of Neurol., [2]Dept. of Psychiatry, Univ. of Wisconsin–Madison, Madison, WI; [3]Sch. of Computing, Newcastle Univ., Newcastle upon Tyne, United Kingdom; [4]Sanford Sch. of Med., Univ. of South Dakota, Sioux Falls, SD; [5]Sch. of Med., St. Louis Univ., St. Louis, MO; [6]Biomed. Engin., Univ. of California, Davis, Davis, CA

Abstract: Neural correlates of consciousness during sleep can be dissociated from those of responsiveness using within-state, no-task paradigms comparing dreaming versus dreamless sleep epochs within similar physiological states. Recent high-density electroencephalography (hdEEG) studies using within-state paradigms during sleep pointed to differences in slow-wave activity (SWA) in posterior cortical areas as most predictive for the presence of dreaming experience. In this study, we used intracranial electroencephalography (iEEG) recordings to further investigate brain activity changes correlated to dreaming sleep. We analyzed iEEG recordings from eight patients with focal epilepsy combined with 17 subjective reports of dreaming and dreamless sleep obtained during these recordings. We collected subjective reports using structured questionnaires following awakenings from non-rapid eye movement sleep performed by nursing staff in the context of clinical care. Only reports deemed reliable were kept

for analysis. Such reports had to meet the following criteria: (1) an awakening from sleep occurred within 15 minutes of the questionnaire administration time, (2) the time of awakening could be clearly determined based on behavioral signs (body movement and eye-opening) on clinical video recordings, and (3) the iEEG delta/beta power ratio dropped at the time of the awakening. We analyzed two minutes of iEEG data leading up to the awakening following the removal of epileptic spikes, comparing dreaming and dreamless sleep using *t*-tests. Analysis focused on SWA (power in the 1-4 Hz range) in predetermined regions of interest (posterior temporal cortex, temporal pole, and prefrontal areas). Results demonstrated a decrease in SWA in posterior temporal cortex during dreaming versus dreamless sleep ($p < .01$). In contrast, we observed an increase in SWA in temporal pole during dreaming sleep ($p < .01$), and no significant difference between dreaming and dreamless sleep in prefrontal areas. These preliminary results are in line with our previous hdEEG findings suggesting a selective reduction of SWA in posterior cortical regions during dreaming compared to dreamless sleep. They also demonstrate the value of iEEG recordings in the investigation of local changes in brain activity during sleep. Future research on a larger patient population will aim to further refine differences in cortical SWA topography predictive of dreaming.

Disclosures: M. Solvik: None. **U. Gorska:** None. **M. Grobbelaar:** None. **C. Kozma:** None. **C. Brace:** None. **J. Azam:** None. **H. Holmes:** None. **M. Kalkach Aparicio:** None. **R. Ciliento:** None. **E. Monai:** None. **C. Papantonatos:** None. **D. Scott:** None. **B. Sevak:** None. **J. Vogel:** None. **G. Tononi:** Other; Intrinsic Powers, Inc., Delix Therapeutics. **M. Boly:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.21/U8

Topic: H.02. Perception and Imagery

Support: Grants: JSPS KAKENHI 21H04896, 23K17462

Title: Vision Transformers Exhibit Innate Understanding of Subjective Distances Through Self-Supervised Learning

Authors: *H. SUZUKI[1], T. YAMAMOTO[2], S. KITAZAWA[1,2,3];
[1]Grad. Sch. of Frontier Biosciences, Osaka Univ., Osaka, Japan; [2]Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan; [3]Ctr. for Information and Neural Networks (CiNet), NICT, Osaka, Japan

Abstract: A recent study on boundary extension (Bainbridge & Baker, 2020, Curr Biol) demonstrated that rapid serial presentation of identical images, separated by scrambled masks, elicits illusions of movement, either closer (boundary extension) or farther(boundary contraction). These illusions correlate strongly with subjective distance ratings of prominent image features. This finding suggests that visual perception involves an instantaneous assessment of feature distance, which is then adjusted in memory based on prior distance distributions,

creating the illusion. In this context, the Vision Transformer (ViT), introduced by Dosovitskiy et al. in 2020, employs an attention mechanism and a class token to aggregate information across an image. Our study, utilizing eye-tracking technology, observed that while a standard ViT's attention patterns differ from human patterns, a ViT trained via the label-free, self-supervised method proposed by Caron et al. (2021) exhibits strikingly similar attention dynamics. This similarity prompted us to investigate whether a self-supervised ViT possesses an inherent understanding of distances—a concept it was never explicitly taught. We tested this by using two sets of 192 images each, identified by Bainbridge& Baker (2020) to induce maximum boundary contraction and extension. We confirmed these illusions by varying the magnification of a subsequent image from -20% to +20%. We then fitted a sigmoid curve to the x-y plot, where x represents the magnification levels and y denotes the probability of the "closer" judgment ranging from 0 to 1. The far group exhibited a -3.2% shift, and the near group a +2.9% shift in the sigmoid curve, affirming the initial findings. We then presented these image sets to a 12-layer self-supervised ViT, recording responses from 384 units above the class token. Using t-distributed stochastic neighbor embedding (tSNE), we observed that the two groups initially intermingled but distinctly separated by the seventh layer. Notably, a simple linear regression applied to the final layer's 384-dimensional responses accounted for 96% of the variance in subjective distance estimates. This result is remarkable as the ViT, trained without explicit distance or feature information, could inherently estimate distances based on attention alone. These findings suggest that the neural mechanisms in ViT's final layer that enable innate distance estimation warrant further investigation.

Disclosures: H. Suzuki: None. **T. Yamamoto:** None. **S. Kitazawa:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.22/U9

Topic: H.02. Perception and Imagery

Support: JSPS KAKENHI 21H04896, 23K17462

Title: Vision Transformers Mimic Human Attention through Self-Supervised Learning but Not Through Supervised Learning

Authors: *T. YAMAMOTO[1], S. KITAZAWA[2];
[1]Grad. Sch. of Med., Osaka Univ., Osaka, Japan; [2]Osaka University/ Grad. Sch. of Frontier Biosci., Suita/ Osaka, Japan

Abstract: The Vision Transformer (ViT), developed by Dosovitskiy et al. in 2020, utilizes an attention mechanism and class token to process whole images. Originally, ViTs trained via supervised learning (SL) excelled in image classification but showed noisy attention patterns. Recent advancements reveal that self-supervised learning (SSL), particularly through the DINO

(distillation with no label) protocol (Caron et al., 2021), significantly refines ViT attention. This study compares the attention patterns of ViTs trained by both DINO and SL protocols with human attention, using 4-, 8-, and 12-layer models. Each model was trained with both protocols, resulting in six configurations. Their attention was analyzed by comparing the peak attentions in each head to human gaze patterns during video viewing, using gaze data from previous studies (Nakano et al., 2010; Costela & Woods, 2019) and multidimensional scaling for analysis. Findings indicate that only 8- and 12-layer ViTs trained via DINO closely mimicked human attention, particularly in the later layers (7th and 8th for 8-layer; 8th and 9th for 12-layer models). Attention heads of these layers autonomously differentiated into two distinct groups, one attending to important visual elements (faces, heads), and the other attending to the background in general, echoing principles of Gestalt Psychology. This suggests that 8-layer ViTs may sufficiently replicate human attention, offering a potential model for exploring the neural basis of attention.

Disclosures: T. Yamamoto: A. Employment/Salary (full or part-time):; Osaka University Hospital. **S. Kitazawa:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.23/U10

Topic: H.02. Perception and Imagery

Title: Ischemic lesions to inferior frontal cortex alter the dynamics of conscious visual perception

Authors: *M. FRITSCH[1], V. WEILNHAMMER[2], J. MICHELY[1], I. RANGUS[3], C. RIEGLER[4], C. NOLTE[4], P. STERZER[5];
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Abstract: In order to adequately navigate our surroundings, ambiguous sensory information has to be transformed into unambiguous conscious experience. The role of prefrontal cortex in such conscious visual perception has been subject to longstanding debate. Recent work, using functional magnetic resonance imaging (fMRI) and repetitive transcranial magnetic stimulation (rTMS), has illustrated a key role of the right inferior frontal cortex (rIFC) in the detection and resolution of perceptual ambiguities. Specifically, inducing a transient virtual lesion in rIFC, with inhibitory rTMS, led to a reduction in spontaneous switches between two possible percepts during viewing of ambiguous visual stimuli. Here, we sought to validate the role of the rIFC in conscious perception by evaluating how loss-of-function in patients with ischemic stroke in this

region influences processing of ambiguous visual information. We hypothesized that patients with ischemic lesions in the rIFC would experience fewer perceptual switches while viewing an ambiguous stimulus, indicated by longer phase-time between switches. **To this end, twenty-three patients (11 female, mean age 70.65 ± 1.2 years) with chronic, right-hemispheric ischemic stroke lesions within the MCA-territory (9 patients with lesions in rIFC = "IFC", 14 without = "non-IFC") performed a computer-based bistable perception task, in which they had to report the perceived direction of rotation of a spherical, random-dot-kinematogram (RDK) that was completely ambiguous. Elapsed phase-time between switches was recorded in seconds. As hypothesized, patients with rIFC lesions showed significantly longer mean phase-times between perceptual switches - resulting in a lower frequency of consciously perceived switches - compared to patients without rIFC lesions ("IFC" 35.3s ± 2.1s vs. "non-IFC" 23.5s ± 1.8s, $T(244.81) = 2.73$, $p < 0.007$). Importantly, this effect remained significant when controlling for age, sex, stroke severity and lesion volume ($T(5.97) = -2.9$, $p = 0.026$). Our results support the notion that the rIFC is critical for resolving perceptual ambiguities, suggesting a key role of frontal cortex in shaping conscious visual experience.**

Disclosures: M. Fritsch: None. **V. Weilnhammer:** None. **J. Michely:** None. **I. Rangus:** None. **C. Riegler:** None. **C. Nolte:** 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.; DZHK (German center for cardiovascular disease), DZNE (German Center for neurodegenerative disease), BIH (Berlin Institute of Health). F. Consulting Fees (e.g., advisory boards); Alexion, AstraZeneca, BMS, Novartis, Pfizer. **P. Sterzer:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.24/U11

Topic: H.02. Perception and Imagery

Title: Perceptual content and non-deterministic outcomes are achieved through probabilistic coding in cortical neural networks

Authors: *E. A. STOLL;
Western Inst. for Advanced Study, Denver, CO

Abstract: While spinal neurons require supra-threshold stimulation to fire, cortical neurons allow random electrical noise to affect the probability of firing. To model this computational process, each extracellular ion is described as an electromagnetic point source, interacting with each region of neuronal membrane. The ion is modeled as having a distribution of possible states, given by probability amplitudes along the x, y, z, time, orbital and spin axes. The voltage state of each neuron is then a function of the mixed sum of all component pure states, or the von

Neumann entropy encoded by the system. If the probabilistic trajectory of any ion affects the membrane potential of multiple computational units, then the system must be computed as a whole, with the state of each neuron being resolved as every component pure state is resolved. This computational process yields a non-deterministic outcome for each computational unit, resulting in synchronous firing. If the membrane of each computational unit is also a charge-detecting polymer substrate that meets the criteria of a holographic recording surface, this computational process will generate a holographic projection of the information being encoded. In short, the constructive and destructive interference of high-dimensional probability amplitudes yields a non-deterministic computational outcome for each neuron. That system state is paired with a multi-sensory percept, which is exclusively accessed by the encoding structure, with available information limited by the range and sensitivity of the sensory apparatus. This model usefully offers a plausible explanation for both perceptual content and non-deterministic computational outcomes emerging from cortical neural network activity.

Disclosures: E.A. Stoll: None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: H.02. Perception and Imagery

Support: IOE Fellowship - R(HR)(loE-IISc)(POF)(CNS)(GKB)-1530

Title: Simultaneously induced slow and fast gamma waves travel independently in primate primary visual cortex

Authors: *B. GAUTHAM[1], S. RAY[2];
[1]Neurosci., IISc, Bangalore, India; [2]Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Introduction: Travelling waves have been reported for a range of neuronal oscillations and have been associated with different neural mechanisms. Previous studies have shown that certain visual stimuli such as large gratings induce two gamma oscillations - slow (25-35 Hz; potentially associated with the somatostatin interneuronal network) and fast (40-60 Hz; potentially associated with the parvalbumin network) in the primary visual cortex (V1). However, whether these stimulus-induced gamma oscillations form travelling waves, and whether these waves move independently or in a coordinated way, remains unknown. **Methods:** Recording from microelectrode arrays (Utah arrays; 81 active electrodes) were obtained from two awake adult female monkeys (maccaca radiata) placed in V1 of right occipital lobe. The macaques performed a fixation task during which full screen stimuli of sinusoidal luminance gratings were presented at full contrast at multiple orientations and spatial frequencies. We used stimulus configurations that produced two distinct gamma waves in the slow and fast gamma ranges. Gamma bursts were identified by Hilbert transform between 25-35Hz (slow gamma) and

40-50Hz (fast gamma) and time points during stimulus period which showed gamma activity in ≥60% of electrodes were used to determine traveling waves. Instantaneous phase extracted using Hilbert transform from these electrodes was subjected to circular linear regression against their location in the grid, to extract the wave fit, direction, duration and velocity. The fit was statistically verified by permutation tests and directions were compared using circular ANOVA. **Results:** Both slow and fast gamma rhythms showed travelling waves, with a mean duration of 0.13±0.091 s and 0.09±0.07 s. Slow gamma waves propagated over larger distances across the grid (90.40±9.31% of electrodes) compared to fast gamma (85.11±12.87%). Interestingly, direction of wave propagation was significantly different between the two rhythms ($p<0.001$), even when they were induced at the same time, suggesting different origins. Mean wave velocities were 0.29±0.07m/s and 0.34±0.17 m/s in the slow and fast gamma bands ($p = 0.02$). While these waves could co-occur, they were not locked to the stimulus. However, the directions remained consistent across trials. **Conclusions:** Slow and fast gamma oscillations in V1 showed unique spatial and temporal wave dynamics signifying that these gamma rhythms are generated in distinct neural circuits and may modulate cortical processing at different levels.

Disclosures: B. Gautham: None. **S. Ray:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.26/U13

Topic: H.02. Perception and Imagery

Title: Temporal changes of electroencephalography oscillation rhythms during consciously induced resting state

Authors: *M. SASHIDA[1], S. IWAMA[2], J. USHIBA[3];
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Abstract: Mindfulness is defined as "the awareness that emerges through paying attention on purpose, in the present moment, and nonjudgmentally to the unfolding of experience moment by moment" and the mindfulness-based cognitive therapy was developed to implement this practice. This treatment approach has shown comparable results to antidepressant medications in a randomized controlled trial and has been recommended in guidelines for the treatment of depression and chronic low back pain. Although mindfulness has been shown to be clinically effective, its neural mechanisms remain unresolved. In the present study, we used electroencephalography (EEG) to estimate the level of arousal during meditation-induced conscious rest and conducted a two-group parallel group comparison between skilled meditators (Meditator) and novices with no meditation experience (Novice). First, they meditated for 30 minutes following an audio instruction, then they meditated for 15 minutes without instruction; during the 15 minutes of meditation, a school chime sound was played as an external disturbance

at the 10-minute mark. Before and after the meditation, a questionnaire on sleepiness and the state and characteristics of relaxation were administered. The EEG data during the 15 minutes of meditation was divided into data every minute, and classified according to how much time it contained high amplitude of specific frequency bands that reflect human brain states. As a result, focusing on the alpha frequency band (8-13 Hz), whose power is enhanced at rest, it was confirmed that the alpha wave occupancy at 9-10 minutes was significantly higher than that at 0-1 and 2-3 minutes of meditation only in the Meditator group (p_{bonf}=0.038, p_{bonf}=0.034; paired Post Hoc Tests). These results suggest that the meditators may have acquired the skill of self-induction to a low arousal state in a stepwise manner through long-term meditation training, and that meditation is an activity to calm the arousal level by self-induction of neural activity under the arousal state. The similarities and differences between the Cz and Fz electrodes were analyzed by simultaneous measurement of the two electrodes and the results were also reported.

Disclosures: M. Sashida: None. **S. Iwama:** A. Employment/Salary (full or part-time):; Keio University. **J. Ushiba:** A. Employment/Salary (full or part-time):; LIFESCAPES Inc., Keio University. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LIFESCAPES Inc..

Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.27/U14

Topic: H.02. Perception and Imagery

Support: NSERC

Title: Neural Mechanisms of Perisaccadic Time Perception: Insights from EEG and Graph Theory

Authors: *A. GHADERI[1], M. NIEMEIER[2], J. CRAWFORD[3];
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Abstract: The impact of saccades on time perception has been extensively studied, yet the underlying neural mechanisms remain poorly understood. Our prior investigations showed that stimulus repetition interacts with presaccadic signals to influence time perception (Ghaderi et al., *Heliyon*, 20220 and this is accompanied by specific sensorimotor network dynamics (Ghaderi et al., *Cerebral Cortex*, 2023). Here we directly investigated the cortical network dynamics associated with correct / incorrect time perception using a multifaceted approach encompassing psychophysics, EEG, sLORETA source localization, and graph theory analysis (GTA). Twenty-one participants were presented with a series of reference stimuli followed by a test stimulus, occurring either just prior to saccades or during sustained fixation. Subsequently, participants assessed the duration of the test stimulus relative to the reference. We then contrasted brain

activity associated with correct trials versus incorrect trials (test duration underestimated) for 4 equal time epochs within the 200 ms pre-saccadic period. Through source localization, we observed temporal cortical activation dynamics, commencing from early visual regions and culminating in higher-order cognitive areas such as the middle frontal and anterior cingulate cortices. Likewise, GTA further delineated the progressive contributions of three distinct groups of brain regions: visual, temporal/parahippocampal, and frontal/ anterior cingulate cortices. Whole-network analysis unveiled significant variations in the topological and dynamical properties of brain networks between trials with underestimated versus accurate judgments. These findings imply the involvement of multiple cortical regions and networks in perisaccadic time perception errors, specifically involving a progression from more sensory areas toward higher-level areas associated with multiple cognitive functions. This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Vision: Science to Applications (VISTA) program.

Disclosures: A. Ghaderi: None. **M. Niemeier:** None. **J. Crawford:** None.

Poster

PSTR245: Perception, Imagery, and Imagination II

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Program #/Poster #: PSTR245.28/U15

Topic: H.02. Perception and Imagery

Support: NIH Grant R00MH117226
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NIH Grant 2T32MH067564

Title: Intracranial recordings reveal dynamics of distributed networks during mental imagery

Authors: *N. L. ANDERSON[1], C. CYR[1], A. M. HOLUBECKI[1], L. SHI[1], V. KOKKINOS[1], J. ROSENOW[1,2,3], S. SCHUELE[1,2], J. SMALLWOOD[4], R. M. BRAGA[1,5];
[1]Neurol., [2]Physical Med. and Rehabil., [3]Neurolog. Surgery, Feinberg Sch. of Medicine, Northwestern Univ., Chicago, IL; [4]Psychology, Queen's Univ., Kingston, ON, Canada; [5]Psychology, Northwestern Univ., Evanston, IL

Abstract: Mental imagery involves the generation of perceptual representations without direct sensory input. Imagery can involve many types of content and is associated with cognitive processes like recollection and prospective thinking. Previous work has shown that different forms of mental imagery are linked to the top-down activation of different brain networks; an outstanding question regards how these large-scale networks are dynamically activated during different forms of imagery, and whether some networks are recruited across many forms of imagery.
Individuals performed a mental imagery task while undergoing intracranial elecroencephalography (iEEG) for surgical planning. Ten epilepsy patients were recruited from

Northwestern Memorial Hospital. In the weeks prior to electrode implantation, patients underwent sessions of functional magnetic resonance imaging (fMRI). Multiple runs of resting-state (passive fixation) were collected. After quality control, each patient provided at least 49 minutes of high-quality, low-motion data (range: 49-161min). This was used to estimate brain networks within each patient using functional connectivity.
During intracranial monitoring patients participated in a novel mental imagery task. Prompts were presented on a screen (such as "Imagine a castle on a hill") for 2s, followed by a 6s imagination period. Then, participants provided separate ratings for the visual and auditory vividness of the imagined content. Participants completed multiple 18-trial runs; after quality control, participants retained a median of 69 trials (range: 36-72). Trials included imagining scenes, faces, and speech. High-frequency broadband (HFB; 70-170 Hz) activity was calculated and averaged across all trials. Electrode contacts were labeled as belonging to different fMRI-defined networks using a winner-takes-all approach by assessing the network overlap with a 3mm-radius sphere centered at each contact.
Preliminary observational results show multiple instances where task-related HFB activity during the imagining task overlapped with individualized fMRI-defined network regions. Contacts in visual or visual attention networks showed rapid activation and return to baseline when stimuli were presented. Contacts in the language network showed elevated but slower responses when text was presented. Finally, responses within the canonical default network showed sustained responses that began toward the end of the prompt period and persisted into the imagining period. Ongoing analyses are investigating differences between types of content and the influence of vividness.

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Poster

PSTR245: Perception, Imagery, and Imagination II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR245.29/U16

Topic: H.02. Perception and Imagery

Support: National Natural Science Foundation of China (32271089)
Ministry of Science and Technology of China (STI2030-Major Projects 2021ZD0204202)
CAS Project for Young Scientists in Basic Research (YSBR-071)

Title: Exploring visual imagery representations in the general population and aphantasia

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Abstract: Visual imagery involves visualizing images in the mind without relying on external sensory input. One central debate in the field of visual imagery is whether imagery is propositional or depictive. The recent discovery of aphantasia has fundamentally challenged this decades-long debate, as those individuals subjectively report an inability to voluntarily generate visual mental images despite being able to perform everyday cognitive tasks. However, the neural mechanism that accounts for this difference in subjective visual experience remains unclear. To investigate this, we designed an fMRI experiment comparing working memory and visual imagery between the general population and individuals with aphantasia. Prior to the main task, participants learned the associations between pseudo-words and pictures of objects from four different categories. On each trial during the fMRI task, participants either viewed a picture of a learned object or a pseudo-word, and were then asked to either retain the previously seen object (working memory) or imagine the corresponding object associated with the pseudo-word cue (imagery) during a delay period. Following the delay they reported their imagery content, vividness, and effort levels. Behaviorally, aphantasic individuals subjectively reported significantly lower vividness and higher effort levels compared with the general population. Moreover, aphantasic individuals performed worse on memorizing the contents of imagery compared to those of working memory, while the general population demonstrated comparable performance for the two. To further explore the neural correlates of these behavioral effects, we trained SVM classifiers to decode object information across the trial time course during each task, in brain regions including the early visual cortex (EVC), the object-selective fusiform cortex (FFC), and the intraparietal sulcus (IPS). Object information was decodable in both groups and tasks in all brain regions of interest. However, when training the classifier with a perception task, results further diverged. While results for the general population remained largely unchanged, results for aphantasia differed across brain regions during the imagery task: object information was still decodable in FFC, decodable but delayed in IPS, and no longer decodable in EVC. These findings suggest that the impaired ability to create voluntary mental images affects multiple aspects of participants' imagery behavior, and the format of object representation in the EVC and IPS differs between aphantasics and the general population during imagery tasks.

Disclosures: T. Wu: None. **Y. Hu:** None. **X. Zeng:** None. **Q. Yu:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.01/U17

Topic: H.03. Decision Making

Support: P50 -MH119569
T32-MH115886
R01-MH123661

Title: Medial prefrontal neuronal dynamics during explore and exploit behavior in a mouse bandit decision making task

Authors: *D. MUELLER[1], E. KNEP[2], A. VELOSA[1], U. MUGAN[3], A. YANG[1], B. EBITZ[6], A. D. REDISH[4], N. M. GRISSOM[5];
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Abstract: The medial prefrontal cortex (mPFC) is known to regulate executive functions, such as decision making and cognitive flexibility. The mPFC has been identified as involved in mediating the tradeoff between exploration and exploitation in complex tasks. We recorded from neural ensembles in mPFC via silicon probes in freely-behaving mice in a two arm restless bandit task, in which the reward probability of each choice changed randomly and independently across trials, and used a hidden Markov model (HMM) to identify explore and exploit action states. We asked whether mPFC ensembles differed between these states to encode different stages throughout the task. Previous research has demonstrated that rule states like exploration and exploitation are reflected in the global activity of the prefrontal cortex and at the level of single prefrontal neurons in primates. As the rules governing rewarded actions are established throughout a session of the bandit task, rule and cue information coded by mPFC neurons should increase, and we expect that correlated ensembles of neurons in this region will develop. Mice (129/b6j F1, 4 M, 4 F) were trained on two arm restless bandit schedules and implanted unilaterally with Cambridge Neurotech silicon probes (2 shank, 32 channels) directed to anterior cingulate and prelimbic cortex (AP +1.8, ML +/- 0.30, DV -2.2); single units were recorded and isolated using kilosort and phy. Units encoding outcomes, valence, and choices have been identified. Preliminary data suggest that in bins of trials surrounding state transitions, there was more correlated activity in exploration-state labeled trials when preceding an exploitation state, and a decorrelation in trials just prior to transitions into exploration. This research will provide insight into the specific decision states and mechanisms mediating complex decision making and individual decision making strategies.

Disclosures: D. Mueller: None. **E. Knep:** None. **A. Velosa:** None. **U. Mugan:** None. **A. Yang:** None. **B. Ebitz:** None. **A.D. Redish:** None. **N.M. Grissom:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.02/U18

Topic: H.03. Decision Making

Support: P50-MH119569
T32-MH115886
R01-MH123661
T32-DA007234

Title: Exploit states, past rewards, and female sex independently reduce action variability in decision making

Authors: D. MUELLER[1], E. M. GIGLIO[1], C. CHEN[2], A. HOLM[3], B. EBITZ[4], ***N. M. GRISSOM**[2];
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Abstract: Sequential reward-guided decision making tasks, such as multi-armed bandit tasks, are well known to engage explore-exploit tradeoffs. Choices in these tasks involve exploit behavior - sustained, repeated selection of rewarding choices, and exploratory behavior - switching between options to determine current values. While significant efforts have gone towards identifying the mechanisms governing choice selection in multi-armed tasks in animal models, little is known about whether the actions necessary to perform these choices also reflect explore-exploit tradeoffs. We took advantage of the fine-grained, continuous measurement of response location coordinates offered by touchscreens in rodent tasks to ask whether explore and exploit states as assigned by hidden Markov model (HMM) significantly impact the location of touches in a mouse restless bandit task. Thirty-two 129/b6j F1 mice (16 male and 16 female) were tested on restless bandit schedules. By employing both Euclidean and Mahalanobis analyses we find that successive touches to the same choice are further apart while an animal is in an explore state than in an exploit state, suggesting greater motor stereotypy when exploiting an option. Further, we have also found sex differences in the area of nosepoke coordinates, suggesting that males in an explore state interact with a larger area of the touchscreen than females in the same state. Additionally, there is an impact of reward on the distance between successive touches, indicating spatial adjustments immediately following trial by trial feedback. This novel analysis capitalizes on the hidden potential for touchscreens to inform not only choice behaviors but the motor actions that generate them, and the neural states that unite movement and cognition.

Disclosures: D. Mueller: None. **E.M. Giglio:** None. **C. Chen:** None. **A. Holm:** None. **B. Ebitz:** None. **N.M. Grissom:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.03/U19

Topic: H.03. Decision Making

Support: NIMH Grant 5P50MH119569
NIDA Grant 5R25DA057802-02

Title: Impairments in decision making following CRISPR-mediated ablation of NMDA receptors in mouse medial prefrontal cortex

Authors: *E. KNEP[1], A. VELOSA[1], D. MUELLER[1], R. DICK[1], B. EBITZ[2], P. E. ROTHWELL[1], N. M. GRISSOM[1];
[1]Univ. of Minnesota, Minneapolis, MN; [2]Neurosciences, Univ. de Montréal, Montréal, QC, Canada

Abstract: Understanding the etiology of cognitive symptoms in schizophrenia is important because these are the most significant symptoms impacting quality of life, but there are currently no medications aimed at treating them. System-wide NMDA receptor antagonists have been shown to produce psychosis-like phenotypes in non-human primates, mice, and humans. Frontal cortex has long been a high-priority target in understanding schizophrenia, and it is assumed that the effects of NMDA receptor antagonists are due to their effects on frontal cortex function, an untested hypothesis. To address this hypothesis, we examined the impact of CRISPR-mediated NMDA receptor ablation in the medial prefrontal cortex on mouse cognition. A key translational method for assessing cognition across mice and humans is a bandit decision making task, where the reward probability of multiple items changes independently and randomly from each other. A key measure is the balance between exploration behavior, switching choices to sample outcomes, versus exploitation behavior, staying on one choice. Explore-exploit balance has been repeatedly seen to be altered in schizophrenia and psychosis. To determine if altered explore-exploit balance is caused by NMDA receptor dysfunction we are targeting anterior cingulate and prelimbic cortex (AP +1.8, ML +/-0.30, DV -2.2), to ablate the obligate GluN1 subunit, coded by the GriN1 gene, of the receptor via cre-dependent activation of CRISPR-CAS9 in the mPFC. 35 cas9-knockin b6j mice (17 GriN1 experimental virus, 17 LacZ control virus) were tested on restless bandit schedules and 8 weeks of viral expression occurred. Preliminary results show that GriN1-deletion mice are able to complete the task, but show impairments in correct performance. Further work will establish if the explore-exploit balance is shifted and whether this changes over expression of the virus. In the end, this novel viral application and combination with a restless bandit task can help us determine if NMDA receptor functionality is important for the etiology of schizophrenia.

Disclosures: E. Knep: None. **A. Velosa:** None. **D. Mueller:** None. **R. Dick:** None. **B. Ebitz:** None. **P.E. Rothwell:** None. **N.M. Grissom:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.04/U20

Topic: H.03. Decision Making

Support: U19 NS113201 (NINDS)
Simons Collaboration on the Global Brain
Helen Hay Whitney Foundation

Title: Neuronal mechanisms of flexible decision-making during foraging

Authors: *L. KINGSBURY, N. UCHIDA;
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Abstract: For most animals, survival depends critically on the ability to forage for resources that are distributed throughout the environment. This involves making decisions about when to harvest from a "patch" of food/water, and when to leave in search of other opportunities. Across different natural environments, animals can use knowledge of resource statistics to flexibly adjust their foraging strategies. This capacity to adapt behavior to different contexts is a core feature of cognition, and while there is some understanding of how the brain instantiates value-based decisions, it remains largely unclear how neural circuits that implement decision computations are contextually reconfigured to alter their functionality. Foraging provides a natural window into the circuit logic and implementation that enable cognitive and behavioral flexibility. In this study, we use structurally equivalent freely-moving and head-fixed virtual foraging tasks to investigate flexible decision-making in mice. Animals navigate different environments to find patches that give water rewards; while *deterministic* patches yield a fixed, predictable reward sequence, *stochastic* patches give probabilistic rewards that diminish over time. Mice learn to adapt their decision process across contexts to flexibly exploit different resource statistics. Building on decision theory and previous work in our lab, we find that an integrator model captures foraging decisions as well as context-driven changes in decision computations. Neural recordings reveal that different environments recruit distinct modes of brain activity, particularly in the dorsal frontal cortex, driving separate population subspaces that encode context-specific decision variables for foraging.

Disclosures: L. Kingsbury: None. **N. Uchida:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.05/U21

Topic: H.03. Decision Making

Title: The role of the rat medial frontal cortex in perceptual decision making

Authors: *J. PALMER[1], K. CHAVEZ LOPEZ[2], M. LAUBACH[2];
[1]American Univ., Washington, DC; [2]Neurosci., American Univ., Washington, DC

Abstract: Our recent study (Palmer et al., 2024) reported that reversibly inactivating the rat medial frontal cortex (MFC) speeds up decision-making without impacting accuracy in a two-alternative forced-choice task. Drift-diffusion models (DDMs) suggested the MFC has a role in maintaining the decision threshold. The task involved choosing between brighter (larger reward) and dimmer (smaller reward) stimuli or detecting the stimuli presented as single offers. Since the brightness of the stimuli was linked to reward size, it was challenging to determine if the MFC is involved in processing perceptual or value-based information specifically. In this study, we

present a variation of the task that isolates the perceptual side of the decision process. Rats initiate cue presentation by holding their nose in a central port. They can sample the cue for as long as they choose. Withdrawing from the central port turns off the cue. To receive a reward (liquid sucrose), they then nosepoke below the location of the presented cue and travel to a reward port on the opposite wall in the behavioral arena. Three cues with varying luminance levels are used. Each cue consists of illuminating 1, 4, or 16 LEDs on an 8x8 grid (randomized by side) for 1 millisecond, creating dynamic stimuli. Behavioral findings so far include: (1) Detection rates increase with brightness. (2) Sampling times are shorter, but choice times are longer on error trials (misses) compared to correct trials (hits). (3) Drift rate positively correlates with luminance. We then used reversible inactivation methods (muscimol) to understand the role of the MFC in perceptual decisions. We found the same main result from the Palmer 2024 study: MFC inactivation speeds up response times but doesn't affect detection rates. It increases drift rate and lowers the decision threshold, without having effects on non-decision time. Neural recordings in MFC found no evidence for stimulus-evoked responses. Instead, neural activity modulated around the animals' actions in the task, especially when they initiated and terminated sampling the stimuli and then made a choice. A 2 Hz "delta" rhythm was observed in LFP recordings as animals initiated trials. This rhythm decreases during cue sampling and is followed by strong inter-trial coherence during the choice period. Synchronization strength tracks the luminance of the previous cue during the period of choice. Delta power is also higher after an error, remaining elevated into the inter-trial interval, and is followed by enhanced inter-trial coherence during cue sampling after an error. These studies suggest a role for the rat MFC in the performance monitoring of perceptual decisions.

Disclosures: J. Palmer: None. **K. Chavez Lopez:** None. **M. Laubach:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.06/U22

Topic: H.03. Decision Making

Support: Lundbeck foundation grant DANDRITE-R248-2016-2518

Title: Encoding of uncertainty and reward by medial prefrontal cortical neurons in mice

Authors: *D. KVITSIANI[1], E. DEMIR[2];
[1]Anat., Southern Illinois Univ., Carbondale, IL; [2]Southern Illinois Univ., Carbondale, IL

Abstract: In reward foraging tasks, animals must consider the frequency of rewards and the uncertainty associated with them as separate variables to maximize their reward harvesting efficiency. Previous research has provided some insights into how individual neurons encode uncertainty and the magnitude of rewards. In our study, we sought to uncover how uncertainty and reward frequency manifest as outcomes of computations within local circuits of the medial

prefrontal cortex. To this end, we trained mice in a task that offered rewards probabilistically, with both reward frequency and uncertainty independently controlled. We then recorded from different classes of neurons, including parvalbumin and somatostatin interneurons, as well as putative pyramidal neurons, using channelrhodopsin-assisted electrophysiological tagging. We will share our findings on how uncertainty and reward frequency are represented by specific cell types in the medial prefrontal cortex.

Disclosures: D. Kvitsiani: None. **E. Demir:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.07/U23

Topic: H.03. Decision Making

Support: JSPS KAKENHI (grant number JP18H05213, JP23H05476)
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Title: Cortical abstraction of cognitive shortcut strategy for efficient decision making

Authors: *A. IBRAHIM ZAHER IBRAHIM[1,2,3,4,5], K. ABDOU[5], M. NOMOTO[1,2,3,4], K. YAMADA-NOMOTO[1,2,3,4], A. CHOUCRY[1,3,6,2], A. CHOUCRY[1], M. FAYED[1,2,3,4], R. OKUBO-SUZUKI[1,2,3,4], K. INOKUCHI[1,2,3,4];
[1]Biochem., Univ. of Toyama, Toyama, Japan; [2]Grad. Sch. of Med. and Pharmaceut. Sciences, Univ. of Toyama, Toyama, Japan, Toyama, Japan; [3]Res. Ctr. for Idling Brain Science, Univ. of Toyama, Toyama,Japan, Toyama, Japan; [4]CREST, Japan Sci. and Technol. Agency (JST), Univ. of Toyama, Toyama, Japan, Toyama, Japan; [5]Dept. of Biochemistry, Fac. of Pharmacy, Cairo University, Cairo, Egypt, Cairo, Egypt; [6]Biochem., Toyama, Japan

Abstract: Creative problem-solving is crucial for our development. Every day we face countless decisions; Some are simple and others are complex. To simplify the decision-making process, we use “Heuristics” or commonly known as “Cognitive shortcuts” to provide us a fast, automatic decision with less effort. Creative heuristics are usually related to idling “Period of Sleep or Rest”. Idling moments are an important factor to help us to inspire insight and solve problems that our conscious minds failed to solve it during the day. However, when and what is the mechanism responsible for the emergence of this insight is still not well understood. Our aim is

to identify how our brain uses learned knowledge during idling to generate creative heuristics. We hypothesize that idling is crucial for generation of Eureka moment brought by replay of existing knowledge and generating new associations to help in creating novel mental shortcuts. We have designed 'Sequences-induced shortcut' "SIS" automated behavior paradigm, in which mice learn different tone sequences with different reward location, but they share a common hidden rule. Mice are tested across different testing stages to apply heuristics using this hidden-rule. These tests could address mouse ability to execute both types of Heuristics; "Time-efficient Heuristics" and "Information-limitation Heuristics". Together, our data shows that sleep and idling moments play a vital role in both, cognition and gist abstraction. Chemo-genetics have shown that Prelimbic cortex have an important role for driving both types of Heuristics. These findings could help in identifying the brain machinery involved in creative problem-solving relying on speedy decision-making

Disclosures: A. Ibrahim Zaher Ibrahim: None. **K. Abdou:** None. **M. Nomoto:** None. **K. Yamada-Nomoto:** None. **A. Choucry:** None. **M. Fayed:** None. **R. Okubo-Suzuki:** None. **K. Inokuchi:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.08/U24

Topic: H.03. Decision Making

Title: Neural correlates of volatile sensory and latent task variables in the anterior cingulate cortex and primary visual cortex in an audio-visual 2-alternative forced choice contextual decision-making task in mice

Authors: *K. SAFARYAN[1], R. LUONG[2], J. LEONOR[2], A. SAATI[2], T. LUONG[2], S. KAISER[2], P. GOLSHANI[1];
[1]UCLA Dept. of Neurol., Los Angeles, CA; [2]UCLA Dept. of Neurol., LOS ANGELES, CA

Abstract: Cognitive flexibility allows animals to make rewarding decisions in rapidly changing environments. Flexible responses to the same stimulus in different contexts require selective attention and continual appraisal of contextual variables. Yet, how distinct cortical circuits encode contextual variables and gate relevant and non-relevant stimuli to derive context-dependent decision making is not understood. To address this problem, mice were trained to perform serial extradimensional shifts (SEDS), where cross-modal stimuli, visual gratings of 45^{o} and 135^{o}, and auditory low(5Hz)- and high(18Hz)- pitch tones, interchangeably define trial outcome or serve as distractor. In this compound discrimination task, where the auditory and visual stimuli are presented simultaneously, mice have to infer which stimulus modality is relevant or serve as the distraction. The shifts between relevant modalities were covert and accompanied by immediate fast decline of the performance with consequent gradual improvement. We estimated performance with parametric sigmoidal model fit to the

performance in each block. Each block on average consisted of 90-100 trials with after-switch recovery periods found to be shorter in the auditory-attend blocks (exponential fit tau = 40.4±11.47 trials) compared to the visual-attend blocks (exponential fit tau = 48.7±17.38 trials). Reaction times were faster in the auditory-attend hit trials (~0.16 sec.) compared to the visual-attend hit trials (~0.22 sec.). The reaction times in missed visual-attend trials were similar to the auditory-attend hit trials signifying perceptual engagement. Here, we study neural correlates of the sensory stimuli across the alternating contexts in the anterior cingulate cortex (ACC) and primary visual cortex (V1). We recorded from multiple units using simultaneous Neuropixels recordings from the ACC and V1. All recorded units were classified as putative excitatory or inhibitory based on mean firing rate and spike waveform features. Separate ratemaps were constructed across trials of the same pairings of audiovisual stimuli in blocks of the SEDS for the expert performance epochs. Both the ACC and V1 units encode a wide range of task variables, whereas encoding of audiovisual stimuli were predominantly seen in the V1. These results show correlated nature of the ACC and V1 responses during contextual decision-making task in mice.

Disclosures: K. Safaryan: None. **R. Luong:** None. **J. Leonor:** None. **A. Saati:** None. **T. Luong:** None. **S. Kaiser:** None. **P. Golshani:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.09/U25

Topic: H.03. Decision Making

Support: NIH R01 DA047870

Title: Dissociable contributions of rat anterior cingulate cortex and orbitofrontal cortex in learning under volatile conditions

Authors: *P. GANUPURU[1], J. L. ROMERO SOSA[2], A. IZQUIERDO[3];
[1]UCLA, Los Angeles, CA; [2]Neurobio., UCLA, Los Angeles, CA; [3]Dept. of Psychology, UCLA, Los Angeles, CA

Abstract: To make successful decisions in unfamiliar contexts, the brain must identify the optimal action given limited information in the reward environment and judge the success of prior actions to guide future learning and decision making. Here we examined how specific frontocortical regions, anterior cingulate cortex (ACC) and orbitofrontal cortex (OFC), mediate flexible adjustments of decision strategy in the face of environmental volatility. To this end, we trained rats to perform a “restless bandit” task in which subjects select between two choices, each associated with a different reward probability (0.80 vs. 0.20), with the reward probability of the choices alternating after a relatively low or high number of trials (m_{low} = 25, SD = 4; m_{high} = 60, SD = 4). We first infused a virus containing inhibitory hM4Di DREADDs on a CaMK promoter targeted at rat ventrolateral OFC (n = 4) or ACC (n = 4). While rats performed this task, we

chemogenetically perturbed ACC and OFC (via administration of 0.1 mg/kg deschloroclozapine, DCZ) to test the role of each region in adapting to volatility. In rats with confirmed well-targeted DREADDs expression with histological verification, we found that there was a significant session by inactivation interaction on probability correct (our main measure of accuracy) for both ACC and OFC hM4Di groups (mixed-effects GLM). After transition to a high volatility condition, ACC perturbation brought about a relatively higher weighting of older evidence whereas OFC perturbation generally decreased the influence of evidence over recent decisions, each compared to a vehicle control administered within-subject. Further, there was a significant effect of OFC, but not ACC, inactivation on hastening reward latencies, and conversely a significant effect of ACC, but not OFC, inactivation on lengthening initiation latencies. These results expand our understanding of how the brain adapts to different reward environments to support adaptive learning and shed light on the frontocortical systems that are necessary for flexible reward learning under conditions of uncertainty.

Disclosures: P. Ganupuru: None. **J.L. Romero Sosa:** None. **A. Izquierdo:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.10/U26

Topic: H.03. Decision Making

Title: Optogenetic inhibition of mPFC projecting insula cell bodies disrupts rule shifting behavior

Authors: *L. L. HAGOPIAN[1], V. S. SOHAL[2];
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Abstract: In many cases, the ability to shift behavior based on environmental cues has been shown to depend on the medial prefrontal cortex (mPFC). Our laboratory tests this ability in mice using a rule shifting task in which mice must learn to shift between texture and odor rules in order to find a hidden food reward (Cho et al., Neuron, 2015). Gamma-frequency activity in parvalbumin (PV) interneurons normally synchronizes between the left and right hemispheres when mice learn a rule shift, and disrupting this synchronization hinders the ability to learn a rule shift and causes perseveration (Cho et al 2020). However, the roles of other brain regions in generating this inter-hemispheric mPFC gamma synchrony is unclear. The insula, known for its potential involvement in cognitive and gustatory function, has bidirectional connectivity with the mPFC (Gogolla 2017). We found that bilateral optogenetic inhibition of mPFC- projecting insula neurons causes significant impairment when mice perform rule shifts specifically from texture-based rules to odor-based ones (number of trials needed to reach the learning criterion = 23.3 ± 6.9 in eNpHR-expressing mice vs. 16.6 ± 5.3 in controls, $p < 0.03$, $n = 18$). This suggests that upstream regions may play sensory cue modality-specific roles in rule shifting. We are currently

using Transmembrane Electrical Measurements Performed Optically (TEMPO), to study the insula's impact on gamma synchrony in mPFC PV interneurons, in order to test whether inhibiting mPFC- projecting insula neurons reduces mPFC gamma synchrony specifically during texture to odor shifts. We are also confirming that the role of the insula is specific to texture-to-order shifts, and exploring gamma synchrony between the mPFC and insula.

Disclosures: L.L. Hagopian: None. **V.S. Sohal:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.11/U27

Topic: H.03. Decision Making

Support: NARSAD YI Grant #31117
R01NS116594

Title: Prefrontal astrocytes enable cognitive flexibility and mediate task-dependent synchronization of gamma oscillations in parvalbumin interneurons

Authors: *L. SHINDY[1], A. J. PHENSY DOS SANTOS[1], L. HAGOPIAN[2], V. S. SOHAL[3]; [1]Psychiatry, Univ. of California, San Francisco, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA, ; [3]Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA

Abstract: Astrocytes have long been considered passive support cells that have a relatively small role in information processing in the brain. However, recent discoveries have revealed that astrocytes are recruited during cognitive behaviors and can directly shape neuronal signaling and influence behavior. Importantly, though astrocyte dysfunction is well documented in many diseases impacting PFC-dependent cognitive function - there is still very little known about how these deficits could causally affect cognitive flexibility. Here, we demonstrate that prefrontal astrocytes actively contribute to cognitive processing through the modulation of neuronal oscillations. We first utilized bulk calcium photometry to reveal that in-vivo activation of the hM3D (Gq) designer receptor led to a robust silencing of prefrontal astrocytes - allowing us to study how loss of astrocyte function impacts cognitive function. We next found that during a rule-shift task used to assess cognitive flexibility in mice, activation of hM3D (Gq) receptors on prefrontal astrocytes impaired mice's ability to perform extradimensional shifts but did not impact performance during the learning of initial associations. Further, hM3D (Gq) activation prevented the synchronization of neural oscillations in prefrontal parvalbumin-positive interneurons, which we have previously found to be critical for cognitive flexibility. Together, these findings suggest that prefrontal astrocytes actively support neural synchrony dynamics and thus may have the potential to enhance neural connectivity and PFC-dependent cognitive function.

Disclosures: L. Shindy: None. **A.J. Phensy dos Santos:** None. **V.S. Sohal:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.12/U28

Topic: H.03. Decision Making

Support: R01NS116594
NARSAD YI Grant #31117

Title: Cross-hemispheric gamma synchrony in prefrontal PVI and subpopulations of projection neurons are modulated differentially in a task-dependent manner

Authors: *A. PHENSY DOS SANTOS[1], L. HAGOPIAN[2], C. COSTELLO[2,3], V. S. SOHAL[4]; [2]Psychiatry, [1]Univ. of California, San Francisco, San Francisco, CA; [3]Psychiatry, Univ. of California, San Francisco, CA; [4]Dept. of Psychiatry, U. California, San Francisco, San Francisco, CA

Abstract: Neural networks in the prefrontal cortex (PFC) are responsible for a number of cognitive processes known to be disrupted in conditions such as schizophrenia. These networks, which are organized by both local microcircuits and inter-regional projections, require precisely tuned neuronal activity to generate appropriate cognitive responses. One way these networks achieve this precision is through synchronization of neural oscillations which may entrain relevant neurons and/or reduce the activity of irrelevant neurons. Gamma-frequency oscillations have been shown to play an important role in prefrontal-dependent cognition. Our lab has previously shown that cross-hemispheric gamma synchrony of parvalbumin positive interneurons (PVI) in the PFC is necessary for mice to shift between strategies to obtain a food reward. Here, we utilize a combination of single and dual-colored genetically encoded voltage indicators (GEVIs) and optogenetics to reveal that during rule shifts, PVI drive gamma synchrony in select subpopulations of PFC projection neurons targeting the MD thalamus in order to support flexible behavior. Notably, this synchrony does not emerge in nearby neurons projecting to the dorsal striatum, highlighting that gamma oscillations can preferentially entrain specific subpopulations of neurons. Further, while PVI are necessary for the emergence of gamma synchrony in MD-projecting cells, peak synchrony occurs in these populations at different times, suggesting a complex interaction that evolves as animals adapt to changes in task structure. This work demonstrates that the PFC engages gamma synchrony across multiple channels to differentially target downstream regions during different task phases.

Disclosures: A. Phensy dos Santos: None. **L. Hagopian:** None. **C. Costello:** None. **V.S. Sohal:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.13/U29

Topic: H.03. Decision Making

Support: Simons Foundation Autism Research Initiative Sex Differences Collaboration

Title: Improving Behavior Decoding from Neural Activity Data with Modified Neural Network Classifiers

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Abstract: A prominent application of machine learning to systems neuroscience has been the use of classifiers to decode distinct behaviors from the activity of neuronal populations recorded in vivo, e.g., using microendoscopic calcium imaging. In particular, we have previously used neural network classifiers to examine how individual/groups of neurons encode social behaviors. By examining the pattern of weights in trained neural networks we can identify ensembles of neurons which encode social vs. nonsocial behavior, then examine how activity levels or correlated activity within these ensembles changes during social interaction. We can also test these networks using data shuffled to preserve or disrupt changes in single neuron activity or correlations between neurons to examine the extent to which changes in synchronized/correlated activity between neurons encode behaviorally relevant information beyond that which is transmitted by changes in single neuron activity levels. Previously, we carried this out using a simple three-layer linear neural network (Frost et al., PLoS Biology, 2021) and found that correlated activity plays an important role in encoding social behaviors. Here we explore how introducing nonlinearities into the activation functions of hidden layer units potentially makes this type of neural network classifier more sensitive to correlated activity and enhances its ability to reliably decode social behavior. Specifically, we have initially focused on the effects of introducing a threshold linear activation for hidden layer units. We trained and tested classifiers to decode social behavior based on calcium imaging data recorded from medial prefrontal cortex (mPFC) neurons during a social interaction assay. Classifiers distinguished frames of calcium imaging data corresponding to either a baseline period in which the subject was alone or social epochs in which the subject was interacting with a juvenile mouse. We observed a significant increase in performance in our classifiers using threshold linear activation functions compared to entirely linear classifiers (threshold linear classifier performance = 0.8626 ± 0.01287 vs. linear classifier performance = 0.7718 ± 0.02421, $p = 0.0002$, $n = 14$ mice). We are currently exploring how this improvement in performance is related to an enhanced sensitivity to coactivity vs. other factors, how these nonlinearities affect generalizability of decoding across multiple social behaviors, how nonlinearity potentially changes the ensembles identified as encoding specific variables, and whether similar nonlinearity-driven changes in performance are associated with distinct, non-social behaviors.

Disclosures: T.E. Bullock: None. **R. Malik:** None. **M. Turner:** None. **V.S. Sohal:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR246.14/U30

Topic: H.03. Decision Making

Title: Chemogenetic modulation of the prelimbic cortex to the nucleus accumbens core circuit reduces cocaine-induced increase of risk choice behavior

Authors: *J. HAN[1], M. KWAK[2], W. KIM[3], J.-H. KIM[4];
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Abstract: Decision-making is critically impaired in individuals with abnormal psychiatric conditions like gambling disorder and substance abuse. These impairments are associated with deficits in top-down executive control governed by the medial prefrontal cortex (mPFC) and its fronto-striatal connections to the nucleus accumbens (NAc), particularly involving the prelimbic (PrL) region of the mPFC and the NAc core. This study employed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), a chemogenetic technique, to examine whether modulating the activity of the PrL to NAc core neural circuits affects risk-taking behaviors. Rats were trained on a rat gambling task (rGT) until their choices among four options, each with varying probabilities of rewards and punishments, showed stable preferences. Subsequently, based on their preferences, the rats were categorized into two groups—risk-averse and risk-seeking—and exposed to two different experimental conditions. One group of rats underwent a cocaine sensitization regime to observe changes in decision-making following cocaine administration and neuronal modulation with Gi and Gq DREADDs. The other group experienced neuronal modulation without cocaine exposure. The results indicated that cocaine typically led risk-averse rats to make riskier choices. Interestingly, this effect was significantly reduced by activating the Gi DREADD in the PrL-NAc core circuit, an outcome not mirrored by activating the Gq protein. Moreover, there were no notable changes in decision-making when neuronal activity was modulated without cocaine. These results indicate that the PrL-NAc core circuit is one of the major target area exacerbated by chronic cocaine leading to risky decision-making and further suggest that this effect can be controlled by neuronal activity modulation to this circuit.

Disclosures: J. Han: None. **M. Kwak:** None. **W. Kim:** None. **J. Kim:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

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Program #/Poster #: PSTR246.15/U31

Topic: H.03. Decision Making

Support: NIH 5P01AI073693-14

Title: Ensemble dynamics in medial prefrontal cortex reflect dominance hierarchy during social behavior

Authors: K. YEUNG[1], A. LARA-VASQUEZ[2], J. GALLAGHER[1], R. DAWKINS[1], P. FUENTEALBA[3,4], P. T. HUERTA[1,5], *J. J. STROHL[1];
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Abstract: Within social groups, decisions are often made through group processes involving individuals at different hierarchical levels. Those higher in the hierarchy might exert more influence over group decisions, potentially leading to skewed outcomes. The medial prefrontal cortex has emerged as a key neural substrate at the intersection of social and spatial cognition. We study how dominance hierarchy regulates social behavior during spatial movement (PMC10879816) by using a goal-directed spatial task in which mice navigate collectively with their littermates foraging for food (located at a designated goal arm in a T maze). We recorded neuronal spikes and local field potentials (LFPs) with tetrodes implanted in prelimbic cortex (PLC) and infralimbic cortex (ILC) in freely moving mice (C57BL/6j males, n = 20, grouped in cages of 4). First, we classified their hierarchy with the tube test which yielded robust social rankings (SR), from dominant (SR1) to submissive (SR4), for each cage. Mice were trained in solo and collective trials. Spikes were sorted using an automated pipeline (PMC8609830) and LFPs were analyzed as canonical bands (in Hz): θ (7-10), β (20-30), γ1 (35-50), γ2 (50-90), and γ3 (90-150). Here, we focus on collective trials and found that the mean firing rate (SR1, 4.2, SR4, 2.2 Hz, $P < 1E-3$) as well as the peak firing rate (SR1, 35.7, SR4, 24.3 Hz, $P < 0.01$, t test) were significantly higher in dominant mice. Notably, PLC-LFP analysis revealed that dominant mice had higher power (values expressed as E-10 mV^2) in β (SR1, 15, SR4, 13, $P < 0.01$), γ1 (SR1, 9.7, SR4, 7.4, $P < 1E-4$), γ2 (SR1, 15, SR4, 9.7, $P < 1E-4$), and γ3 (SR1, 11, SR4, 6.6, $P < 1E-4$). The ILC-LFP data showed a similar pattern with the dominant mice having much higher power in β (SR1, 17, SR4, 10, $P < 1E-4$), γ1 (SR1, 9, SR4, 6.8, $P < 1E-4$), γ2 (SR1, 12, SR4, 8.6, $P < 1E-4$), and γ3 (SR1, 10, SR4, 7, $P < 0.05$; Mann-Whitney U test). Remarkably, we found that when dominant mice led others (and chose the correct arm), the θ band had the highest power in PLC (45E-10) and ILC (60E-10). Conversely, when dominant mice followed others (but still chose the correct arm), the γ3 band had the weakest power in PLC (8.7E-10) and ILC (7.2E-10). Our results thus reveal that, during social behavior, both neuronal firing rates and ensemble band power are strongly associated to social rank, with dominant mice showing higher β, γ1, γ2, and

γ3. Even more remarkably, we find that social decision-making in dominant mice is strongly associated with high θ power and low γ3 power. In summary, these findings might help bridge neuronal properties of the prefrontal cortex ensembles and decision-making in social contexts.

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Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

Location: MCP Hall A

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Topic: H.03. Decision Making

Support: KAKENHI 24K00516
KAKENHI 21K18562
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The Takeda Science Foundation
The Uehara Memorial Foundation
The Naito Foundation

Title: Distinct locus coeruleus projections to the prefrontal cortex and hippocampus regulate memory flexibility during reversal learning

Authors: *S. AMEMIYA, T. J. MCHUGH;
RIKEN Ctr. for Brain Sci., Saitama, Japan

Abstract: In changing environments adaptive decision making relies on the referencing and updating of memories. Locus coeruleus noradrenergic neurons (LC-NA) are known to be involved in these flexible memory processes, as are their postsynaptic target regions, the prefrontal cortex (mPFC) and hippocampus. However how these circuits differentially contribute to these mnemonic processes remains poorly understood. In this study, we investigated the LC-NA circuits involved in reversal learning, a task requiring memory flexibility. We first examined the anatomical organization of LC-NA projections to the mPFC and the hippocampal CA1 region using retrograde tracing with adeno-associated virus vectors encoding different fluorescent proteins. Consistent with previous data, we found largely non-overlapping populations of LC-NA project to the mPFC and the CA1. Subsequently, we assessed noradrenergic activity in the mPFC and the CA1 during reversal learning in a T-maze. Subject mice were trained on the T-maze with one arm baited with pellets and the other having no reward for three consecutive days, then the reward contingency was reversed, and mice ran the reversal condition for four consecutive days. During the task, noradrenaline release in the PFC and the CA1 was monitored by fiber photometry and GRABNE sensors. We observed that the noradrenaline release patterns across maze locations in PFC and the CA1were different, suggesting that the distinct populations of LC-NA neurons projecting to each region were

differentially engaged during the task. However, noradrenaline release in both regions increased when mice exhibited head orienting behavior ("vicarious trial-and-error" (VTE)) at the choice point, a behavioral correlate of memory-guided decision making. Finally, we examined the effects of chemogenetic inhibition of mPFC-projecting LC-NA neurons during the reversal phase of the task and observed impaired reversal learning and suppressed VTE. In summary, our data suggest that distinct subpopulations of LC-NA projecting to the mPF

Disclosures: S. Amemiya: None. **T.J. McHugh:** None.

Poster

PSTR246: Prefrontal Cortex in Non-Primate Mammals

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Program #/Poster #: PSTR246.17/U33

Topic: H.03. Decision Making

Title: Frontal dynamics underlying flexible decision making in mice

Authors: *F. ABELA[1], X. ZHAO[2], J. J. BOS[3], F. P. BATTAGLIA[4], P. H. TIESINGA[4], L. MA[5];
[1]Radboud Univ., Nijmegen, Netherlands; [2]Radboud Univ., Nijmegen, ; [3]Radboud Univ. Nijmegen, Nijmegen, ; [4]Radboud Univ. Nijmegen, Nijmegen, Netherlands; [5]Psychology, York Univ., Toronto, ON, Canada

Abstract: Humans and other animals require flexibility to make decisions appropriate for an ever-changing environment. The precise mechanisms by which mammalian brains achieve this flexibility remain unclear. Here we used the probabilistic reversal learning (PRL) task to assess behavioral flexibility and how positive and negative feedbacks influence decision making. We trained C57BL/6 mice on PRL and conducted ultra-high-density single unit recordings using Neuropixels probes from the medial prefrontal cortex in well-performing animals. The animals had binary choices (e.g., left vs right) associated with different reward probabilities (e.g., 80% vs 20%), which they discovered via trial and error. Once a consistent choice was established, the contingencies were reversed, so the previously highly rewarded side became unfavorable and vice versa. After one month of training, mice were able to flexibly switch contingencies multiple times in one session. We observed that their behavioral performance was not consistent in different blocks within the same training session. This inconsistency led us to hypothesize that the animals use different strategies during learning, and that the alternation of these strategies is reflected both in their behavioral performance and neural activity. We aim to identify and characterize the different learning strategies employed by the mice and investigate how changes in the neural activity correlates with them. We will also characterize the activity of prefrontal neurons associated with stable behavioral performance prior to contingency switches as well as during active learning phase.

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Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.01/U34

Topic: H.03. Decision Making

Support: Leon Levy Foundation postdoctoral scholarship

Title: The effect of hunger on value-based decision making in humans

Authors: *S. BERMAN, D. SHOHAMY;
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: How does the body's physiological state affect the human mind? Expressions like "listen to your gut" are often disregarded as metaphors. However, growing evidence suggests that gut-brain interactions do impact our thoughts and actions. Hunger, in particular, has been shown to have a consistent and dramatic effect on motivation with homeostatic hunger signals modulating the so-called reward system. This modulation increases the incentivizing value of food and drives food-seeking and consumption. Nevertheless, many open questions remain about how hunger affects the human mind. In particular, the reward system is not unique to food behavior, raising the question: are there broader modulatory effects of hunger on valuation and value-guided cognition, beyond food-related behavior?
To characterize hunger-modulated valuation processes, 70 Healthy participants were recruited online on two separate days, once when fasted and once shortly after a meal. The participants performed subjective valuation and value-based decisions involving both food and non-food rewards. To test the effect of hunger on food, participants rated and chose between visual food cues. For non-food rewards, participants made choices for information, in the forms of answers to trivia questions. Participants rated their curiosity about the trivia questions and later chose whether to view the answer in exchange for a cost (money or time). Results demonstrate that, as expected, hunger increases the value of food. Hunger also was found to change food choices, yielding choices of food that is higher in fat and protein. Finally, we found that hunger had a varied effect on information-seeking and valuation. Specifically, the effects on information seeking varied across participants (Fig. 1). Those who reported more "intuitive eating" showed a relationship between ratings of food and of curiosity. The opposite relationship was found in participants with lower intuitive eating. Though preliminary, these results suggest that differences in sensitivity to physiological signals may mediate the effect of hunger on behavior.

Disclosures: S. Berman: None. **D. Shohamy:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

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Program #/Poster #: PSTR247.02/U35

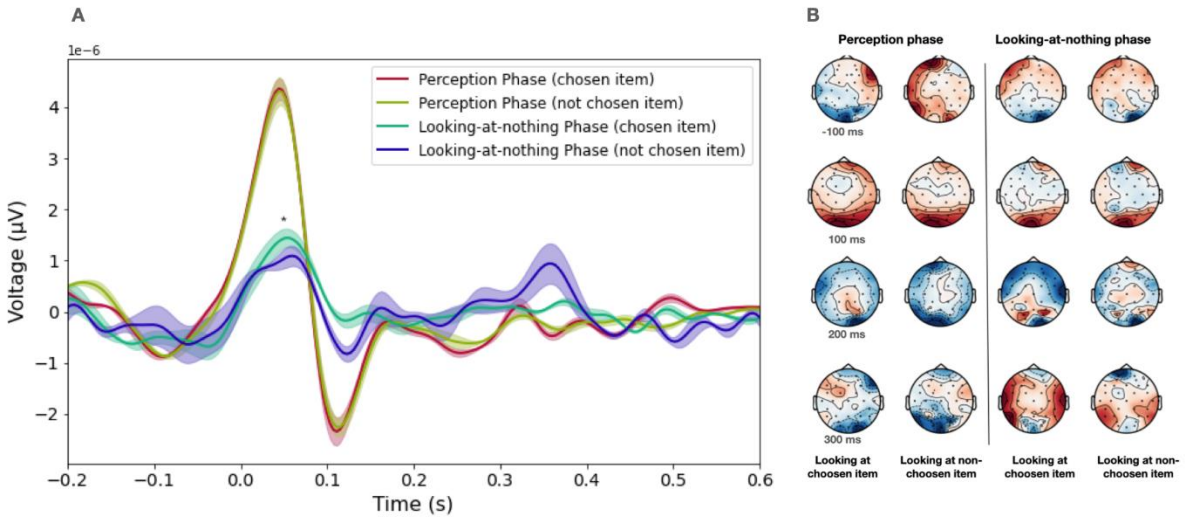
Topic: H.03. Decision Making

Support: FONDECYT N° 3210646

Title: Neural signatures of Mental Imagery During Decision Deliberation: EEG and Eye-Tracking Analysis

Authors: *K. M. PADILLA[1], C. MURUA[2], S. A. MADARIAGA[1,2], P. E. MALDONADO[1,2]; [1]Neurosci., Univ. de Chile, Santiago, Chile; [2]Ctr. Nacional de Inteligencia Artificial (Cenia), Santiago, Chile

Abstract: We navigate through numerous daily decisions, but how do we weigh our choices? While current decision models often prioritize gaze-driven evidence accumulation, they overlook mental imagery's role. Prior research suggests mental imagery is linked to eye movements. People tend to look back at spatial locations of visually encoded information when they recall it through visual imagery, a phenomenon called looking-at-nothing (LAN). Our study explores this interplay, proposing neural networks simulate mental states during deliberation. Combining the LAN paradigm with a preferential choice task, we examined 30 participants (17 women, 13 men). In the perception phase, subjects viewed two food options presented briefly in different screen locations. Subsequently, during a 3-second LAN phase, they contemplated an empty screen before choosing. This design enabled us to scrutinize neuronal correlates during mental imagery, capturing EEG and eye movement data in both phases. In addition, we assessed the vividness of mental imagery using an adapted version of the Vividness of Visual Imagery Questionnaire (VVIQ). We find that during the LAN phase, participants spend more time looking at the empty position where the item to be chosen was presented compared to the portion of space of the item not selected, comparable to the eye movement patterns observed in the presence of stimuli (Song et al., 2019). Analyzing eye-fixation-related potentials (EFRPs) across phases, we observed significant ERP activity even in the absence of stimuli, with the P1 component showing enhanced amplitude when subjects fixated on the chosen item ($F=17.91$, p-value=0.0001). We also found differences according to the VIVIQ score. The increased activity observed in frontal and temporal areas suggests the potential engagement of brain regions associated with mental simulations (Schmidt et al., 2019). Our findings provide a valuable approach to studying the role of mental simulations during deliberation, particularly in conditions where future-oriented cognition is impaired, offering new avenues for research.



Disclosures: K.M. Padilla: None. **C. Murua:** A. Employment/Salary (full or part-time):; Enjoy S.A. **S.A. Madariaga:** None. **P.E. Maldonado:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.03/V1

Topic: H.03. Decision Making

Support: Moonshot R&D JPMJMS2294

Title: Exploring Neurotransmitter Receptors Underlying Social, Food, and Monetary Reward Processing

Authors: *K. MATSUMORI[1], K. MATSUYANAGI[2], K. MATSUMOTO[3];
[1]Tamagawa Univ., Machida, Japan; [2]Brigham Young Univ. Online High Sch., Provo, UT; [3]Brain Sci. Inst., Tamagawa Univ., Machida, Japan

Abstract: Abstract: The neural mechanisms governing reward processing rely on neurotransmitter systems such as dopamine, opioids, serotonin, and oxytocin. However, existing human neuroimaging methods lack the ability to fully elucidate neurotransmitter substrates or assess multiple receptors comprehensively due to their limitations. In this study, we employed newly developed PET-derived receptor density maps and microarray receptor-related gene expression maps to investigate the neurotransmitter receptors implicated in social, food, and

monetary reward processing. Using linear support vector machines, we analyzed whole-brain neural activity patterns elicited by cues related to these rewards. Furthermore, we explored the correlation between neural activity patterns distinguishing between reward conditions and neurotransmitter maps based on both PET-derived receptor density and gene expression data. Our findings indicate distinct receptor systems driving food reward processing compared to social or monetary rewards. This research offers insights that may inform future pharmacological interventions by uncovering the molecular basis of reward representation using non-invasive neuroimaging methods.

Disclosures: K. Matsumori: None. **K. Matsuyanagi:** None. **K. Matsumoto:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

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BK21 FOUR Program Grant No. 5199990314123 funding from the Seoul National University Creative Pioneering Researchers Program
Seoul National University Artificial Intelligence Graduate School Program Grant No. 2021-0-01343

Title: Neural Correlates of Risk and Reward Signals in Smokers Using an MRI-Compatible Vaping Device: A Preliminary Study

Authors: *E. LEE[1], J.-H. LEE[1], J. W. BROWN[2], W.-Y. AHN[1];
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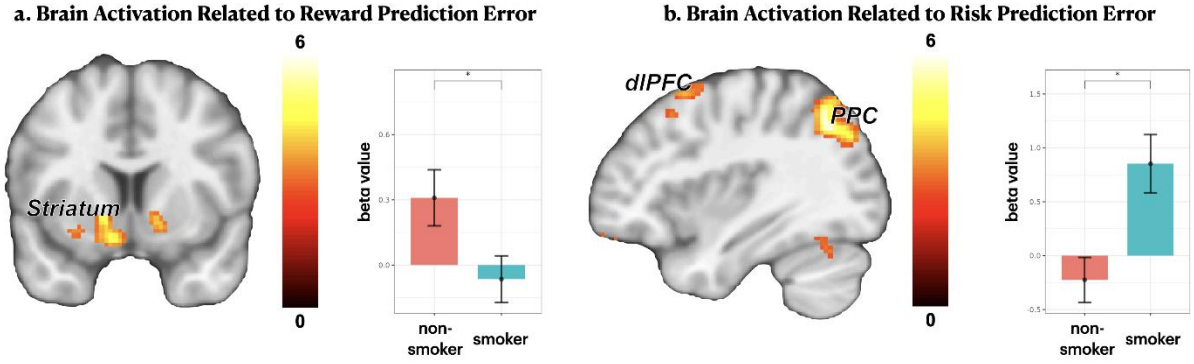
Abstract: Investigating the neural mechanisms of reward and risk processing is crucial for understanding the impaired decision-making in addiction, where the evaluation of reward and risk is often distorted. While it's crucial to utilize primary (drug) rewards to capture the drug-specific neural processes, less is known about reward and risk encoding in the context of actual drug use. This study aims to fill this gap by investigating how reward and risk processing differ between smokers and non-smokers, using both monetary and nicotine rewards in a structured gambling task and a novel MRI-compatible vaping device. We employed functional magnetic resonance imaging (fMRI) to examine the neural correlates of reward prediction errors and risk prediction errors. Preliminary fMRI results with monetary rewards indicated that significant activation in the striatum correlated with reward prediction errors. Additional findings highlighted risk prediction error-related activations in the posterior parietal cortex (PPC) and the

dorsolateral prefrontal cortex (dlPFC). Notably, our results show that smokers exhibited distinct neural responses from non-smokers, suggesting their altered reward and risk encoding. Specifically, the attenuated response of the striatum supports the hypothesis of addiction-related reward system desensitization. Furthermore, the increase on PPC activation during risk prediction error processing in smokers may suggest altered risk encoding in nicotine addiction, necessitating further investigation to unravel the interplay between risk processing and addictive behaviors. These preliminary findings using monetary rewards set the groundwork for forthcoming experiments with the novel MRI-compatible vaping device to directly assess neural responses to nicotine rewards. The use of a direct nicotine reward would further allow for a more accurate depiction of the addiction-specific neural processes. It is our expectation that this study will shed light on our understanding of the distinct neural processing of primary drug rewards.



Disclosures: E. Lee: None. **J. Lee:** None. **J.W. Brown:** None. **W. Ahn:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

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Topic: H.03. Decision Making

Support: Office of Naval Research N00014-15-18-2136
NIH Research Project Grant Program (R01)

Title: Multi-attribute decision-making strategies: exhaustive and filtering

Authors: *W. LEONG[1], J. G. ELSEY[2], E. NIEBUR[3], V. STUPHORN[4];
[2]The Zanvyl Krieger Mind/Brain Inst., [3]Neurosci., [4]Mind/Brain Inst., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: In daily life, most choices between options consist of a number of shared or distinct attributes. In such scenarios, making optimal choices requires integrating and comparing a substantial amount of information simultaneously. The strain on humans' limited cognitive resources prompts them to focus attention on crucial information subsets, driving our

investigation into sampling strategies in multi-attribute decision making (MADM). To better understand the sampling strategies in MADM, we conducted a series of experiments in which we systematically varied the complexity of the choice menu by increasing the number of attributes, or options, or both. We recruited groups of participants to engage in different versions of a MADM task, varying in the presentation of options and attributes, utilizing eye position as an indicator of overt attention while they made decisions among sets of gambles that varied systematically in complexity. Attributes' magnitudes were masked by colored circles, revealed only upon active fixation. Participants freely inspected them without time constraints before indicating their choice via keypress. The resulting eye movements provide temporal and spatial insights into participants' focus of attention during the decision-making process. Using principle component analysis (PCA) and hierarchical clustering based on the order of sampling, we find that participants primarily use two strategies for sampling decision-related information. The *exhaustive* strategy is employed when options are few and involves sampling all attributes of each option independently before moving to the next option (2-Option, 2-Attribute: 68% of participants, 2-Option, 4-Attribute: 66% of participants). The *filtering strategy* is more common when more options are present and entails first comparing the most important attribute across all options to narrow the choice menu to 1-2 options, followed by sampling all attributes of the remaining candidate options (4-Option, 2-Attribute: 75% of participants, 4-Option, 4-Attribute: 68% of participants). While the latter strategy reduces information processing load, it can lead to sub-optimal choices. Notably, the choice of strategy depended on the number of options rather than the number of attributes, highlighting differences in attentional strategy and their role in comparing options when tackling increasingly complex decision problems.

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Poster

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NIH Grant AA027381

Title: A mediating role for delay discounting in the effect of episodic future thinking on demand

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of Michigan Sch. of Med., Ann Arbor, MI; [6]Dept. of Psychiatry and Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada; [7]Advanced Recovery Res. Ctr., Roanoke, VA

Abstract: Reinforcer Pathology proposes that the demand for reinforcers is impacted by the temporal window over which future rewards are evaluated. When the temporal window is short, immediate, brief, and reliable reinforcers are selectively preferred. When the temporal window is long, then protracted and more variable reinforcers of lower intensity become preferred. Altering the duration of one's temporal window may be accomplished by having people vividly envision plausible future events. This manipulation, known as episodic future thinking (EFT), has been shown to change both the duration of the temporal window, as measured through delay discounting (DD), and demand for immediate rewards. Reinforcer Pathology suggests a causal link between the effect of EFT on DD and demand. As the discounting rate is reduced (i.e., the temporal window is lengthened), a corresponding change in the evaluation of reinforcers should occur. Demand for immediate reinforcers such as drugs of abuse and high-calorie foods, should be reduced in proportion to the impact of EFT on DD.
We tested this hypothesis by performing secondary data analysis on papers that assessed the effects of EFT on both DD and demand. We hypothesize that EFT should reduce delay discounting and demand and that the effect of EFT on demand should be mediated by changes in DD. We performed a systematic literature search to identify publications that used EFT and assessed changes in DD and demand. We report mediation analyses for individual studies and at the meta-analytic level.

Disclosures: A. Gervassi-Saga: None. **R. Fontes:** None. **R. Freitas-Lemos:** None. **D. Tomlinson:** None. **J. Mackillop:** None. **J. Stein:** None. **S.M. McClure:** None. **A. Tegge:** None. **W.K. Bickel:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.07/V4

Topic: H.03. Decision Making

Support: RF1-AG067011

Title: Risk for Financial Exploitation and Psychosocial Factors Modulate Age-Related Differences in Social Reward Processing

Authors: *J. WYNGAARDEN[1], C. SHARP[1], A. DACHS[1], D. SAZHIN[1], T. TROPEA[1], I. KOHLI[1], T. GIOVANNETTI[1], D. S. FARERI[2], J. JARCHO[1], D. V. SMITH[1];
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Abstract: Positive social interactions, such as receiving praise from peers, engage neural reward circuitry similar to that activated by monetary rewards, primarily including the ventral striatum (VS). However, social rewards can also evoke activation and connectivity between these areas

and other brain regions more commonly associated with social processing, including regions like the posterior temporoparietal junction (pTPJ) and components of the default mode network (DMN). While these findings have been well documented in young adults and adolescents, less is known about how they change in older adulthood. It could be the case that age-related changes in these neural mechanisms contribute to vulnerability to maladaptive outcomes like risk for financial exploitation. To examine these relationships, the current ongoing study is leveraging matched monetary and social reward tasks in which participants (N = 101; ages 21-80 years; mean = 43.26 years) choose between two stimuli in search of a reward (Quarmley et al., 2019, Frontiers in Neuroscience). In the monetary task, participants choose between two doors to find a monetary prize and avoid a monetary cost (win = $1.00 gain; loss = $0.50 loss). In the social task, participants choose between the faces of two peers who have purportedly indicated whether they like or dislike the participant (win=peer 'like;' loss=peer 'dislike'). Our pre-registered hypotheses aim to investigate how both neural reactivity and effective connectivity during experiences of social reward vary as a function of age and risk for financial exploitation, and the extent to which psychosocial factors moderate these relations (https://osf.io/npgtv). Preliminary findings first show robust striatal activation for both social and monetary rewards, but no significant age-related striatal response to social vs. monetary reward. We also did not find support for our hypothesis that risk for financial exploitation would modulate DMN-TPJ connectivity during social reward. Exploratory analyses do show a significant interaction between age and risk for financial exploitation in relation to VS-inferior temporal lobe connectivity, such that individuals at risk for financial exploitation show decreased connectivity as they get older. These findings enhance our understanding of how the brain's reward circuitry responds to different classes of rewards across adulthood, with implications for psychological processes like social functioning and vulnerability to exploitation.

Disclosures: J. Wyngaarden: None. **C. Sharp:** None. **A. Dachs:** None. **D. Sazhin:** None. **T. Tropea:** None. **I. Kohli:** None. **T. Giovannetti:** None. **D.S. Fareri:** None. **J. Jarcho:** None. **D.V. Smith:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.08/V5

Topic: H.03. Decision Making

Support: NIMH ZIA MH002928
NARSAD Young Investigator Grant 30892

Title: Ventral frontostriatal circuitry mediates the computation of reinforcement from symbolic gains and losses

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Abstract: Reinforcement learning (RL), particularly in primates, is often driven by symbolic outcomes. However, it is usually studied with primary reinforcers. To examine the neural mechanisms underlying learning from symbolic outcomes, we trained monkeys on a task in which they learned to choose options that led to gains of tokens and avoid choosing options that led to losses of tokens. We then recorded simultaneously from the orbitofrontal cortex (OFC), ventral striatum (VS), amygdala (AMY), and the mediodorsal thalamus (MDt). We found that the OFC played a dominant role in coding token outcomes and token prediction errors. The other areas contributed complementary functions with the VS coding appetitive outcomes and the AMY coding the salience of outcomes. The MDt coded actions and relayed information about tokens between the OFC and VS. Thus, OFC leads the process of symbolic reinforcement learning in the ventral frontostriatal circuitry.

Disclosures: H. Tang: None. **R. Bartolo:** None. **B.B. Averbeck:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.09/V6

Topic: H.03. Decision Making

Support: ZIA MH002928

Title: Large-scale functional organization of cortex and basal-ganglia during reinforcement-learning

Authors: *F. GIARROCCO[1], B. B. AVERBECK[2];
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Abstract: Reinforcement learning (RL) refers to learning to maximize reward and avoid punishment. This process relies on the brain's ability to build a value representation of stimuli and actions, and to use this representation to select appropriate behaviors. The cortico-basal ganglia circuitry (CBGC) is one of the most important systems of the brain mediating these functions. Traditionally, this circuitry is conceptualized as pathways controlling oculomotor, skeletomotor, and motivational functions. Current RL theories suggest that a single value representation in nodes within the motivational pathway, including the amygdala and the ventral striatum (VS), drives learning within motor systems. Here we challenge this view. We hypothesize that motivational regions encode distinct value representations pertaining to motor systems, and that these extend through the CBGC controlling the oculomotor and skeletomotor systems. To test this hypothesis, we are conducting large-scale neuronal recordings across the circuitry in two male macaque monkeys while they perform a RL task that requires learning the value of visual stimuli through either saccades or arm reaching movements. Preliminary results show a behavioral dissociation in learning via reaching and saccades, mirrored by different RL model estimates of stimulus- and action-values related to the two motor systems. In one monkey,

we recorded the activity of over 2500 neurons across the dorsal premotor cortex (PMd), ventrolateral prefrontal cortex (PFCv), caudate (Cd), putamen (Put), lateral VS, medial VS, globus pallidus pars interna (Gpi), and the amygdala. Crucially, among motivational regions, the lateral VS shows predominant representation of stimulus value, action value, and choice direction for reaching movements. The medial VS, amygdala, Put, and GPi show different representation of stimulus and action values for the two motor systems, whereas the Cd primarily shows differences related to the action value. In the cortex, both the PMd and PFCv exhibit distinct action value coding associated with the two motor systems, with PMd also differently encoding stimulus value and choice direction. Additionally, we are measuring how information is transferred within the CBGC. Preliminary analysis suggests that the Put integrates reaching-related information from PFCv and PMd, and saccade-related information from PFCv. The Gpi mainly receives reaching-related information from the Put. Thus, our preliminary results challenge traditional views of how the CBGC supports RL and suggest that the learning process likely relies on the dynamic interaction between multiple value representations across the CBGC.

Disclosures: F. Giarrocco: None. **B.B. Averbeck:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

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Program #/Poster #: PSTR247.10/V7

Topic: H.03. Decision Making

Support: NIH Grant ZIA MH002928 (BA)

Title: Neural representations of symbolic rewards and motivation in the non-human primate limbic system

Authors: *D. BURK, A. TANGEN, B. B. AVERBECK;
NIMH/NIH, Bethesda, MD

Abstract: Deficits in motivation are a core feature of clinical depression and other psychiatric diseases. The limbic system has been implicated in both reinforcement learning and motivation. In many paradigms learning and motivation are confounded, which makes it difficult to disambiguate the contribution of brain areas to these processes. We have developed a behavioral paradigm and computational model that allow us to examine these processes independently. In the task, monkeys learn to make choices to earn symbolic reinforcers in the form of tokens. The reinforcers accumulate across trials and are periodically exchanged for primary rewards. We have shown recently that motivated behaviors, including trial initiation times, choice reaction time, and willingness to complete trials, can be linked to motivational features of the task including accumulated tokens and trials until delivery of primary rewards. Here, we used a task variant that involves two token types that lead to delivery of preferred (water) and non-preferred (mint-flavored water) fluid rewards, to further probe motivational processes. We then used

simultaneous, multi-site neural population recordings in limbic circuitry including the insular cortex (InCtx), ventral striatum (VS), ventral pallidum (VP), lateral hypothalamus (LH), and amygdala (Amyg) to examine the way in which computations underlying learning and motivation are differentially implemented in these areas.Analysis of behavioral data indicates that monkeys prefer blue tokens that lead to water over green tokens that lead to mint-flavored water and that reaction times for choice of non-preferred options are slower, indicating motivational shifts. Computational modeling of choice behavior shows that mint tokens have little value despite a possible contribution to the monkey's satiety. Preliminary neural data show differential responses to delivery of preferred and non-preferred tokens and rewards across recording areas. The significance of our study lies in understanding how symbolic reinforcers drive motivation and how signals related to motivation and learning vary differentially across the limbic system.

Disclosures: D. Burk: None. **A. Tangen:** None. **B.B. Averbeck:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.11/V8

Topic: H.03. Decision Making

Support: ZIA MH002928 (BA)

Title: Investigation of the relationship between pupil diameter, learning, and motivation from symbolic reinforcement

Authors: *A. C. TANGEN, D. BURK, H. TANG, B. B. AVERBECK;
NIMH/NIH, Bethesda, MD

Abstract: Pupil diameter is a noninvasive measure that can be correlated with arousal, cognitive load, and motivation. An increase in pupil size, or dilation, is often associated with an increase in arousal. Arousal and motivation can help reach rewards in the future, while learning directs future behavior. In most reinforcement learning (RL) tasks, the objective is to maximize rewards over time. However, we can also make choices to obtain symbolic reinforcers (e.g. money) that are later exchanged for primary rewards. Here, we investigate the relationship between pupil diameter, learning, and motivation during a RL task with symbolic reinforcers. In the task, two monkeys (one male, one female) learned to maximize rewards by choosing images associated with gaining tokens, and avoiding choosing images associated with losing tokens. Accumulated tokens were exchanged for drops of juice every four to six trials. Monkeys were motivated by larger accumulated token counts and gaining more tokens. Previous work has shown that pupil dilation can increase during the learning phase of a new task and correlate with exploring choice options. Therefore, we hypothesize that pupil size would be larger during the learning of new images and decrease as the monkeys learn the image values. Preliminary data indicates that the

pupil size was modulated by token count. During the image presentation, pupil size positively correlated with the value of the to-be-chosen option, and this effect also developed as learning progressed. By investigating the effects of learning and motivation on pupil diameter, we aim to gain better insights into neuropsychiatric disorders that involve symbolic reinforcement, such as gambling addiction.

Disclosures: A.C. Tangen: None. **D. Burk:** None. **H. Tang:** None. **B.B. Averbeck:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

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Program #/Poster #: PSTR247.12/V9

Topic: H.03. Decision Making

Support: NIMH 3R01 NS116623-01S1

Title: Prefrontal dynamics of attention and valuation during decision making

Authors: *N. T. MUNET[1], J. D. WALLIS[2];
[1]Univ. of California, Berkeley, Berkeley, CA; [2]U.C. Berkeley, Berkeley, CA

Abstract: Deliberative decision making is a dynamic process in which the decision maker must represent the relevant decision variables, compute each option's overall value, and weigh these values to select the best option. Prior work from our lab shows that the orbitofrontal cortex (OFC) dynamically represents the value of each of the choice alternatives during deliberation, alternating back-and-forth between the chosen and unchosen values. While the time spent in each value state has been shown to predict choice behavior, the functional role of OFC's alternating dynamics remains unclear. One possibility is that fluctuations in value may be driven by shifts in top-down attention between options. Conversely, the dominant value signal may direct attention to the associated option, with shifts in the value state leading to shifts in attention. To test these hypotheses, we recorded from OFC and nearby lateral prefrontal cortex (LPFC), a region central to attentional control, during a novel experimental paradigm to identify and isolate distinct neural signals for attention and valuation at single-trial resolution. At the single-unit and population scale, we find that both LPFC and OFC encode correlates of space and value in a prioritized manner that may underlie attention and decision making. Moreover, we find evidence that these representations generalize across task contexts, suggesting a task-general code for spatial attention. Single-trial decoding analyses further reveal that PFC represents the locations and values of available options dynamically, which may be pertinent to the time course of the decision. I will discuss how these dynamics relate to choice behavior, as well as whether the dynamics of covert attention and valuation signals are correlated in PFC. Such a correlation would be consistent with an interaction between attentional and evaluative processes that may be important for gathering and weighing evidence over the course of a decision.

Disclosures: N.T. Munet: None. **J.D. Wallis:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.13/V10

Topic: H.03. Decision Making

Support: NIMH R01-MH117763
NIMH R01-MH121448

Title: Fronto-striatal mechanisms underlying bias and self-control in non-human primates

Authors: *T. ELSTON[1], J. D. WALLIS[2];
[1]Neurosci., U.C. Berkeley, Berkeley, CA; [2]U.C. Berkeley, Berkeley, CA

Abstract: We often have an initial impulse based on prior assumptions which must be inhibited through self-control. However, the neural mechanisms enabling self-control over rapid, bias-driven responses remain unclear. We addressed this question by performing high-density, neuropixel recordings from the caudate nucleus of the striatum (CdN) and orbitofrontal cortex (OFC) from two rhesus monkeys trained to perform a contextual reasoning task. The task required the animals to flexibly apply different sets of value mappings to a fixed set of choice options. Critically, the task reliably elicits initial biased responses which are subsequently corrected within single trials. This took the form of a "double take" saccade pattern: initial saccades were biased towards the option that would have been best in an alternative context (but is worst in the present context). Then, a second saccade would occur as the animals corrected their mistake. These "double takes" only occurred when there was conflict between two value mappings associated with the same stimulus and they did not occur when only one value mapping was possible. Thus, our behavioral results suggest that biases and subsequent control over them may arise due to conflicting value-associations.

We report evidence that the expression of bias and its subsequent control depends on interactions between the CdN and OFC. Using a single-trial population decoding approach, we were able to observe the conflicting value-associations in the OFC. Specifically, on trials where the animals exhibited a "double take", as the animals gazed at the low value option they will ultimately reject, the OFC represented the (high) value of that stimulus in the alternative context. Then, prior to the animal shifting its gaze to correct the initial biased response, the OFC population code switched to representing the contextually appropriate value. Interestingly, CdN activity primarily reflected the final value encoded by the OFC, suggesting a downstream effect of conflict resolution in OFC.

We also found evidence that alpha coherence between the deep layers of the OFC and the ventromedial aspect of CdN was elevated during instances of successful self-control.This heightened coherence is a candidate mechanism whereby OFC value signals may be transmitted to the CdN, facilitating the selection of contextually appropriate actions and enabling the exertion of self-control over bias.

Disclosures: T. Elston: None. **J.D. Wallis:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

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Program #/Poster #: PSTR247.14/V11

Topic: H.03. Decision Making

Support: NIH Intramural Funding

Title: Characterizing the interhemispheric connections in the superior colliculus during value decisions using optical tools.

Authors: *A. GOPAL P A[1], X. YU[2], C. MEJIAS-APONTE[3], K.-I. INOUE[4], M. TAKADA[5], O. HIKOSAKA[6];
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Abstract: Identifying objects of the highest value in the environment requires comparing the value generated from local visual signals to the value of inputs from across the visual field. Our research using saccadic eye movements showed that neurons in the sensorimotor structure Superior Colliculus (SC) are sensitive not only to the value of objects present in the receptive field but also to the value of objects presented in the opposite visual field during a two-alternative forced choice task. We hypothesized that the value information from the opposite visual field reaches these SC neurons, likely through the interhemispheric connections, such as the direct reciprocal connections between the hemispheres of SC. Here, we investigated the neural basis of this information transfer using optogenetics and by mapping the pattern of functional connections between the two hemispheres, which was previously untenable with simple anatomical or electrophysiological techniques. We injected anterograde (AAV2-CMV-ChR2) or retrograde(AAV2Retro-hSyn-ChR2) viral vectors in one of the SC hemispheres and stimulated the neurons in the opposite hemisphere. With anterograde viral vectors, we assessed the downstream effect of intercollicular connections. We found that neurons were excited as well as inhibited when the axon terminals on the opposite hemisphere of the injection were stimulated. With retrograde viral vectors, we assessed the nature of neurons that send information to the opposite hemisphere and found that all three functional subtypes of SC neurons, i.e., visual, visuomotor, and motor neurons, project to the other side. To investigate the anatomical bases of these observations, we processed the tissue procured from the animal in which anterograde viral vectors were injected and found axonal fibers in the hemisphere opposite to the injection site. These fibers were localized in the intermediate and the deep layers of the opposite SC. We are now in the process of identifying potential excitatory and inhibitory synapses on these interhemispheric fibers that will corroborate our findings. Combining

optogenetics anterograde/retrograde strategies) with electrophysiology and anatomical tools will help to elucidate the detailed functional connectivity profile of the intercollicular pathway and its role in value-based decisions.

Disclosures: A. Gopal P A: None. **X. Yu:** None. **C. Mejias-Aponte:** None. **K. Inoue:** None. **M. Takada:** None. **O. Hikosaka:** None.

Poster

PSTR247: Value-Based Decision Making in Humans and Non-Human Primates

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR247.15/V12

Topic: H.03. Decision Making

Support: 1ZIA EY000415

Title: Relative value representation in the macaque frontal eye field, with a comparison to the substantia nigra pars reticulata

Authors: *A. YOSHIDA, O. HIKOSAKA;
Lab. for Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: We primates flexibly change our choice behavior depending on the situation. Reward is an essential factor in this process, and we change our behavior to obtain reward. To investigate such brain functions, choice tasks have been used in which correct choices are rewarded, and incorrect choices are not rewarded or are rewarded less. In this world, however, we are not only faced with a choice between "yes" and "no" rewards. In some situations, the value of the choice can be relative. We created a choice task based on relative values to investigate how the brain represents such relative values. This task involved five options (fractal objects) associated with different reward values. During a trial, two of these five objects were presented sequentially. Initially, a background image (Scene) indicated which two objects would appear. Monkeys made a selection by performing a saccade towards an object. If they maintained fixation on the object for a specified period (over 400 ms), this was considered “accepted,” and a corresponding reward was given. If the monkeys did not execute a saccade towards the object or did not maintain fixation after a saccade, the object was “rejected,” and another object was randomly presented. This random presentation of objects continued until the monkeys made a definitive choice.We recorded neuronal activity from the frontal eye field (FEF), which is the main efferent projection source to the superior colliculus (SC) involved in saccade control, in order to reveal the functions of the FEF in making relative value-based decisions. Neurons in the FEF increased neural activity when monkeys accepted relative good objects and attenuated the activity when the monkeys rejected relative bad objects. When monkeys accepted relative bad objects, FEF neurons increased neuronal activity to the same extent as when monkeys accepted relatively good objects. These results suggest that the neuronal activity in the FEF represents chosen actions rather than relative values.Previously, we recorded neural activity from the substantia

nigra pars reticulata (SNr) in the basal ganglia, which is another efferent projection source to the SC, while monkeys performed the same task and showed that SNr neurons represented the relative value of the presented object, not the chosen actions. Taken together, the SC may receive relative value information from the SNr-SC pathway and action information to choose from the FEF-SC pathway, which may be integrated to select the final action.

Disclosures: A. Yoshida: None. **O. Hikosaka:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

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Program #/Poster #: PSTR248.01/V13

Topic: H.08. Learning and Memory

Support: DFG Grant 327654276 (SFB 1315)

Title: Scratching the Surface: Understanding the role of Calbindin-containing pyramidal cells in the superficial-layer of the CA1 and their roles in learning and memory in mice

Authors: *A. VOIGT[1], D. SCHMITZ[2];
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Abstract: Based on the timing of their neurogenesis, pyramidal cells within sublayers of the CA1 have been shown to develop unique morphological, electrophysiological and genetic properties (Soltesz and Losonczy et al. 2018). But what do these differences imply for the roles of these cells within the hippocampus? To understand the implications of pyramidal cell sub-layering in hippocampal microcircuits, and its purpose in the broader context of learning and memory, we studied a cell population containing a defining genetic marker: Calbindin-containing pyramidal cells within the superficial layer of the CA1. Using a Calb1-Cre mouseline and Cre-dependent viruses, we introduced optogenetic and chemogenetic tools into the Calbindin-containing cells of the CA1. Potential microcircuits within the hippocampal CA1-Subiculum (SUB) structures were studied using optogenetic activation in combination with single-cell and multi-cell patch-clamping in brain slices. To complement those findings, we employed retrograde rabies tracing. By injecting a Cre-dependent rabies virus into the SUB of VGlut2-Cre mice we were able to study whether the bursting cell subpopulation of the SUB, marked by VGlut2, is preferentially innervated by the CB-containing sublayer of the CA1. Whether any of these potential microcircuits play a role in learning and memory was investigated by studying mice in a Barnes maze setup. Calb1-Cre mice were first injected with Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) into the CA1 sublayer, and then given CNO prior to learning or recalling the escape location on a circular platform (Barnes Maze). We thereby tested whether the inhibition of these cell subpopulations would lead to changes in search strategies or learning rates in the affected animals. Using these approaches, based on stereotactic injections in the CA1 pyramidal cell layer, we can refine our understanding

of local and functional compartmentalization in the hippocampus and sublayer-specific projections to a downstream processing area.

Disclosures: A. Voigt: None. **D. Schmitz:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.02/V14

Topic: H.08. Learning and Memory

Title: Hippocampal behavioral timescale synaptic plasticity (BTSP) in a non-spatial olfactory working memory task is reduced with lateral entorhinal inhibition

Authors: *C. C. DORIAN[1], J. TAXIDIS[2], P. GOLSHANI[3];
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Abstract: Behavioral timescale synaptic plasticity (BTSP) is an *in vivo* plasticity process triggered by plateau potentials in hippocampal CA1 which leads to the rapid formation of place fields during spatial navigation. Whether a BTSP-like process also leads to formation of non-spatial representations in the hippocampus is poorly understood. Inspired by research suggesting that entorhinal cortical inputs to the hippocampus drive large calcium plateau potentials that can generate place fields in CA1 after a single trial, we asked if this form of plasticity can also be elicited during an odor-cued working memory task, and whether lateral and medial entorhinal cortex (LEC and MEC) play a role in driving BTSP events. We conducted 2-photon calcium imaging of CA1 pyramidal neurons expressing GCaMP8f while mice performed an olfactory-based delayed non-match to sample working memory task with a 5 second delay between odor cues. We recorded BTSP-like events characterized by calcium responses 10 standard deviations above baseline and lasting for more than one second during performance of the task in expert mice. While only ~2% of these putative calcium plateau potentials generate odor-specific fields, nearly all successful events occurred just after odor offset and resulted in formation of stable and odor-specific responses during odor presentation. This backward drift of the peak field from the plateau-like event on the induction trial is similar to the backward drift observed in spatial tasks. To investigate the role of LEC and MEC on the induction of these events, we inhibited LEC or MEC using PSAM chemogenetic inhibition during expert task performance combined with 2-photon calcium imaging of CA1 pyramidal neurons. Neither LEC or MEC inhibition caused behavioral impairments, but both significantly affected BTSP events in CA1. MEC inhibition significantly reduced the number of putative calcium plateau potentials from 0.5 events per cell per session to 0.3 events per cell per session ($p < 0.02$). However, MEC inhibition did not affect the success rate of plateau-like events for generating an odor-specific field. In contrast, LEC inhibition had no effect on the number of plateau-like events but significantly reduced the

success rate of these events forming an odor-specific field from 2% to less than 1% ($p < 0.01$). Our findings suggest that BTSP-like events occur from olfactory sensory inputs during a non-spatial working memory task and are modulated by entorhinal inputs.

Disclosures: C.C. Dorian: None. **J. Taxidis:** None. **P. Golshani:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

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Topic: H.08. Learning and Memory

Support: CIHR grant MOP-137072
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Title: Unveiling the role of hippocampal CA1 VIP interneurons in contextual fear memory encoding

Authors: *S. TAMBOLI[1,2], S. SINGH[1,2], D. TOPOLNIK[1,2], L. TOPOLNIK[1,2];
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Abstract: Contextual fear memory (CFC) serves as a widely utilized framework for examining the neurobiological underpinnings of fear acquisition and extinction within the rodent hippocampus. While previous investigations predominantly concentrated on hippocampal pyramidal neurons (PNs), limited attention has been given to exploring the potential involvement of interneurons (INs) in CFC. Within the CA1 region of the hippocampus, vasoactive intestinal polypeptide-expressing (VIP) INs establish intricate connectivity patterns, exerting regulatory control over PNs and other INs, and have been implicated in memory formation. This study employed a combination of in vivo calcium imaging and optogenetic interventions in freely behaving mice to elucidate the contributions of VIP-INs to contextual fear memory. Furthermore, we investigated whether VIP INs modulate the activity of their downstream targets by conducting calcium imaging of PNs, as well as two major types of INs in CA1 - somatostatin (SST) and parvalbumin (PV) expressing INs during CFC task in mice. Analysis of calcium activity recordings of VIP-INs during conditioning revealed a consistent increase in activity following the presentation of aversive stimuli. Intriguingly, both PNs and PV-INs exhibited robust increases in calcium transients in response to shock, while SST-INs' activity remained largely unaltered. Additionally, we assessed the impact of optogenetic manipulation of VIP activity on fear memory encoding. Taken together, our findings suggest that CA1 VIP-INs are responsive to aversive stimuli and may play a role in supporting the encoding of fear memories.

Disclosures: S. Tamboli: None. **S. Singh:** None. **D. Topolnik:** None. **L. Topolnik:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.04/V16

Topic: H.08. Learning and Memory

Support: EMBO Postdoctoral Fellowship ALTF 365-2022
The Kavli Foundation
Research Council of Norway

Title: Relevance of entorhinal cortex input to CA1 neuronal activity and behavioral performance in olfactory and visuospatial associative memory

Authors: *A. DE LANDETA[1], L. A. DESCAMPS[2], C. G. KENTROS[2];
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Abstract: The hippocampus and entorhinal cortex (EC) are known to be crucial to support episodic memory - a memory of what happened, where and when. Notably, the main excitatory input to the hippocampus is provided by the superficial layers of EC via the perforant path, particularly from neurons projecting from EC layer II: Stellate cells in medial EC and Fan cells in lateral EC. Although episodic memory has been widely studied, little is known about the contribution of these two cell-types on its processing. To study this, we use calcium imaging to record CA1 pyramidal cells in a mouse line expressing an inhibitory chemogenetic receptor in EC layer II Stellate and Fan cells, as animals perform a paired olfactory and visuospatial associative episodic memory task (Muzzio et al., 2009. doi: 10.1371/journal.pbio.1000140). Both variants of the task are identical except for the strategy the animals need to employ. Animals experience four odors which pseudo-randomly vary between trials in four cups at different positions in an environment, one of which is rewarded. The only difference between the two variants lies in the feature associated with the reward: a particular odor in the olfactory variant or a location in the visuospatial variant. Thus, in the olfactory variant animals need to pay attention to a specific odor and ignore its location in the environment; while in the visuospatial task they must pay attention to the reward location and ignore the odors. By silencing EC layer II projection neurons at distinct stages of the task, we investigate the role of EC input both in olfactory and visuospatial associative memory acquisition and retrieval. Also, we evaluate and compare the neuronal activity of CA1 pyramidal cells as animals are executing these paired associative episodic memory tasks with or without input from EC layer II projection neurons. Our results will increase our understanding of how distinct kinds of episodic memory are processed by the hippocampal-entorhinal circuit.

Disclosures: A. de Landeta: None. **L.A. Descamps:** None. **C.G. Kentros:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.05/V17

Topic: H.09. Spatial Navigation

Support: NRF-2021R1A4A2001803
2019R1A2C2088799
2022M3E5E8017723

Title: Robust and Differential Reactivation of Task-related Neural Firing Patterns of Place Cells in the Subiculum and CA1 in a Visual Scene Memory Task

Authors: *J. SEOL, S.-M. LEE, I. LEE;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Robust and Differential Reactivation of Task-related Neural Firing Patterns of Place Cells in the Subiculum and CA1 in a Visual Scene Memory Task Jae-Min Seol, Su-Min Lee and Inah Lee
It is essential to process both spatial and non-spatial representations for episodic memory in the hippocampus. It has been hypothesized that spatial representations of place cells are reactivated during sharp-wave ripples (SWRs). However, whether non-spatial, task-related representations are also reactivated in SWRs is unclear. We examined this by reanalyzing the data used for our previous study (Lee et al., 2022) in which rats (n=5) were trained to perform a scene memory task in a T-maze while single units were recorded from the CA1 (n=168) and subiculum (SUB; n=135). As reported previously, individual place fields were defined by spiking phases in relation to theta rhythm. For individual SWRs (n=1899 in CA1, n=5899 in SUB), we further analyzed a group of place cells co-activated during a single SWR during inter-trial intervals. In the SWRs from the SUB and CA1, we found specific reactivation patterns in which cells with similar task-related information were reactivated together. Among the cell ensembles that showed significant reactivations during SWRs, task-related reactivation occurred more in SUB (61.5%) than in CA1 (41.5%), whereas spatial replay occurred more in CA1 (20%) than in SUB (6%) ($\chi 2$ test, $p<0.0001$). We also found a unique type of task-related reactivation that represented different types of task-related information simultaneously, which was also more prevalent during SWRs in SUB than in CA1 (18.5% and 14.7% of the task-related reactivations in SUB and CA1, respectively). Next, we compared the participation rate of each cell group during task-related reactivations. SUB cells with multiple fields in relation to theta phase (MF cells) had a higher SWR participation than single-field cells (SF cells), while CA1 MF cells did not show such higher participation rate. These MF cells have higher non-spatial correlates than SF cells and comprise the majority of SUB cells. These results show that SUB cells with multiple fields are the primary source of the higher proportion of task-related reactivations, and also of task-related reactivations with multiplexed information. Our results suggest that non-

spatial correlates of place cells are reactivated during SWRs as robustly as spatial correlates, and SUB may play key roles in amplifying and conveying such task demands.

Disclosures: J. Seol: None. **S. Lee:** None. **I. Lee:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.06/V18

Topic: H.09. Spatial Navigation

Support: NRF 2019R1A2C2088799
NRF 2021R1A4A2001803
NRF 2022M3E5E8017723
NRF 2022R1I1A1A01069756

Title: Subicular neurons flexibly alter their spatial firing patterns to keep track of the event boundaries in a hippocampal memory task in a virtual environment

Authors: *S.-M. LEE, I. LEE;
Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The subiculum has long been recognized as a critical interface between the hippocampus and cortical regions, but its roles in remembering episodic events are poorly understood. We previously reported that a class of subicular neurons organized their firing fields according to the structure of a hippocampal task (Lee et al., J Neurosci 2018). Building on this finding, we posit that the subiculum restructures the fine-tuned positional information transmitted from the hippocampus into behaviorally meaningful representations aligned with task demands. To verify the prior results in a more controlled experimental setting, we trained male Long-Evans rats (n=3) in a VR-based memory task that required contextual learning. Specifically, the rat ran on a circular track to obtain honey water as a reward (40μL) while experiencing two virtual environments constructed with distinct visual landmarks. The reward was given at a fixed location (0°), but the starting point was pseudo-randomly chosen from one of three positions (90°, 180°, 270°) for each trial (n≥90). Once the rat reached the target location, he had to lick one of the two water ports to obtain a reward. Each water port was associated with one of the VR environments for reward. Each trial ended as the rat returned to the starting point along the circular track after receiving the reward. While the rat was learning the task, single-unit spiking activities and local field potentials were recorded in the CA1 and the subiculum simultaneously by a multi-electrode drive equipped with 24 tetrodes. Our preliminary data showed that some subicular neurons encoded task events that seemed critical to define the structure of the task by demarcating event boundaries (e.g., the start of the trial). These cells flexibly shifted their firing fields as the event boundaries changed with the starting location along the track across trials. Interestingly, such subicular neurons whose firing patterns were correlated

with the event boundaries or the task structure were observed even before the rat acquired the association between the VR contexts and behavioral responses. Furthermore, those neurons showed context-dependent rate modulation in their event-bound firing fields, which corroborates the findings in the prior study. After the rat successfully learned the task, subicular neurons altered their firing fields more dynamically according to the VR contexts and starting locations. Combining our results with previous literature reporting spatial boundary coding in the subiculum, we propose a generalized function of the subiculum in episodic memory that represents behaviorally significant boundaries in space and events.

Disclosures: S. Lee: None. **I. Lee:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.07/Web Only

Topic: H.09. Spatial Navigation

Support: NRF-2021R1A4A2001803
2019R1A2C2088799
2022M3E5E8017723

Title: Dynamic multiplexing of spatial and non-spatial information by single units in the nonhuman primate hippocampus during contextual object discrimination in a VR environment

Authors: *S. SHIN[1], J. SEOL[2], Y. NAYA[3], J. LEE[4], I. LEE[2];
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Abstract: It has been hypothesized that neurons in the hippocampus represent objects and the environments in which they appear to support episodic memory. Unfortunately, the class of neural correlates underlying object-in-environment memory has never been rigorously tested in nonhuman primates (NHPs), presumably because of the limited environmental manipulations possible in the head-fixed recording paradigm in NHPs. We used state-of-the-art VR technology to characterize the neural firing patterns in the hippocampus while NHP performs an object recognition task across different VR environments. Specifically, we recorded single units in the hippocampus while NHPs (n=2) performed a context-dependent object discrimination task in a VR environment. In the task, NHPs navigated a linear track in one of two distinct VR environments (forest and city) using a joystick. During navigation, they were stopped at one of 8 different trial locations and encountered a pair of objects (chosen among six objects). The animals must choose one of the objects rewarded in the current environment to obtain a water reward (0.2-0.4 ml). Both NHPs successfully learned the task (> 96% accuracy). Among the single units analyzed (n=143), we observed event-related units (n=105, 73%) that significantly

increased their firing rates at one of the trial locations between object-onset and choice events. Some hippocampal units exhibited positional correlates; that is, 66% (n=94) of single units were putative spatial units with a spatial information score (SI) of 0.5 or higher (bit/spike) in at least one VR environment. Interestingly, 71% of cells showing event-related firing exhibited putative spatial correlates, indicating conjunctive coding of spatial and non-spatial variables by single units in the hippocampus. We also observed a shift between spatial and non-spatial event-related firing patterns according to the environment in some conjunctive neurons. These results suggest that spatial and non-spatial variables are multiplexed in the firing patterns of a large population of single neurons in the hippocampus of NHPs in a contextual manner.

Disclosures: S. Shin: None. **J. Seol:** None. **Y. Naya:** None. **J. Lee:** None. **I. Lee:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.08/V19

Topic: H.08. Learning and Memory

Support: TMS Grant

Title: Postnatal cajal-retzius cell ablation induces learning deficits in young adult mice

Authors: *I. L. GLÆRUM[1], R. P. MACHOLD[2], R. H. SALIH[3], M. J. NIGRO[4], Y. ROUDI[5], G. QUATTROCOLO[6];
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Abstract: Postnatal ablation of Cajal-Retzius cell induces learning deficits in young adult mice. Glærum I. L., Salih R., Machold R., Nigro M. J., Roudi Y. & Quattrocolo G. The hippocampus is a brain structure renowned for its role in memory formation, learning and the representation of space. The hippocampal circuitry is comprised of a diverse set of excitatory and inhibitory neurons, including Cajal-Retzius cells (CR), a transient population of reelin^{+} glutamatergic neurons. In contrast to neocortical CR-cells, which disappear soon after birth, hippocampal CR-cells persist for an extended period during postnatal development. In previous work (Glærum et al., 2024), we showed that early postnatal ablation of hippocampal CR-cells leads to layer-specific changes in the number of spines and dendritic branches of CA1 pyramidal cell, significant alterations in synapse-related genes and a complementary loss of synapse-related proteins. This demonstrates that CR cells play a fundamental role in the establishment of the hippocampal circuit. However, the impact of CR cells on hippocampal function remains to be determined.To assess their functional role, we use a combination of a double transgenic mouse

line and viral vectors to specifically ablate hippocampal CR cells at birth. In a simple spatial navigation task, the T-maze, characterized by a low reference and working memory load, we found that CR cell ablation induced a delayed learning curve. Then we challenged the mice with an 8-arm radial maze, a task with high reference and working memory load. In this case, the CR cells ablated animals failed to reach the same level of performance as control animals, committing more errors than control group. Interestingly, we observed goal-directed hyperactivity in CR cell ablated animals, which was not present outside the confines of the task. This indicates a change in strategy to prioritize speed at the cost of performance accuracy. Taken together, our findings indicate that CR cell ablation leads to deficits in hippocampal function and thus implicates CR cells in the establishment of hippocampal spatial memory.

Disclosures: I.L. Glærum: A. Employment/Salary (full or part-time):; Kavli Institute for Systems Neuroscience and Center for Algorithms of the Cortex, Norwegian University of Science and Technology (NTNU), Trondheim 7491, Norway.. 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.; Mohn Research Center for the Brain, Norwegian University of Science and Technology (NTNU), Trondheim 7491, Norway. **R.P. Machold:** None. **R.H. Salih:** None. **M.J. Nigro:** None. **Y. Roudi:** None. **G. Quattrocolo:** A. Employment/Salary (full or part-time):; Kavli Institute for Systems Neuroscience and Center for Algorithms of the Cortex, Norwegian University of Science and Technology (NTNU), Trondheim 7491, Norway..

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.09/V20

Topic: H.09. Spatial Navigation

Support: JSPS KAKENHI 21K15611
JST CREST JPMJCR23N2

Title: Learning spatial and conceptual representations through hippocampal attractor dynamics and synaptic homeostasis

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Ctr. for Information and Neural Networks, Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: Place cells in hippocampus have been considered as a basis of cognitive maps for spatial navigation and memory. Place cells are sequentially activated during sleep (replay), reflecting hippocampal memories of past spatial experiences. Furthermore, hippocampus contributes to computations of not only physical spaces but also non-spatial semantic concepts. Concept cells in hippocampus (and other related regions) are activated by specific concepts such

as specific persons and semantic groups. However, computational mechanisms behind the formation of these spatial and conceptual representations, and the role of replays during sleep for the learning process are still unclear. Here I propose a computational model for learning spatial and conceptual representations in hippocampus, called as “contrastive learning by attractor dynamics (CLAD)” model. CLAD model is derived from the theory of contrastive embedding which is often used for self-supervised representation learning in machine learning. CLAD model optimizes the objective function of contrastive embedding by repeating hippocampal learning processes during awake and sleep periods of hippocampus. During awake periods, the network model is driven by external inputs and synaptic potentiation occurs. In sleep periods, neural activities are internally generated by an attractor network model (replay), and synaptic depression occurs. This process is biologically plausible because synaptic potentiation during awake periods and depression during sleep periods have been experimentally observed in hippocampus and other brain regions (synaptic homeostasis). Through simulation experiments in 1-D and 2-D spatial structures, I found that spatial representation learning by this CLAD model generates place-cell-like representations during awake periods and sequential replays during sleep periods. Furthermore, by applying CLAD to text data which contains rich semantic information, the model generates neurons that selectively respond to words related to specific concepts, which correspond to concept cells. Consistently with the role as cognitive maps, those neural activities represent 1-D and 2-D spatial structures and semantic structures of words at the population level. CLAD model suggests that the combination of synaptic homeostasis and attractor dynamics for replay can realize the formation of experimentally observed hippocampal representations for spaces and concepts. Furthermore, that mechanism is tightly related to the modern representation learning method, thus those representations are possibly useful for many realistic tasks.

Disclosures: T. Haga: None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.10/V21

Topic: H.08. Learning and Memory

Support: NIH grant R01MH119346

Title: Cellular and network alterations in Plxna2 knockout mice, an in vitro study

Authors: *W.-C. CHANG[1], K. STANGIS[2], X.-F. ZHAO[3], R. J. GIGER[4], G. G. MURPHY[5]; [1]Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]MBNI, Univ. of Michigan Molec & Behav Neurosci Inst. (MBNI), Ann Arbor, MI; [3]Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [4]Neurology/Cell and Developmental Biol., Univ. of Mchigan, Ann Arbor, MI; [5]MNI/MIP, Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Genome-wide association studies have repeatedly linked the semaphorin receptor Plexin A2 (encoded by *PLXNA2*) with a number of psychiatric diseases, including schizophrenia. In mice, deletion of *Plxna2* interrupts progenitor cells' migration from the dentate neuroepithelium to the hippocampal fissure, which results in the redistribution of granule cells and the distorted dentate gyrus in mice; the mossy fibers also aberrantly innervate to other strata in CA3 over the striatum lucidum. Importantly, *Plxna2* knockout mice showed deficiency in consolidating fear memory, prepulse inhibition, and female mice lacked sociability, which depicts a trait of schizophrenia. In this study, we aim to explore the cellular and network mechanisms underlying the ectopic behaviors of *Plxna2* knockout mice. Whole-cell recordings were performed on the granule cells from the suprapyramidal blade and infrapyramidal blade of the dentate gyrus. Surprisingly, *Plxna2* knockout did not alter the passive properties or the excitability of the granule cells, albeit excitatory inputs to the granule cells were less in *Plxna2* knockout mice. To interrogate network stability in the *Plxna2* knockout mice we induced sharp wave ripples (SPW-Rs) in the horizontal hippocampal slices using no magnesium high potassium ($[K^+]_o$= 6 mM) artificial cerebrospinal fluid, and found CA3 from the *Plxna2* knockout mice was incapable of generating large or pace-making SPW-Rs. Ongoing experiments are focused on further characterizing the anomalous SPW-Rs and determining the underlying mechanisms.

Disclosures: W. Chang: None. **K. Stangis:** None. **X. Zhao:** None. **R.J. Giger:** None. **G.G. Murphy:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.11/V22

Topic: H.09. Spatial Navigation

Support: NIH/NIA grant # R01AG064066

Title: Spatial memory impairment in anti-NMDAR encephalitis mouse model: neuronal-glial mechanisms and electrophysiological insights

Authors: *H. YUCEER KORKMAZ[1,2,3], V. YILMAZ[1], E. TUZUN[1], S. HUSSAINI[2,3];
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Abstract: Anti-N-methyl-D-aspartate receptor (NMDAR)-antibody encephalitis is an autoimmune disorder associated with diverse neurological symptoms including memory deficits. The molecular and electrophysiological mechanisms underlying the spatial memory problem observed in anti-NMDAR encephalitis have not been fully elucidated. In this study, we aimed to clarify the effect of NMDAR antibodies on neuronal and glial cells in spatial memory impairment using a passive transfer mouse model. Purified IgGs from sera of both anti-NMDAR

encephalitis patients and healthy controls were administered intracerebroventricularly to C57BL/6J mice for 14 days using osmotic pumps. Behavioral assessments were conducted prior to IgG administration and at intervals of 3, 10, and 17 days following the initiation of IgG infusion. In-vivo multi-region electrophysiology recordings were collected from the CA1 region of the mouse hippocampus. Immunofluorescence staining was conducted post-mortem for all experimental groups. IgG infusion in mice significantly reduced the discrimination index in both novel object location and novel object recognition tests and spontaneous alternation in T-maze in the NMDAR group. Multi-region electrophysiology studies revealed notable reductions in average firing rate as well as increase in sparsity and information in wide-spiking single units with NMDAR IgG infusion. Conversely, increased field sizes were observed in narrow-spiking single units of NMDAR group as compared to the control group, indicating a decline in spatial memory performance. Immunofluorescence studies showed reduced hippocampal NMDAR expression and increased astrocyte proliferation in the NMDAR group. Our study findings revealed that the administration of NMDAR IgG enhances astrocyte activity while concurrently reducing NMDAR expression in parallel to an impairment in spatial memory. These results may imply that both neuronal and glial mechanisms mediate NMDAR IgG-induced spatial memory loss and associated electrophysiological manifestations. Further work is ongoing to unravel the complex neuronal and glial mechanisms of cognitive dysfunction observed in anti-NMDAR encephalitis.

Disclosures: H. Yuceer Korkmaz: None. **V. Yilmaz:** None. **E. Tuzun:** None. **S. Hussaini:** None.

Poster

PSTR248: Hippocampal Circuit Mechanisms of Memory

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR248.12/V23

Topic: H.08. Learning and Memory

Support: NIH 1ZICES103330-06

Title: Motor Behavior, Cognition, and Hippocampal Gene Expression: Impacts of Developmental Low-Dose Chlorpyrifos Exposure

Authors: *D. KENDRICKS[1], D. THOMAS[1], J. STALLONE[2], L. R. WILSON[3], J. D. CUSHMAN[4];
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Abstract: Prenatal chlorpyrifos exposure in murine models alters neural development and produces long-term alterations in behavior. While long-term impacts of moderate-to-high doses of this neurotoxicant, known to inhibit cholinesterase inhibition, have been widely reported, the

deleterious effects of exposure to doses insufficient to inhibit cholinesterase activity have received much less attention. The aims of the current study were to characterize the long-term outcomes of low-dose prenatal chlorpyrifos exposure on murine behavior and link observed behavior deficits to alterations in hippocampal gene expression. C57BL/6J mice were exposed to 0, 0.5, or 5 mg/kg chlorpyrifos from gestation days 6.5 - 17.5. At the end of exposure, cholinesterase activity was assessed in plasma and brain of dams and brains of pups. Brains of animals were taken at weaning for assessment of gene expression within the hippocampus utilizing 10x Visium Spatial Transcriptomics. Remaining offsprings' behavior was assessed beginning in young adulthood. Motor behavior was assessed using a functional observation battery, open field, and the Noldus PhenoTyper. Animal's cognition was assessed in a spatial discrimination and reversal procedure conducted in the PhenoTyper. Exposure to 0.5 mg/kg/day reliably did not alter cholinesterase activity compared to controls but produced long-term changes in behavior. Outcomes of this study provide evidence of the importance of studying the impacts of organophosphate pesticide exposure in the absence of overt alterations in cholinesterase activity.

Disclosures: D. Kendricks: None. **D. Thomas:** None. **J. Stallone:** None. **L.R. Wilson:** None. **J.D. Cushman:** None.

Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR249.01/V24

Topic: H.08. Learning and Memory

Support: NIH Transformative Award
NIH Early Independence Award DP5 OD023106-01
Ludwig Family Foundation
Chan-Zuckerberg Initiative
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Center for Systems Neuroscience and Neurophotonics Center at Boston University 10:35
Air Force Office of Scientific Research (AFOSR) under award number FA9550-21-1-0310

Title: Prefrontal cortex arbitrates memory-guided conflict resolution

Authors: *A. CABAN[1], M. SURETS[2], S. RAMIREZ[1];
[1]Boston Univ., Boston, MA; [2]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Flexible memory-guided decisions are fundamental to our daily lives. Such decision-making often requires a strategic balance between motivational drives including the pursuit of reward and avoidance of danger. Here, we aim to understand the underlying mechanisms that

support such flexibility during memory-guided conflict resolution. To this end, we developed a novel decision-making task in which animals strategically combine the drive to approach a reward and simultaneously avoid a footshock without compromising either goal. During training, mice learn that a 30s light cue indicates the availability of reward at a nose port (i.e. sucrose water), and a 30s auditory cue predicts the onset of a footshock which they can avoid by stepping onto a platform. After animals learn the reward and punishment contingencies, the light and auditory cues are delivered at the same time, creating a conflict between reward-seeking and punishment avoidance. Over 7 days, we find that female mice initially are more risk taking than males but then converge over days on the same resolution to this conflict, i.e. seek reward early (away from the time of shock delivery) and avoid later (seconds closer to the time of shock delivery). To mechanistically study the brain regions recruited during this flexible decision-making process, we then combined whole-brain inmuno-staining of the activity-dependent immediate early gene cFos with the theoretical framework of graph theory. By applying topological measures of centrality to correlation networks created from cFos expression we identified several regions (i.e. Hubs) in our networks, with the prelimbic cortex (PL) playing a key role. We confirmed this critical role by chemogenetically perturbing valence-specific engrams in PL which all resulted in a complete disruption of flexible behavior. Finally, population calcium imaging of PL during memory guided decision-making found that PL bidirectionally encodes avoidance and reward seeking behaviors. Our current work leverages single-cell calcium imaging to better probe how ensembles recruited during memory-guided conflict resolution relate to ensembles recruited for valence specific memory-guided behavior. Lastly, we aim to leverage activity dependent labeling and anatomical tracing to mechanistically understand the downstream targets of PL cells recruited during conflict resolution. Overall, our findings suggest the PL has a critical role in arbitrating positive and negative memories during memory-guided decision making.

Disclosures: A. Caban: None. **M. Surets:** None. **S. Ramirez:** None.

Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR249.02/V25

Topic: H.08. Learning and Memory

Support: Air Force Office of Scientific Research (AFOSR) under award number FA9550-21-1-0310
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Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Prefrontal engrams modulate behavioral flexibility in a reversal learning task

Authors: *M. SURETS[1], A. J. CABAN[2], S. RAMIREZ[1];
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Abstract: Behavioral flexibility is critical for survival, though how the brain accommodates unpredicted changes to guide subsequent decision making and behavior is not fully understood. The prefrontal cortex (PFC) is widely recognized as a crucial node for the integration of stimulus-related information with context to guide action selection. To study its cellular mechanisms, we developed a probabilistic reversal learning task that engages the PFC and tested whether PFC-mediated engrams modulate the behavioral flexibility required for the task. In this behavior, mice nose-poke for reward (sucrose water) on one of two sides based on the reward probabilities associated with each side. Our initial line of work shows that PFC engrams drive stimulus-driven behavior when there is certainty of reward (Surets 2024). In our modified version of the task, the probability of reward is 90% on one side and 10% on the other side, resulting in mice developing a preference for the side with the higher probability of reward over days. Once the preference has been established, the probabilities are reversed on the following day, requiring mice to flexibly adapt to a newly learned rule. In order to identify how PFC cell ensembles recruited by rule learning (90/10) relate to the ensembles recruited during reversal learning (10/90), our ongoing experiments combine cFos-based activity-dependent tagging strategies and projection-specific anatomical tracing to capture and visualize cells and axonal outputs recruited between the two ensembles. We hypothesize that the cellular overlap between the two ensembles (i.e. the two reward probabilities) will inversely correlate with performance on the reversal learning, while improved flexibility will come from increased strength of PFC projections to the rest of the cortex and decreased connections between PFC and deeper brain areas. Overall, our study will further our understanding of behavioral flexibility, which is often impaired in various psychiatric and neurological disorders such as addiction and obsessive-compulsive disorder.

Disclosures: M. Surets: None. **A.J. Caban:** None. **S. Ramirez:** None.

Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

Location: MCP Hall A

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Program #/Poster #: PSTR249.03/V26

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Ludwig Family Foundation
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Chan-Zuckerberg Initiative
Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Engram dynamics before and after learning in CA1

Authors: *A. MONASTERIO[1], S. COELLO[1], G. K. OCKER[2], S. RAMIREZ[3], B. B. SCOTT[4]; [1]Boston Univ., Boston, MA; [2]Dept. of Mathematics and Statistics, Boston Univ., Boston, MA; [3]Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA; [4]Psychological & Brain Sci., Boston Univ., Boston, MA

Abstract: A fundamental question in neuroscience is how memory formation shapes brain activity at the level of populations of neurons. Recent studies of hippocampal 'engram' cells, identified by immediate-early genes (IEGs) induced by learning, propose that these populations act as a neuronal substrate for memory storage. The current framework for engram formation proposes that cells join ensembles based on increased intrinsic excitability and that after initial learning they co-activate to support memory retrieval. However, direct evidence of how engram population dynamics evolve across learning is limited. Here we combined activity-dependent genetic tagging and two-photon calcium imaging to characterize CA1 engram population activity before and after learning. We observed that spontaneous activity two days before learning predicted genetic tagging, consistent with a model in which spontaneous fluctuations bias cells into forming engram assemblies. Surprisingly, we did not detect increased spontaneous activity rates or pairwise correlations amongst tagged CA1 neurons after learning. However, these results were consistent with computational network models that incorporate strong and specific inhibitory connections, supporting the idea that excitatory/inhibitory balance in CA1 may play a key role in engram dynamics. These results highlight a putative role for slow time scale excitability fluctuations in driving engram formation and suggest that excitatory-inhibitory balance may regulate engram cell co-activation. Our ongoing experiments investigate additional tagging strategies (TRAP2) as well as recording engram activity during conditioned stimulus mediated retrieval after trace fear conditioning. Together, these results demonstrate a role for spontaneous activity in allocation of engram populations and highlight key future directions for interrogating hippocampal engram circuit physiology.

Disclosures: A. Monasterio: None. **S. Coello:** None. **G.K. Ocker:** None. **S. Ramirez:** None. **B.B. Scott:** None.

Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

Location: MCP Hall A

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Title: Artificially manipulating memories among neuronally drifting ensembles

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Abstract: Memories unify an organism's experience over time. Decades of research have studied discrete cellular ensembles in the hippocampus as correlates of memory, often referred to as an engram; however, their real-time dynamics remain relatively understudied. Furthermore, recent findings suggest that neuronal representations are in a constant flux as experience and time accrue in the brain, yet the mechanisms by which neurons uphold the balance between flexibility and stability of our memories remains unknown. Here, we ask how memories remain stable despite their unstable cellular correlates. To investigate this, we use a combination of large-scale two-photon calcium imaging, optogenetic stimulation, and activity-dependent genetic strategies to investigate how the activity of memory-bearing (i.e. engram populations) and non-memory bearing cells drift over time. To that end, we first used a cFos-based tagging strategy to identify dentate gyrus (DG) and CA1 cells processing a fear memory while expressing a pan-neuronal calcium sensor GCaMP7f in CA1. With this design, we optogenetically stimulated DG-mediated engrams while simultaneously imaging downstream CA1 dynamics in engram and non-engram cells. Our preliminary experiments provide a technological proof of principle in which we successfully activate DG-mediated engrams while imaging tagged and untagged cells in downstream CA1, with light-on epochs producing decreases in firing rates in untagged cells and stable firing rates in tagged cells. We predict that engram cells will exhibit slower drift rates and less cell turnover in artificially reactivated DG populations across days compared to non-engram cells.Together, these experiments investigate the physiological and cellular principles enabling an enduring memory from dynamic biology in the brain, with implications for therapeutic interventions targeting conditions characterized by memory decline.

Disclosures: A. Ito: None. **S. Ramirez:** None. **J.A. White:** None.

Poster

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Title: Psilocybin Enhances Performance on a Perceptual Evidence Accumulation Task

Authors: *C. DELGADO SALLENT[1], S. AHMED[1], S. ANALOUI[1], A. KHAWAJA-LOPEZ[1], Z. OZTURK[1], A. RATNAKAR[2], B. B. SCOTT[3], S. RAMIREZ[1];
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Abstract: Psychedelics are effective in treating psychiatric disorders, often producing sustained effects after a single dose. However, the neural mechanisms underlying their action is poorly understood. Moreover, perceptual changes are frequent and robust features of the psychedelic experience and therefore investigation into how they alter how they alter cognitive performance is crucial in order to understand their mechanisms. Here we characterize psilocybin therapeutic properties by combining a free response pulse-based perceptual evidence accumulation task in mice and brain-wide mapping of activity-dependent gene expression in drug-responsive populations. In our perceptual integration task, first find that psilocybin administration at medium doses (1 mg/kg) slowed subjects' response times (RT) and increased their accuracy. Drift diffusion model (DDM) fits suggest that these behavioral changes were due to an increased boundary separation. Additionally, RTs were well fit by the DDM, and parameter fits suggested that mice use a multi-flash accumulation strategy. To further characterize which brain regions are activated by psilocybin administration (1 mg/kg), our ongoing work combines whole-brain immuno-staining of the activity-dependent immediate early gene c-fos with the theoretical framework of graph theory. Based on our previous data suggesting that drugs such as ketamine increase network topology and recruit the orbitofrontal cortex (OFC), our psilocybin experiments bi-directionally perturb and image OFC activity during the perceptual evidence accumulation task. Taken together, our results indicate that psilocybin improves perceptual evidence accumulation by raising the decision threshold and allowing the integration of more evidence before the decision.

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Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

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Title: Prefrontal cortex glioblastoma alters neuronal-astrocytic dynamics and promotes age-dependent dysregulation of acute stress responses

Authors: *H. LEBLANC[1], S. RAMIREZ[2], E. RUESCH[3], M. BUZHARSKY[4];
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Abstract: Glioblastoma is the most common malignant tumor of the central nervous system with poor prognosis and debilitating impact on quality of life. Despite significant efforts in the past three decades, the gold standard for treatment still leaves five-year survival rates at less than 5%, and even lower in patients within the age range of the cancer's peak incidence. Along with the rapid physical decline, patients often suffer progressive psychiatric symptoms and cognitive decline that not only predicts their survival prognosis, but heavily impacts the quality of their survival. There is therefore a need to identify the mechanisms underlying these neuropsychological changes and to develop targeted therapeutic strategies that can serve as an adjuvant to conventional anticancer treatment.
To better address this gap, we developed a frontal lobe model of glioblastoma by introducing murine, high grade glioma cells into the medial prefrontal cortex of young (7-8 week old) and aged (85-86 week old) mice, and subjected them to a battery of behavioral tasks (e.g. the tail suspension test, elevated zero maze, Y-maze) to assess the effects of tumor burden and age on cognition and behavior. We found age-dependent differences in stress coping strategies, with aged tumor-bearing mice showing increases in passive coping behaviors, and young tumor-bearing mice showing contrasting decreases in passive coping behavior. Additionally, both young and aged tumor-bearing mice exhibited similar decreases in risk assessment and anxiety-related behaviors in the elevated zero maze. Our ongoing work seeks to assess the impact of frontal tumor burden on 1) brain-wide neuronal activation patterns associated with the stress coping and anxiety-related behavioral alterations of young and aged mice and 2) characterize tumor-induced changes to extratumoral neuron-astrocyte calcium dynamics, a cellular interface critical to sustaining proper behavioral function, in brain areas particularly susceptible to psychiatric and aging-related dysfunction (i.e. hippocampus, medial prefrontal cortex). Together, our work provides new insight into the mechanisms underlying glioblastoma-induced psychopathology that may be targeted for quality-of-life enhancing therapeutics.

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Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

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Program #/Poster #: PSTR249.07/V30

Topic: H.08. Learning and Memory

Title: Neuroinflammation induces age-related decrease in oscillatory power of hippocampus and cortex

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Abstract: Neuroinflammation (NI) is a pathology common to most neurodegenerative and psychiatric disorders. In the hippocampus (HPC) and anterior cingulate cortex (ACC), two areas integral to learning and memory, neuroinflammation has been associated with increased microglial activation, cytokine expression, and cerebral microhemorrhages. However, very little is known about how it contributes to cognitive symptoms. Also, while NI effects are more pronounced in older individuals, it remains unclear if and how its age-related impact is reflected in both HPC and ACC's functional activity. To address this, we investigated the effect of induced inflammation on neural oscillatory activity in the HPC and ACC of aged and young mice to understand neuroinflammation's functional impacts. We compared HPC and ACC oscillatory activity from sessions before and after 7 days of poly (I:C) administration. This TLR3 receptor agonist provokes robust neuroinflammation but only causes minimal sickness behavior. Animals were exposed to novel and familiar environments during each session, while activity was continuously sampled. We recorded sessions before injections (baseline), and at different time points after injections stopped (1,7,14,21,60 days after). We found significant decreases in oscillatory power across multiple frequency bands (theta, beta, slow gamma) persisting for up to 2 weeks in the HPC and ACC networks. At very distant time points, 60 days after NI induction, activity had returned to baseline levels in all frequency bands and for all animals. Notably, our younger cohort had much weaker effects that were detectable with machine learning-based methods, but not when canonical frequency band power was compared with significance tests, unlike aged animals. Our data reveals an enduring and heightened age-related disparity in the functional response to NI. It presents a possible explanation behind the difference in the rate at which cognitive decline progresses among older adults diagnosed with brain disorders. Particularly, it emphasizes the need to reassess the therapeutic strategy deployed to address neurodegenerative diseases in aged populations.

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Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

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Topic: H.08. Learning and Memory

Support: NIH Grant R01AG062762

Title: Acc reward location information is carried by hippocampal theta synchrony and suppressed in a type 2 diabetes model

Authors: G. BHASIN[1], L. A. CREW[2], E. FLORES[3], R. A. WIRT[4], A. A. ORTIZ[5], J. W. KINNEY[6], ***J. M. HYMAN**[3];
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Abstract: The anterior cingulate cortex (ACC), part of the medial prefrontal cortex, is important for higher order cognitive functions, emotional responses, and the monitoring of internal states. In turn, ACC neurons encode expected and actual reward values, track reward likelihood, and integrate diverse context information. ACC dysfunction has been implicated in an array of psychiatric and neurodegenerative disorders, which have a bidirectional relationship with the metabolic disorder Type 2 diabetes (T2D). T2D is a chronic disease linked to obesity and is characterized by hyperglycemia, loss of insulin signaling and neuroinflammation, which in turn affects multiple vital body organs increasing morbidity and mortality chances. Interestingly, patients with certain psychiatric disorders have as much as a 60% increased likelihood to develop T2D. ACC dysfunction has been reported in patients and preclinical animal models of psychiatric and neurodegenerative disorders, but what is the ACC response to T2D? To better understand the functional effects of T2D on ACC activity, we delivered an intermittent, low dose streptozotocin (STZ) protocol which led to lasting hyperglycemia and recorded single units during a delayed alternation task. We recorded from 569 neurons during 34 sessions total from both chronically hyperglycemic (n=5) and healthy control animals (n=3). We observed higher spatial information scores in the hyperglycemic group units ($p<.001$), however overall mean firing rates remained comparable ($p>.05$). The biggest differences were found in the allotment of spatial coding assets, as hyperglycemic ACC neurons differentiated right vs. left trials mainly during reward approach, while in controls the distribution was flat across the population ($p<.01$). Ensemble analysis found that state space separation and decoding accuracy only differed between groups at the reward location and were higher for controls ($p<.001$). Hyperglycemia

was muting reward representations. Furthermore, in control animals hippocampal theta phase-locked cells had stronger reward coding than ACC theta synchronized, or non-theta synchronized cells ($p<.0001$). Therefore, hyperglycemia led to a deficit in reward related information in hippocampal theta phase-locked ACC cells, which created an overall muted reward representation in hyperglycemic animals. T2D infers a nuanced and layered effect on ACC activity, leading to reward coding deficits and a differential change in spatial coding properties. Lastly, we found that ACC reward location information is largely carried by hippocampal theta phase-locked neurons.

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Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

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Topic: H.08. Learning and Memory

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NINDS K99NS128718

Title: Identifying midline thalamic cells that project to the dorsal and ventral hippocampus

Authors: *T. D. VIENA[1], **G. LOPEZ**[2], L. X. CONDOR[2], T. A. ALLEN[2];
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Abstract: The hippocampus is a critical node for learning and memory and receives significant diencephalic input from the midline thalamus. Current evidence suggests dorsal hippocampus (dCA1) and ventral hippocampus (vCA1) contribute differently to memory, showing variations in gene/neurotransmitter patterns, electrophysiological properties, anatomical connections, and behavioral responses (spatial navigation vs emotional processing) (Strange et al., 2014). However, recent work proposes a more unified hippocampus across the dorsoventral axis (Loureiro et al., 2011). Both the dorsal midline thalamus (paraventricular, PVT; paratenial, PT) and ventral midline thalamus (reuniens, RE; rhomboid nucleus, RH) project to dorsal and ventral hippocampus (Vertes et al., 2015). However, whether individual neurons from midline thalamus simultaneously project to both dorsal and ventral hippocampus is unclear. Such dual-projecting neurons could serve as common synchronizing input and help integrate across dorsoventral hippocampus memory functions. Here, we used a dual retrograde adeno-associated virus (AAV) to map midline thalamic cells projecting to dCA1, vCA1, or both. Rats received bilateral retro AAV-CAG-TdTomato injections aimed at dCA1 (β:-4.3mm, DV:-/+2.4) at two lateral coordinates (L:-/+2.75,-3.75) and bilateral retro AAV-CAG-GFP injections in vCA1 (β:-5.6, L:-/+5.8) at three ventral coordinates (DV:-6.0,-6.5,-7.0). After four weeks of viral expression, rats were perfused, and brain tissue was collected for microscopy imaging. We found PVT-vCA1

projecting cells were expressed across the anteroposterior axis (6 levels) but in lower amounts towards the posterior axis (~40-70/level). PVT-dCA1 projecting cells followed a similar pattern but represented about 20% of PVT-vCA1 labeled cells. In RE and RH, vCA1 projecting cells were also present across the anteroposterior axis but in much lower quantity than PVT-vCA1 cells (~8-12/level). In contrast, RE/RH-dCA1 projecting cells were found in larger numbers, mostly in the posterior axis (~25-50/level). Dual projecting cells were observed in meager numbers across the axis in PVT, interanteromedial nucleus, RH, and RE (~1-7/level). No hippocampal projecting cells were observed in paratenial, centromedial, or lateral mediodorsal nuclei. These findings demonstrate midline thalamus projects to hippocampus through largely distinct, non-overlapping dCA1- and vCA1-projecting populations. The presence of a few dual projecting cells suggests midline thalamus might not be essential but could still be involved in integrating memory representations across the dorsoventral axis of hippocampus.

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Poster

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Topic: H.08. Learning and Memory

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Title: The role of the nucleus reuniens of the thalamus in the retrieval of old interval time memories

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Abstract: The nucleus reuniens (RE) is a ventral midline thalamic structure that bidirectionally connects the hippocampus (HC) and agranular medial prefrontal cortex mPFC (Cassel et al., Prog Neurobiol 2013; Dolleman-van der Weel et al., Learn Mem 2019; Vertes et al., Curr Opin Behav Sci 2023). RE is associated with several memory functions, including extinction and reversal learning, memory consolidation, context-dependent memory retrieval/expression, and behavioral flexibility. (e.g., Jayachandran et al., Cell Rep 2019; Xu & Sudhof, Science 2013; Cholvin et al., J Neurosci 2013; Dolleman-van der Weel et al., Brain Struct Funct 2009). For example, damage or inactivation of RE function impairs spatial working memory (Hallock et al., J Neurosci 2016; Maisson et al., Neurobiol Learn Mem 2018; Viena et al., Hippocampus 2018), sequential context memory (Jayachandran et al., Cell Rep 2019), contextual fear memory (Ratigan et al., Nat Commun 2023; Ramanathan et al., Nat Commun 2018), as well as impair the retrieval of remote spatial memories (Loureiro et al., J Neurosci 2012). Here, we further explored RE's role in retrieving old fixed-interval time memories. First, water restricted rats were trained to nose poke within a short time interval (5 or 10s) signaled by an auditory cue (white noise) to

obtain a small water delivered for the first poke after the interval had elapsed (daily 60-trial sessions for weeks). Rats demonstrated interval timing memory by poking predominately at the time of the expected reward. Next, we switched the rats to longer intervals (20, 40 or 80 sec) (daily session for weeks). The longer time interval followed a 4:1 ratio, allowing for good time discrimination in memory (Leak & Gibbon, J Exp Psychol Anim Behav Process 1995). Across sessions nose poke activity accumulated at the new interval time and responses to the previously learned time interval decreased, until only the most recent time memory was evident in the behavioral profiles. To probe the role of RE in memory retrieval the potent $GABA_A$ agonist muscimol or saline infusions were performed for both the short and the long intervals trained (pre-session infusions). Results show that muscimol infusions targeting RE decreases timing accuracy and precision at the shorter time and longtime intervals, suggesting a role for RE in interval time memory generally. Interestingly, we also observed a return of older interval memory in addition responses to the recent longer time interval. This result supports the idea that activity in RE contributes directly to memory retrieval by suppressing old irrelevant interval timing memories.

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Poster

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Title: Developmental emergence of oscillatory coupling and spatial memory capacity in the hippocampal-neocortical network

Authors: *L. MA[1], D. KHODAGHOLY[2], J. N. GELINAS[3];
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Abstract: Mature hippocampal-neocortical networks are central to multiple cognitive processes, such as learning and memory. Expression and coupling of hippocampal sharp-wave ripples (SWRs) and cortical oscillations facilitate and further differentiate different memory stages. However, neither precisely coordinated oscillatory activity nor spatial memory capacity are innate properties of nascent neural networks. We hypothesized that hippocampal and cortical oscillations require early developmental interactions to accompany emergence of spatial memory maturation. To test this hypothesis, we performed recordings simultaneously from hippocampus and neocortex of unanesthetized mouse pups during early development, using soft, conformable and implantable interface devices. We used an open-field spatial location memory task to assess the functional maturation of the developing hippocampal-neocortical networks. We found that

ripple-band oscillations emerged concurrently in hippocampus and cortex. These oscillations robustly recruited local neural firing, and developed temporal coupling patterns. These results suggest the importance of hippocampal-cortical communication in the maturation of neural networks, with implications for memory processes.

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Program #/Poster #: PSTR249.12/V35

Topic: H.08. Learning and Memory

Title: A modulatory role of the nucleus reuniens in hippocampal-prefrontal communication

Authors: *S. SHAO[1], J. J. FERRERO[1], T. KAWANO[2], L. MA[3], D. KHODAGHOLY[1], J. GELINAS[4];
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Abstract: Memory consolidation requires functional synchrony between the hippocampus (HC) and the medial prefrontal cortex (mPFC). The nucleus reuniens (RE) has recently been reported as a structural and functional hub between HC and mPFC. The role of the RE in hippocampal-prefrontal communication during memory consolidation remains unclear. Here, we used *in vivo* electrophysiology to monitor the hippocampal-thalamic-prefrontal network in rats during non-rapid eye movement (NREM) sleep. We identified ripple-like activity in the RE in the 100-180 Hz frequency range with an average duration of 45 ms, and was associated with robust recruitment of thalamic neural spiking. RE ripple activity was phase-locked to both hippocampal sharp wave-ripples (SWR) and mPFC ripples. Hippocampal ripples are known to couple with cortical ripples for facilitating memory consolidation. Although ripples were independently detected in all three structures, we found that coupling between HC and mPFC ripples necessarily involved co-occurring RE ripples. The coupling strength among HC, RE and mPFC ripples modulated with the memory demand. These results suggest a modulatory role for the RE in oscillatory synchronization between the HC and the mPFC, with potential implications for network mechanisms of memory consolidation.

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Poster

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Program #/Poster #: PSTR249.13/V36

Topic: H.08. Learning and Memory

Title: Spatial control of conductive polymer doping for conformable complementary internal ion-gated transistors

Authors: *D. WISNIEWSKI[1], D. KHODAGHOLY[2];
[1]Electrical Engin., Columbia Univ., New York, NY; [2]Columbia Univ., New York, NY

Abstract: Bioelectronic devices capable of accurately and safely interfacing with physiological systems for the capture, monitoring, processing, and stimulation of neural systems are required. Because of the ionic flux-based communication system used by neurons in the brain tissue signal transduction is required to observe neural activity with an electrical interface. This creates the need for organic mixed-conducting based transistors and sensors for measurement and recording of neuronal signals and systems. To use these sensors effectively conformable and scalable systems must be created for interfacing with both biology and existing technology. Current integrated circuit technology relies on a complementary approach of balanced N-type and P-type devices for improvements in power efficiency and scalability. Here we demonstrate a method to create transistors with N-type functionality, transistor action in the first quadrant and resistor like in the third quadrant, from P-type materials which usually express the opposite behavior, modulating in the third quadrant and acting as a resistor in the first quadrant. These opposing behaviors enabled a complementary logic device similar input and output voltage dynamics. We further demonstrate that this methodology can be applied to both electron and hole conducting materials. Additionally, the use of a single channel material and vertical geometry resulted in equal impedances between the two transistors yielding a higher power efficiency and fabrication density. Transistors were characterized in terms of asymmetry, voltage required for complementary operation, speed, and gain. Using designs informed by the characterization of channel contact asymmetry we then fabricated circuits including amplifiers for chronic in vivo recording in animal models. The high stability and signal to noise ratio of the devices allows for chronic mouse pup recording utilizing a fully conformable CIGT amplifier probe.

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Topic: H.08. Learning and Memory

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Title: Amyloid β-induced dopamine dysfunction in the lateral entorhinal cortex impairs associative memory

Authors: *T. NAKAGAWA[1], J. XIE[1], M. SAVADKOHI[2], H. JUN[1], J. Y. LEE[1], S. SOMA[1], T. C. SAIDO[3], K. M. IGARASHI[1];
[1]Univ. of California, Irvine, Irvine, CA; [2]Univ. of California, Irivne, Irvine, CA; [3]RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: Alzheimer's disease (AD) is the most common cause of dementia. Previous fMRI studies show that the lateral entorhinal cortex (LEC) is the primary site of dysfunction in early-stage AD patients, but it remains unclear what type of memory is affected by LEC dysfunction. We previously found that the LEC neurons in healthy animals are critically involved in associative memory encoding and dopamine facilitates associative memory encoding (Lee et al., Nature 2021). To test whether this LEC activity is impaired in AD, we used in vivo electrophysiology to examine LEC neurons in amyloid precursor protein knock-in (APP-KI) mice performing an odor cue-reward association task. APP-KI mice showed impaired memory performance in our associative memory task, and their LEC neurons showed disrupted associative memory encoding. Photometry recordings revealed that LEC dopamine was decreased at novel rewarded odor. Optogenetic stimulation of LEC dopamine fiber rescued associative memory. These results suggest that dysfunction of LEC-projecting dopamine neurons underlies memory impairment in AD from early stages, pointing to a need for clinical investigation of LEC dopamine in AD patients.
Reference: Lee JY, Jun H, Soma S, Nakazono T, Shiraiwa K, Dasgupta A, Nakagawa T, Xie JL, Chavez J, Romo R, Yungblut Y, Hagihara M, Murata K, and Igarashi KM (2021)Dopamine facilitates associative memory encoding in the entorhinal cortex ***Nature,*** 598:321-326

Disclosures: T. Nakagawa: None. **J. Xie:** None. **M. Savadkohi:** None. **H. Jun:** None. **J.Y. Lee:** None. **S. Soma:** None. **T.C. Saido:** None. **K.M. Igarashi:** None.

Poster

PSTR249: Learning and Memory: Hippocampal-Cortical Interactions II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR249.15/V38

Topic: H.08. Learning and Memory

Title: Lateral entorhinal cortex and prefrontal cortex neurons co-dependently encode associative memory

Authors: *J. LEE, H. JUN, A. ICHII, J. DONOHUE, K. M. IGARASHI;
UC Irvine Sch. of Med., Irvine, CA

Abstract: Associating a novel stimulus with an expected outcome is a fundamental component of learning. During olfactory associative learning, neurons in layers 2/3 of lateral entorhinal cortex (LEC) detect odors associated with reward and relay temporally coordinated outputs to the hippocampus. Hippocampally-processed signals return to layers 5/6 of the LEC ($LEC_{L5/6}$) and are further transmitted to neocortex broadly. The roles of $LEC_{L5/6}$ and its target neocortical areas in associative learning have been largely unexplored. Since $LEC_{L5/6}$ neurons both project to and receive axons from the medial prefrontal cortex (mPFC) forming a loop circuit, we recorded from $LEC_{L5/6}$ neurons and their target neurons in mPFC during associative learning. We found that neurons in $LEC_{L5/6}$ and mPFC encode cue-outcome associations in a mutually dependent manner. As mice learned odor cue associations, $LEC_{L5/6}$ and mPFC neurons classified novel and familiar cues into dichotomic groups of rewarded vs. punished cues. When $LEC_{L5/6}$ neurons were optogenetically suppressed, tangled mPFC representations of novel cues failed to discriminate rewarded vs. punished cues, impairing associative learning behavior. Conversely, when mPFC neurons were suppressed, $LEC_{L5/6}$ representations of individual cues failed to group meaningfully, disrupting both the learning and the retrieval of associative memories. These results suggest the critical role of the unexplored $LEC_{L5/6}$-mPFC bidirectional circuit in encoding associative memory as a cognitive map of outcome rules.

Disclosures: J. Lee: None. **H. Jun:** None. **A. Ichii:** None. **J. Donohue:** None. **K.M. Igarashi:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.01/W1

Topic: H.08. Learning and Memory

Title: Blockade of NMDA receptors affects the learning process during the serial-feature positive discrimination conditioning of eyeblink response in rats

Authors: *N. YAMAGAMI, R. UCHIDA, K. USUI, S. KAWAHARA;
Fac. of Engin., Univ. of Toyama, Toyama, Japan

Abstract: Several paradigms of eyeblink conditioning depend on the activities of forebrain, including the hippocampus and the prefrontal cortex, and are impaired by administration of NMDA antagonists. In the serial-feature positive (SFP) discrimination task, rats learn to express

the conditioned response (CR) when the light cue stimulus is delivered several seconds before the tone conditioned stimulus (CS) but not without the preceding light cue (Tokuda et al., 2014). The analysis of hippocampal local field potential (LFP) revealed a positive trial-by-trial correlation between an increase in the relative hippocampal theta power before the CS and the subsequent CR expression, suggesting a hippocampal contribution to anticipation of the coming CS. In the present study, we investigated the effects of noncompetitive NMDA antagonist MK-801 on the SFP discrimination task, during which the hippocampal LFP and the prefrontal electrocorticograms (ECoG) were recorded. Wistar/ST male rats were administered saline or MK-801 (0.1 mg/kg, *i.p.*) before the daily conditioning. We found that MK-801 group acquired the SFP discrimination as well as the control group. However, this discrimination, but not CR expression, was significantly suppressed after switching to saline injection with a tendency of relearning during 3 days of saline-injection session. Control group also showed a severe impairment of the SFP discrimination, as well as the CR expression, after switching to MK-801 injection. These behavioral data suggested a difference in learning process between control and MK-801 groups. Analysis of the hippocampal LFP and the prefrontal ECoG revealed that MK-801 suppressed the cue-induced increase in the relative theta power in the prefrontal cortex but not in the hippocampus during the acquisition sessions. In addition, switching of the injected solution after sufficient leaning affected the hippocampal theta oscillation more than the prefrontal one both in control and MK-801 groups. Further analysis revealed a cue-induced increase in the prefrontal-hippocampal theta coherence, associated with the subsequent CR expression, in control group. This increase in coherence tended to be suppressed in MK-801 group due to an elevation of basal theta coherence. These neural data, together with the behavioral ones, suggest that under the blockade of NMDA receptors rats could learn the SFP discrimination task but with a somewhat different neural mechanism.

Disclosures: N. Yamagami: None. **R. Uchida:** None. **K. Usui:** None. **S. Kawahara:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.02/W2

Topic: H.08. Learning and Memory

Title: The role of prefrontal spatial coding in supporting a contextual rule-switching task

Authors: *A. CUMPELIK[1], J. L. CSICSVARI[2];
[1]IST Austria, Klosterneuburg, Austria; [2]Inst. of Sci. and Technol. (IST) Austria, Klosterneuburg, Austria

Abstract: The medial prefrontal cortex (mPFC) plays a broad role in decision making, cognitive flexibility, and executive function, as well as long-term memory consolidation. These distinct functions align with the mPFC's role in context-dependent decision making, which requires evaluating and selecting representations relevant to specific tasks or goals.

The hippocampus and prefrontal cortex exhibit coordinated activity during hippocampal theta and sharp-wave ripples (SWRs). This synchrony correlates with performance during tasks requiring spatial information for accurate decisions. The mPFC has been shown to encode spatial information, and its assemblies reactivate during sleep coincidentally with hippocampal SWRs. Additionally, recent work indicates that the mPFC replays temporally organized spatial sequences in well-trained animals during awake immobility when performing a spatial rule-switching task. Our research aims to identify learning correlates in the mPFC in naive animals. We use 32-tetrode microdrives to record hippocampal and mPFC activity while rats learn to associate specific food cues with designated reward locations in a radial eight-arm maze. Two paired cue-reward associations are learned simultaneously. To find the reward, the rats must adapt their behavior flexibly based on the presented cue, representing the "context" they find themselves in. During training, we observed a sudden jump in performance after 6-7 days of training. This shift may coincide with the refinement of spatial representations and trajectory replay in the mPFC.
Determining the time course of mPFC spatial representations and trajectory replay during learning will provide insight into how behavioral demands may drive the refinement of task-relevant information in the mPFC.

Disclosures: A. Cumpelik: None. **J.L. Csicsvari:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.03/W3

Topic: H.08. Learning and Memory

Support: Z1A DA000587

Title: Schema signaling by ensembles of orbitofrontal neurons is suppressed by hippocampal output during learning of new problems.

Authors: *W. ZONG[1], J. ZHOU[2], M. P. GARDNER[3], Z. ZHANG[4], K. M. COSTA[6], G. SCHOENBAUM[5];
[1]NIH, NIDA IRP, Baltimore, MD; [2]Natl. Inst. of Biol. Sci., Beijing, China; [3]Concordia Univ., Montreal, QC, Canada; [4]Nation Inst. on Drug Abuse, [5]NIDA IRP, BALTIMORE, MD; [6]Psychology, Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: The roles of both the orbitofrontal cortex (OFC) and hippocampus (HC) in the formation of cognitive maps and their subsequent generalization into schemas are well-established. Yet, the precise nature of their interaction in facilitating this cognitive function remains an area of ongoing inquiry. Previously, we have presented data showing that single OFC units generalize representations of task structure or schema common across the two mazes. Optogenetic inactivation of the ventral subiculum had no impact on the prevalence of such

schema cells in the OFC for previously experienced maze pairs. However, when inactivation was applied during the learning of new maze pairs, there was a significant enhancement in the development of schema cells in the OFC. In this presentation we will describe results of population level ensemble analyses comparing coding to single units. Using PCA to assess task representations across mazes, we observed discrepancies between the Control and Ventral Subiculum inactivation groups during the transfer to new problems but not during the performance on established problems. LDA clustering analysis effectively differentiated positions but posed challenges for the GtACR2 group during learning across multiple days. Analysis of across-trial learning dynamics using non-negative Tensor Composition Analysis (TCA) revealed distinct patterns in the Control group, while the GtACR2 group exhibited components linked to learned rewards during the learning of new problems. Overall, our population-level analyses mirrored the results of single-unit approaches, highlighting significant effects of inactivation on OFC representations during learning of new problems, indicative of accelerated formation of generalized representations.

Disclosures: W. Zong: None. **J. Zhou:** None. **M.P. Gardner:** None. **Z. Zhang:** None. **K.M. Costa:** None. **G. Schoenbaum:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.04/W4

Topic: H.08. Learning and Memory

Support: STI 2030-Major Projects (2022ZD0207500 to J.Z.)
Beijing Nova Program (Z211100002121029 to J.Z.)

Title: Hippocampal and orbitofrontal neurons contribute to complementary aspects of associative structure

Authors: *H. LIN[1,2], J. ZHOU[1];
[1]Chinese Inst. for Brain Res., Beijing, Beijing, China; [2]Peking University, Beijing, China

Abstract: The ability to establish associations between environmental stimuli is fundamental for higher-order brain functions like state inference and generalization. Both the hippocampus and orbitofrontal cortex (OFC) play pivotal roles in this, demonstrating complex neural activity changes after associative learning. However, how precisely they contribute to representing learned associations remains unclear. Here, we trained head-restrained mice to learn four ‘odor-outcome’ sequence pairs composed of several task variables—the past and current cues, sequence structure of ‘cue-outcome’ arrangement, and the expected outcome; and performed calcium imaging from these mice throughout learning. Sequence-splitting signals that distinguished between paired sequences were detected in both brain regions, reflecting associative memory formation. Critically, we uncovered differential contents in represented

associations by examining, in each area, how these task variables affected splitting signal generalization between sequence pairs. Specifically, the hippocampal splitting signals were influenced by the combination of past and current cues that defined a particular sensory experience. In contrast, the OFC splitting signals were similar between sequence pairs that shared the same sequence structure and expected outcome. These findings suggest that the hippocampus and OFC uniquely and complementarily organize the acquired associative structure.

Disclosures: H. Lin: None. **J. Zhou:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.05/W5

Topic: H.08. Learning and Memory

Support: NIH Grant R01-MH112688

Title: Examining multiple moments of decision-making through prefrontal, hippocampal, and dorsolateral striatal interactions in a complex spatial task

Authors: *U. MUGAN[1], S. HOFFMAN[2], A. D. REDISH[3];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]NIH, Natl. Inst. on Drug Abuse (NIDA), Rosedale, MD; [3]Dept. Neurosci, Univ. of Minnesota, Minneapolis, MN

Abstract: Naturalistic behaviors often occur in uncertain and complex environments. Prior research suggests that three regions make distinct contributions to the decision-making process: hippocampus (HC), encoding flexible action plans, dorsolateral striatum (DLS), encoding cached action plans, and medial prefrontal cortex (mPFC) encoding strategy. Studies have found that mPFC and HC coordinate through oscillatory synchronization at important decision-points in decision-making tasks, which is interpreted as enhancing information transfer and integration between mPFC and HC. However, past studies have largely focused on representations of future outcomes at high-cost choice points (CP) in simple mazes, such as T-junctions, thus ignoring potential planning that may be preceding it.
To fill this gap, we examined neural activity in rats on a left/right/alternation foraging task by simultaneously recording from dorsal HC, DLS, and mPFC with silicon probes and inactivating mPFC with DREADDs in a separate cohort of rats. Rats ran through a complex central path and either turned left or right for a food reward, where rules changed twice throughout each 45 min session.
In addition to well-studied reorientation behaviors at choice points, rats also displayed pause and reorientation behaviors at the start of the maze (SOM) that significantly increased after rule switches, periods hypothesized to be driven under the deliberative system. Furthermore, the prominence of the reorientation behaviors at SOM and CP were significantly correlated. HC

nonlocal sequences at SOM and CP elongated under uncertainty, and were correlated with deliberative behaviors, such as exploration and reorientation. We found increased DLS activity at the SOM and CP after automation, which is thought to indicate the beginning and end of cached action sequences. Notably, there was an inverse relationship between HC sequence length and DLS bursting at both SOM and CP. mPFC activity carried information about the extent of nonlocal HC coding occurring at SOM over longer behaviorally relevant timescales, much like at CP. There was a significant compensatory increase in both vicarious trial and error (VTE) events at CP and reorientation behaviors at SOM following mPFC inactivation on the previous day. Reorientation behaviors at SOM and CP VTE were significantly more correlated in control than active virus animals. Our findings reveal that in more naturalistic environments planning may also be occurring at multiple locations in the environments, each of which are similarly modulated by mPFC activity that controls the initiation and extent of deliberative sequences.

Disclosures: U. Mugan: None. **S. Hoffman:** None. **A.D. Redish:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.06/W6

Topic: H.08. Learning and Memory

Support: e NatNIMH Intramural Research Program: ZIA MH-002920

Title: The development of hippocampal-neocortical connectivity inhuman infants

Authors: *S. AUDRAIN[1], J. BAFFOE-BONNIE[2], J. WILSON[2], S. MILLEVILLE[3], S. J. GOTTS[4], A. MARTIN[5];
[1]NIMH, Bethesda, MD; [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]NIMH/IRP/LBC, NIMH/IRP/LBC, Bethesda, MD; [4]Lab. of Brain and Cognition, NIMH/NIH, Bethesda, MD; [5]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: As adults, we do not remember events experienced as infants. Infancy is a critical period of development for the human memory system, yet we know little about the functional neural changes that occur during this time. In adults, there are notable differences in functional connectivity along the long-axis of the hippocampus with the neocortex, and such hippocampal-neocortical interaction is needed for establishing long-term memories. Using resting-state scans collected as part of the Baby Connectome Project, we investigated functional connectivity along the long-axis of the hippocampus with the neocortex in 212 infants over the first two years of life. We conducted a seed-to-voxel connectivity analysis, predicting connectivity as a function of age and long-axis while controlling for motion and temporal signal to noise ratio (tSNR) across participants. We identified numerous cortical regions that showed a significant interaction in connectivity between long-axis and age. Hierarchical clustering of these cortical regions based on the direction of the interaction revealed several canonical cortical networks, including medial

temporal, posterior medial, cingulo-opercular, and dorsal frontoparietal networks. Thus, we found that hippocampal connectivity along the long-axis with the neocortex is still maturing over the first two years of life. This maturation of connectivity was not restricted to classic memory areas, rather, cortical networks not canonically associated with memory were also identified based solely on their connectivity with the hippocampus along its anterior-posterior axis with development. The development of hippocampal-cortical connectivity during the earliest years of life may provide important clues for understanding infantile amnesia and cognition more broadly.

Disclosures: S. Audrain: None. **J. Baffoe-Bonnie:** None. **J. Wilson:** None. **S. Milleville:** None. **S.J. Gotts:** None. **A. Martin:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.07/W7

Topic: H.08. Learning and Memory

Support: Swiss National Science Foundation Grant P00A-106701
Swiss National Science Foundation Grant PP00P3-124536
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NIH Grant MH041479
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California National Primate Research Center Grant OD011107

Title: Postnatal structural development of the monkey perirhinal and parahippocampal cortices

Authors: J. VILLARD[1], L. J. CHAREYRON[1], P. A. BANTA LAVENEX[2], D. G. AMARAL[3], ***P. LAVENEX**[1];
[1]Univ. of Lausanne, Lausanne, Switzerland; [2]UniDistance Suisse, Brig, Switzerland; [3]Univ. of California Davis, Sacramento, CA

Abstract: The perirhinal and parahippocampal cortices are key components of the medial temporal lobe memory system and essential for the processing of spatial and declarative memory. In humans, major changes in declarative memory capacities occur within the first seven years of life, but until recently, the neurobiological substrates underlying these changes remained hypothetical. Previous studies have shown that distinct regions, layers and cells of the hippocampus and entorhinal cortex exhibit different profiles of structural and molecular development during early postnatal life. Accordingly, the differential maturation of distinct hippocampal circuits is thought to underlie the differential emergence of specific "hippocampus-dependent" memory processes. To better understand the maturation of the primate medial temporal lobe memory system, we implemented design-based stereological techniques to characterize the structural development of the different layers and subdivisions of the perirhinal

and parahippocampal cortices in macaque monkeys at different postnatal ages. We found different developmental changes in neuronal soma size, neuron number and volume of the distinct layers and subdivisions, which overall suggest an earlier maturation of the parahippocampal cortex compared to the perirhinal cortex, and an earlier maturation of the superficial layers relatively to the deep layers. These findings are consistent with studies showing the differential maturation of the rostral and caudal entorhinal cortex, which are interconnected with the perirhinal and parahippocampal cortices, respectively. This study provides fundamental information on the normal development of the primate medial temporal lobe memory system and defines critical periods of maturation that might be sensitive to perturbation and contribute to developmental disorders.

Disclosures: J. Villard: None. **L.J. Chareyron:** None. **P.A. Banta Lavenex:** None. **D.G. Amaral:** None. **P. Lavenex:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.08/W8

Topic: H.08. Learning and Memory

Support: New Frontiers in Research Fund

Title: Human saccadic eye movements, hippocampal ripples and memory

Authors: *I. SKELIN[1,2], M. ISTASY[3], C. KATZ[5], Q. CHU[6], T. BIBA[4], T. A. VALIANTE[1]; [1]Univ. Hlth. Network, Toronto, ON, Canada; [2]CRANIA, Toronto, ON, Canada; [3]Temerty Fac. of Med., [4]Dept. of Psychology, Univ. of Toronto, Toronto, ON, Canada; [5]Inst. of Biomaterials and Biomed. Engin., Univ. of Toronto Univ. Hlth. Network, Toronto, ON, Canada; [6]Univ. Hlth. Network - Univ. of Toronto, Toronto, ON, Canada

Abstract: Inhibition of return (IOR) is a phenomenon whereby the saccadic eye movements show a lower probability of returning to previously explored parts of a scene. IOR likely depends on the interactions between the neuronal populations involved in eye movement planning and short term memory. However, the brain circuitry level understanding of mechanisms underlying IOR, including the involvement of the electrophysiological activity implicated in memory (theta oscillations, sharp-wave/ripples (SWRs)), is still incomplete. To address this question, we took advantage of a wide electrode coverage in patients with epilepsy undergoing pre-surgical evaluation of epileptic foci to measure population level activity (high frequency activity, HFA; 70-200 Hz) distinguishing saccades that return to previously explored areas (return saccades), relative to saccades directed towards non-explored areas (non-return saccades). On individual trials, participants explored naturalistic scenes, searching for hidden targets. The analysis was done on 10602 saccades (16 datasets, 15 subjects), and a total of 536 electrodes across the temporal, frontal, parietal and occipital lobes. Overall, 12.3 ± 0.6% of saccades were classified as

‘return’, defined as terminating within an arbitrary radius of 150 pixels (15%/11% of the scene height and width, respectively) from any of the previous saccade end points during the same scene exploration. A distributed network of local populations (~23% of tested electrodes) shows significant HFA modulation during peri-saccadic window (+/-200 ms), depending on the saccade category (return or non-return). Saccade category-dependent HFA modulations on individual electrodes are almost exclusively unidirectional (increase or decrease, but not both), with the majority of electrodes showing lower HFA on return saccades. Preliminary analysis shows the higher local theta power temporally overlapping with saccade type-dependent HFA modulations, regardless of the HFA modulation direction. In addition, hippocampal SWRs tend to precede the saccade onsets, with the stronger modulation on non-return saccades. These findings suggest the role of SWRs and theta oscillations in memory-based saccadic planning.

Disclosures: I. Skelin: None. **M. Istasy:** None. **C. Katz:** None. **Q. Chu:** None. **T. Biba:** None. **T.A. Valiante:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.09/W9

Topic: H.08. Learning and Memory

Title: How well can Tolman-Eichenbaum Machine explain neurons in the hippocampal-entorhinal cortex?

Authors: *D. KAWAHARA[1], S. FUJISAWA[2];
[1]Riken Ctr. for Brain Science: RIKEN Noshinkei Kagaku Kenkyu Ctr., Wako/Saitama, Japan;
[2]RIKEN Ctr. For Brain Sci., Wako, Japan

Abstract: In neuroscience, there is growing interest in approaches to understanding information processing in neural networks of the brain from machine learning, for example, the similarities between CNN and the visual cortex of the brain.The Tolman-Eichenbaum Machine proposed by Whittington et al. (2020) has attracted attention as a machine learning model to reproduce, for example, place cells, grid cells, and remapping in the hippocampal-entorhinal cortex. However, it is still unclear to what extent the Tolman-Eichenbaum Machine and the hippocampal-entorhinal cortex network are similar. In this study, we conducted simulation experiments of several tasks to investigate the degree of similarity between the Tolman-Eichenbaum Machine and neurons in the hippocampal-entorhinal cortex. The results showed that certain types of neurons could not be reproduced by the Tolman-Eichenbaum Machine. We also discuss a model that explains neurons in the hippocampal-entorhinal cortex in a more unified manner by improving the Tolman-Eichenbaum Machine.

Disclosures: D. Kawahara: None. **S. Fujisawa:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.10/W10

Topic: H.08. Learning and Memory

Support: NIH UG3/UH3 grant UG3MH125273

Title: Effect of Closed-Loop Auditory Stimulation of Cortical Slow Oscillation on Cortical Spindles, Hippocampal Sharp Wave Ripples, and Memory Consolidation

Authors: *A. DA COSTA SOUZA, C. VARELA;
Psychology, Florida State Univ., TALLAHASSEE, FL

Abstract: The neural oscillations that characterize sleep are essential for memory consolidation. The temporal coordination of non-REM cortical Slow Oscillations (SO), spindles, and hippocampal Sharp Wave Ripples (SWR) is thought to facilitate memory consolidation.
The online closed-loop manipulation of SO up and down states (US, DS) - a higher and lower excitability phase, respectively - allows for the investigation of SO manipulation on memory consolidation; for instance, auditory closed-loop stimulation of SO US has been shown to improve memory in humans.
Recent studies using closed-loop auditory stimulation (CLAS) experiments targeting SO or SWR in rodents have found variable results. They found no change in SO[1] and a decrease in SWR[1,3] and spindle activity[1]; or an increase in SO and spindle activity[2]; no change (US targeting[1,2]) or worsening (DS[1], SWR targeting[3]) of memory performance. These results suggest that non-REM sleep oscillations can be influenced online to regulate memory consolidation, but that we do not yet understand how the specific parameters of those manipulations can alter memory consolidation.
To investigate how SO manipulation affects spindles and SWRs, we performed a CLAS experiment targeting the US or the DS of the SO in rats. We also investigated how US and DS CLAS targeting affects memory in an Object Place Recognition (OPR) task.
Rats were implanted with tetrode arrays in the retrosplenial cortex and hippocampus and recorded during an OPR task. The experimental session consisted of: 1 hour of baseline (BL), 10 minutes of training, 2 hours of post-training (PT), and a 10-minute test. During BL, no auditory stimuli were presented, while during PT, CLAS was delivered targeting the SO US or DS for 2 hours; or no stimulation was delivered. An open-loop control was also included. We will present results to answer the following questions: Does SO US or DS CLAS change spindle or SWR power, rate, or phase-coupling? Does it evoke or suppress SO? How do these results correlate with memory performance? With these answers, we will gain insights into what parameters of CLAS regulate sleep oscillations (spindles, SWR) and memory consolidation.
References: 1. S. Aksamaz et al., Eur J Neurosci. 59, 595-612 (2024).2. C. G. Moreira et al., eLife. 10, e68043 (2021). 3. K. Salgado-Puga, G. Rothschild, bioRxiv, in press, doi:10.1101/2023.11.22.568283.

Disclosures: A. da Costa Souza: None. **C. Varela:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.11/W11

Topic: H.08. Learning and Memory

Support: NIH Grant 1R01MH136197-01

Title: Causal Evidence of the Hippocampal Engagement by Parietal Stimulation

Authors: *Z. LI[1,2], K. WU[1,2], B. D. UITERMARKT[3], J. E. BRUSS[3], N. TRAPP[1], H. OYA[4], A. D. BOES[3], J. JIANG[1,2];
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Abstract: The hippocampus is an important subcortical region for human memory and other cognitive functions. It has long been demonstrated to be structurally and functionally connected with cortical regions in the parietal lobe. However, despite existing evidence indicating the impact of neural stimulation of the parietal lobe on human memory performance, the precise causal modulation of neural responses in the hippocampus by the parietal lobe remains unclear. To address this issue, we employed state-of-the-art perturbation-measure methodologies. We stimulated the right parietal cortex with transcranial magnetic stimulation (TMS) while concurrently recording neural responses in the hippocampus using invasive (intracranial electroencephalography, iEEG) or non-invasive neuroimaging techniques (functional magnetic resonance imaging, fMRI). In Study 1, we delivered single-pulse TMS to the right parietal cortex of three epilepsy patients during their pre-surgical monitoring, with simultaneous recording of their hippocampal responses by iEEG (n = 18 electrodes). Results showed that TMS stimulation evoked a predominant response in 44.4% of the hippocampus electrodes. This TMS-induced response was significantly stronger compared to that in the sham stimulation condition. Furthermore, the amplitude of the TMS-induced response for each electrode positively correlated with its resting-state functional connectivity to the corresponding simulation site (Spearman r = .70, p = .002), suggesting the potential of utilizing functional connectivity to guide the stimulation in the parietal lobe for better targeting the hippocampus. In Study 2, we adopted concurrent TMS-fMRI to examine this TMS-induced response in neurotypical populations (N = 43 participants). Consistent with Study 1, the blood-oxygen-level-dependent (BOLD) signals of the right hippocampus were significantly correlated with its resting-state functional connectivity to the individual's stimulation site in the right parietal lobe (Spearman r = .33, p = .032). Together, our study revealed the hippocampal engagement to parietal TMS stimulation and highlighted the feasibility of a functional connectivity-based strategy to target the hippocampus. These findings extend the knowledge of the parietal-hippocampus circuit, offering valuable insights for developing non-invasive neuromodulatory therapies for improving hippocampus-related cognitive functions in clinical populations.

Disclosures: Z. Li: None. **K. Wu:** None. **B.D. Uitermarkt:** None. **J.E. Bruss:** None. **N. Trapp:** None. **H. Oya:** None. **A.D. Boes:** None. **J. Jiang:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.12/W12

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH130529

Title: Positive and Negative Retinotopic Codes Structure Hippocampal-Cortical Interactions

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Abstract: Position-dependent activity in response to stimulation of the retina, or retinotopic coding, has recently been observed in memory-related cortical regions like the Default Network in both humans (Szinte and Knapen 2020; Knapen 2021) and non-human primates (Klink et al., 2021), and even the hippocampus (Silson et al., 2021; Knapen 2021). Yet, the role of retinotopic coding in memory systems is unclear. In cortex, retinotopy outside of canonical visual regions often takes the surprising form of decreasing signal during visual receptive field stimulation (-pRFs), and the shared retinotopic code between these -pRFs and classic positively responding voxels (+pRFs) appears to structure perceptual-mnemonic interactions (Steel*, Silson* et al. 2024). What are the characteristics of the retinotopic code in the human hippocampus, and how does it structure interactions with cortex? Here we characterize positive and negative retinotopic responses to visual stimuli in the hippocampus using high-resolution (7T, 1.8mm isotropic, n = 7) pRF mapping data from the Natural Scenes Dataset (Allen et al. 2022). We found that the hippocampus contained a relatively high proportion of negative pRFs, consistent with our prediction. On average across participants, 25% of hippocampal voxels exhibited a significant ($R2 \geq 0.08$) retinotopic response, and 53% of these voxels were negative in valence. Negative voxels had similar pRF sizes to their positive counterparts. Consistent with prior work, positive pRFs showed a significant contralateral visual field bias (i.e., left hippocampus tended to represent the right visual field) ($p < 0.05$), while negative pRFs were less eccentric and did not evidence the contralateral bias. Interestingly, resting state functional connectivity analyses suggest positive and negative hippocampal pRFs are differentially coupled to cerebral cortex, co-fluctuating more strongly with congruently signed cerebral pRFs. This work suggests the importance of visual coding in structuring the interaction between the hippocampus and cerebral cortex, and adds support for the view that negative pRFs may play an important role in hippocampally-dependent cognitive processes like episodic memory and scene construction.

Disclosures: P.A. Angeli: None. **A.D. Steel:** None. **E.H. Silson:** None. **C.E. Robertson:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.13/W13

Topic: H.08. Learning and Memory

Support: R01NS101108

Title: Performance of rats in learning and memory tasks not impacted despite unilateral traumatic brain injury affecting the hippocampus

Authors: *M. HABIB[1], L. LOVE[1], E. MIRZAKHALILI[1], C. D. ADAM[2], J. A. WOLF[1]; [1]Dept. of Neurosurg., Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania Neurosci. Grad. Group, Philadelphia, PA

Abstract: Learning and memory deficits are common following traumatic brain injury (TBI), yet the mechanisms underlying these deficits are poorly characterized. Previously we showed that rats subjected to a lateral fluid percussion injury (L-FPI) had decreased oscillatory power, reduced theta-gamma phase-amplitude coupling, and disrupted spike-filed coherence in hippocampus ipsilateral to the injury site. While these results provide a potential pathophysiological mechanism underlying learning and memory dysfunction following TBI, cognitive deficits in the L-FPI model are inconsistent. TBI deficits in the 8 Arm Radial Maze (Radial Arm Maze; RAM) have been reported following L-FPI in rats, but most studies focused solely on learning or memory. Our RAM task permitted simultaneous evaluation of learning and memory as rats navigated the maze. To this end, Long-Evans rats were food restricted and trained to navigate to three baited arms on the RAM. Rats were run on consecutive days (30 trials/day; 1 trial involved visiting all three baited arms) to assess memory of the baited arms. During trials they could make reference memory errors (RMEs) by entering an unbaited arm or working memory errors (WMEs) by revisiting a previously rewarded arm. Rats then underwent a reversal session to assess learning, wherein one of the three baited arms remained constant while the other two changed locations. Following this, rats were subjected to either L-FPI or sham surgery and run through subsequent reversal and probe sessions to assess learning and memory post-injury. Interestingly, with the contralateral hippocampus intact, we found no significant differences in the number of WMEs or RMEs between sham (n=3) and injured (n=3) rats. To address concerns about potential striatal dependence, we introduced delays of 2, 4, 8, and 16 seconds between reward retrievals to increase hippocampal involvement. However, there were no performance differences between sham (n=1) and injured (n=3) rats, although both groups made more WMEs at longer delays. While preliminary, these results suggest that a unilateral hippocampal injury may not disrupt learning and memory on this task. This aligns with prior lesion studies, indicating that unilateral hippocampal inactivation does not affect performance on hippocampal-dependent tasks, while bilateral inactivation impairs performance. We are currently investigating whether an excitotoxic lesion of the contralateral hippocampus reveals learning and memory deficits on this task in rats subjected to L-FPI. Such findings would imply that compensatory mechanisms from the contralateral hippocampus may impact learning and memory in the L-FPI model.

Disclosures: M. Habib: None. **L. Love:** None. **E. Mirzakhalili:** None. **C.D. Adam:** None. **J.A. Wolf:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.14/W14

Topic: H.08. Learning and Memory

Support: LF grant R310-2018-3611
LF experiment R436-2023-471
LF experiment R436-2023-855

Title: Endopiriform neurons projecting to ventral CA1 are a critical node for recognition memory

Authors: N. YAMAWAKI[1,2,3], H. LOGIN[1,2,3], S. ØSTERGAARD FELD-JAKOBSEN[1], B. M. MOLNAR[1], M. ZIPPOR[1], M. MOLTESEN[1], A. OKRASA[1], J. M. RADULOVIC[4,1,2,3,5], ***A. TANIMURA**[1,2,3];
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Abstract: The claustrum complex, an evolutionarily conserved brain region found across mammalian species, reptiles, and birds, is hypothesized to serve as a node for establishing higher order cognitive functions by coordinating neuronal activity on a global scale. Some of these functions include sensory perception and attention, which may affect memory processing, including working memory, associative memory, and recognition memory. However, the circuit organization and function of its neuroanatomical subregions are not well understood. We demonstrated that some of the key roles of the claustrum complex can be attributed to the connectivity and function of a small group of neurons in its ventral subregion, the endopiriform (EN). One of distinct targets of EN, the ventral CA1 (vCA1), is known to coordinate in a range of behaviors related to exploration and recognition memory, and these functions are thought to be regulated by its afferent inputs in a domain-specific manner. We, therefore, hypothesized that EN is a key node for establishment of recognition memory. To address this, we set out to characterize circuit and function of EN neurons defined by their projection to vCA1 (EN $^{vCA1\text{-}proj.}$ neurons) using genetic tools and mouse models of social and object recognition memory. We found EN $^{vCA1\text{-}proj.}$ neurons innervated multiple components of the limbic system except amygdala and prefrontal cortex and produced potent feedforward inhibitory control over vCA1 pyramidal neurons. During recognition memory test, activity of EN $^{vCA1\text{-}proj.}$ neurons were condensed around conspecifics or objects where mice spent most time on. However, disruption of EN $^{vCA1\text{-}proj.}$ activity only impaired memory-guided exploration of novel stimuli without affecting innate

exploration induced by novelty. These findings demonstrate that EN subserves some of the key functions required for recognition memory governed by specific limbic system.

Disclosures: N. Yamawaki: None. **H. Login:** None. **S. Østergaard Feld-Jakobsen:** None. **B.M. Molnar:** None. **M. Zippor:** None. **M. Moltesen:** None. **A. Okrasa:** None. **J.M. Radulovic:** None. **A. Tanimura:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: H.08. Learning and Memory

Support: the Fundamental Research Funds for the Central Universities

Title: Dynamic changes in hippocampal representations during recall predict false memories induced by misinformation in eyewitnesses

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Abstract: The recollection of an eyewitness to a criminal event may be influenced by subsequent misinformation, which may result in the formation of false memories. As eyewitnesses repeatedly attempt to recall events, their recollections are systematically distorted by misinformation. It has been demonstrated that neural representations in the hippocampus support the recall of narrative events (Barnett et al., 2023; Chen et al., 2017; Reagh et al., 2023; Shao et al., 2023). Despite the growing body of memory research on the hippocampus, there is still much to be learned about how it represents events during repeated recall before and after the exposure of misinformation. In this study, we used the functional magnetic resonance imaging (fMRI) technique to collect neural data from thirty-two participants across four stages: witnessing the original events, first free recall, reading post-event information, and second free recall. Each of the four stages lasted approximately half an hour, with a three-hour interval between the first-recall and post-event stages. One day later, participants completed a final recognition test outside the brain scanner. They were requested to recall and recognize information pertaining to the original events. In the hippocampus, neural pattern similarity was calculated between the original-event and first-recall stages, between the original-event and second-recall stages, and between the post-event and second-recall stages, for the corresponding items and the non-corresponding items in the same event, for each memory type in the recognition test, separately. During both recall stages, the hippocampus reinstated the pattern of neural activity that encoded the original events, which subsequently led to correct recognition of control items (i.e., correct memory for accurate information). However, during the second recall stage but not the first recall stage, the left hippocampus reinstated an opposite pattern of neural activity that encoded the original events, which subsequently led to false recognition of critical

items (i.e., false memory induced by misinformation). These findings provide support for the trace transformation theory and highlight the key role of the hippocampus in reconstructive memories.

Disclosures: X. Shao: None. **B. Zhu:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

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Topic: H.08. Learning and Memory

Support: BBSRC DTP Grant

Title: Ventral hippocampal functional inhibition disrupts repeated reversal learning, whereas disinhibition disrupts expression of the previous response

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Abstract: Reversal learning, a form of cognitive flexibility disrupted in many brain disorders, including schizophrenia, involves switching from one response to another when the reward contingencies of the responses are reversed. The hippocampus, especially ventral hippocampus (VH), projects to fronto-striatal circuits regulating reversal learning. Previous studies reported that hippocampal lesions impaired, whereas chemogenetic activation of VH facilitated, acquisition of reversal learning in animal models. However, there is limited evidence on how VH activity affects distinct stages of reversal learning. Therefore, we examined how VH functional inhibition or disinhibition, via infusion of a GABAA receptor agonist (muscimol) and antagonist (picrotoxin), affects repeated reversal learning and reversal learning acquisition, in young adult male Lister hooded rats performing a two-lever discrimination task. To study repeated reversals, rats were trained to acquire a spatial discrimination (press left or right lever) followed by four reversals, to achieve stable performance levels. Then, the impact of VH muscimol, picrotoxin and saline infusions on repeated reversals was compared within-subjects (n=13). To study reversal learning acquisition, we examined the impact of VH muscimol, picrotoxin or saline on spatial discrimination and three reversals, using a between-subjects sequential design, with half of the study completed at present (n = 5-7 per group). VH functional inhibition, but not disinhibition, impaired repeated reversal learning, indicating that repeated reversal learning requires VH activity, but not balanced levels of VH activity. In contrast, VH disinhibition, but not inhibition, impaired expression of the previous response during reminder trials preceding reversal trials, suggesting such expression does not require VH activity but may be disrupted by

aberrant activation of projection sites. Our study of reversal learning acquisition is ongoing. With half of the sequential design completed, VH disinhibition tended to improve reversal acquisition, but this may reflect impaired expression of the previous response during reminder trials. A Bayesian trial-by-trial analysis is on the way to examine changes in behavioural strategies that may underlie the impact of VH manipulations on repeated reversal and reversal acquisition.

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Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

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Support: JST ERATO JPMJER1801
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Title: Postnatal maturation of layer 2 regular spiking neurons in the granular retrosplenial cortex

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Abstract: Memories acquired in adulthood persist, while those from childhood often fade in adulthood. However, it has been shown that childhood memories are retained in an inaccessible state. Although the study of memory persistence has primarily focused on hippocampal activity, recent research in mice has shed light on the involvement of extrahippocampal cortices, particularly the retrosplenial cortex (RSC). Within the RSC, the granular retrosplenial cortex (gRSC) receives inputs from both the subiculum and the neocortex and is thought to be critical for memory consolidation. However, it is unclear how the gRSC contributes to differences in memory persistence between infancy and adulthood. We hypothesized that the neural circuits formed by the neocortex and hippocampus within the gRSC are critical for establishing stable memories in adulthood. The gRSC contains two types of neurons in layer 2/3: regular spiking neurons and late spiking neurons. Regular spiking neurons receive strong inputs from the neocortex, whereas late spiking neurons receive strong inputs from the subiculum. To investigate whether these neuron types in layer 2 of the gRSC are associated with persistent memory in mice, we used the whole-cell patch-clamp technique on acute brain slices to compare morphology and intrinsic membrane properties. We distinguished cell types by current injection and then visualized their morphology by biocytin-based reconstruction in the intrapipette solution. Our observations revealed that the dendritic complexity of regular spiking neurons in

adult mice, particularly in layer 3, exceeded that of their infant counterparts. Conversely, no noticeable differences were observed in late spiking neurons. Our data suggest a robust synaptic input from the subiculum to regular spiking neurons of the gRSC in adulthood compared to infancy, as evidenced by the concentration of subicular axons in layer 3. In the future, we plan to investigate this suggestion using optogenetic techniques. In addition, we plan to examine the response of hippocampal activity, such as sharp-wave ripples, under physiological conditions.

Disclosures: H. Mizuno: None. **Y. Ikegaya:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.18/W18

Topic: H.08. Learning and Memory

Support: CRC 1416

Title: Identifying and mobilizing neural resources for recognition memory in young and old populations

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Abstract: Previous studies indicate that successful performance on high demand recognition memory tasks requires medial temporal lobe (MTL) and prefrontal cortex (PFC)'s functional integrity. The MTL and the PFC are composed of vastly distinct functional subregions, including the hippocampal (CA1 and CA3) and parahippocampal subfields (PER, POR, LEC, MEC), and the Anterior cingulate (ACC), Prelimbic (PrL) and Infralimbic (IL) cortices. The extent to which each of these subareas might serve as a neural resource for recognition memory in aging organisms is however not well-understood. To address this question, we first compared brain activity between high and low performers in young and older mice (2 and 18 months-old, respectively) performing an object-in-place task, sensitive to hippocampal and PFC lesions (Barker and Warburton, 2015). Second, we investigated if cognitive training (repeated training in the object-in-place task: 4 times) improved performance on the task and studied the changes in brain activity in young and older mice with and without cognitive training. To do so, we detected the expression of the RNA of the immediate early genes Arc and Homer1A, commonly used to map cognitive functions (Guzowski et al., 2001; Sauvage et al., 2013; 2019; Nakamura and Sauvage, 2016), and compared between groups the proportion of cells that had been activated during both memory formation and retrieval (i.e. Arc/Homer1A+ reactivated cells). Preliminary results show that a limited number of areas could serve as neural resources for recognition memory in older compared to younger adults. In addition, cognitive training improved memory

performance in both young and older mice but at a different rate. Finally, brain activity patterns following the final training session, reveal that cognitive training might lead to changes at the neural level that favor a reversion of the effect of aging on brain integrity. Ongoing experiments focus on investigating whether the benefit resulting from the cognitive training can be transferable to other tasks. These findings might be instrumental in developing targeted interventions to slow down or restore age-related cognitive decline.

Disclosures: E. Atucha: None. **M. Sauvage:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

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Topic: H.08. Learning and Memory

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Title: Offline neuronal dynamics generate bias in emotional experience

Authors: *S. UM[1], M. KHALED HANAFY[2], R. OKUBO-SUZUKI[3], M. NOMOTO[4], E. MURAYAMA[5], K. CHOKO[5], K. YAMADA-NOMOTO[5], H. ASAI[6], A. SUZUKI[7], A. CHOUCRY[8], K. INOKUCHI[9];
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Abstract: Despite the proposed theory of higher-order processing of emotion, neuronal mechanism of how cognitive distortions such as overgeneralization, biased negative memory replay and self-reflection are generated is still not fully understood. Here we established a simple behavioral paradigm to prove that negative mood generates emotional bias when recalling episodic experiences. By implementing PCA/ICA dimensionality reduction method to track memory-related ensembles from chronic 1P Ca^{2+} imaging across the days, we detected an emergence of specific neuronal representations in hippocampus which likely reflect emotional state of episodic experiences. These state-dependent populations display emotional schema-like activity when mice experience new emotional event. Furthermore, negative mood guides offline co-activation of two separate emotional memory-related representations during sleep after new

experience and closed-loop optogenetic silencing of negative experience-specific ensemble during sleep prevented emotional bias in episodic experiences. Finally, we have found a prefrontal-hippocampal circuit regulating such emotional bias in episodic experiences. Together, we provide a new conceptual insight of how mood generates specific neuronal representation in memory network and higher-order area to encode emotional state of experiences and offline population dynamics may underlie overgeneralization of emotional events at the cognitive level.

Disclosures: S. Um: None. **M. Khaled Hanafy:** None. **R. Okubo-Suzuki:** None. **M. Nomoto:** None. **E. Murayama:** None. **K. Choko:** None. **K. Yamada-Nomoto:** None. **H. Asai:** None. **A. Suzuki:** None. **A. Choucry:** None. **K. Inokuchi:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.20/W20

Topic: H.08. Learning and Memory

Title: Electrophysiological correlates of recent and remote memory retrieval in the entorhinal cortex and CA1 and CA3 hippocampal subfields

Authors: *H. MULLA-OSMAN[1], S.-P. KU[1], E. ATUCHA[1], S. CALABRESE[1], A. REBOREDA[1], K. ALLEN[2], M. YOSHIDA[1], M. SAUVAGE[1];
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Abstract: The electrophysiological mechanisms underlying memory retrieval as memories age are still not well understood. We have previously shown that, while the CA1 hippocampal subfield is recruited for memory retrieval independently of the age of the memory, CA3's role in this process is limited to the most recent memories (i.e. up to 1-month-old early remote memories; Lux et al., eLife, 2016). In addition, we reported that parahippocampal areas such as the medial entorhinal cortex (MEC) provide support to CA1 for retrieving very remote memories (i.e. up to 1-year-old; Atucha et al., Cell Reports, 2023; SFN poster 8237). This selective 'disengagement' of CA3 over time suggests a shift from the contribution of the trisynaptic (EC-DG-CA3-CA1; TS) and the temporoammonic (EC-CA1; TA) pathway for the retrieval of recent memories to a preferential engagement of the TA pathway for recalling most remote memories. We have recently shown that the synchronization of population spiking activity with theta oscillations (spike-to-theta phase-locking) across brain regions can predict memory performance (Ku et al., Cell Reports, 2024; SFN poster 8262). Here, we test the hypothesis that the largest difference in the coordination of electrophysiological signals between recent and very remote memory retrieval will be observed between MEC and CA3, i.e. pertains the TS loop. To do so, we recorded single units and LFP signals *in vivo* simultaneously in CA1, CA3 and MEC during contextual fear memory retrieval 1 day, 1 month and 1 year after memory formation in distinct groups of mice and compared firing rates at the single cell and population level, theta power and

cross-regional interactions. Findings emerging from this project will provide further insights into the contribution of the TS and TA pathways to memory retrieval as memories age and into the coordination of electrophysiological signals in these two pathways within this framework.

Disclosures: H. Mulla-Osman: None. **S. Ku:** None. **E. Atucha:** None. **S. Calabrese:** None. **A. Reboreda:** None. **K. Allen:** None. **M. Yoshida:** None. **M. Sauvage:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

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Program #/Poster #: PSTR250.21/W21

Topic: H.08. Learning and Memory

Support: RO1NS125250

Title: Ripples orchestrate inter-regional dynamics for serial order memory in humans

Authors: *E. ERGIT, B. C. LEGA;
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Abstract: Representation of serial order constitutes a key feature of episodic memory, requiring that inter-item relationships (context information) are maintained via map-like representations to facilitate recall. Rodent models of spatial and non-spatial order implicate key hippocampal mechanisms in supporting order memory, such as theta phase coding and ripple events. However, in humans, both lesion studies and neurophysiological data suggest that areas beyond the hippocampus, particularly the orbitofrontal cortex and medial parietal regions, play a significant role in representing order information. To investigate the neurophysiological mechanisms of order information in humans, we used direct brain recordings from neurosurgical patients performing a serial recall task. Our unique dataset included the simultaneous acquisition of data from the hippocampus, orbitofrontal cortex, and posterior cingulate regions. Focusing on ripple events, we demonstrate distinct inter-regional dynamics during successful order memory encoding using precise temporal information. The relative timing of co-rippling events implicates theta oscillations in cross-regional integration, and we show that ripple rate (but not ripple duration) correlates with the amount of information being encoded. Finally, motivated by mechanisms of order memory described in non-human primates, we show that ripple events exhibit a reciprocal temporal relationship with elevations in beta oscillatory power. We link our findings with computational and anatomical models of episodic memory processing as well as general theories of temporal binding facilitated by co-rippling events. These results point towards multiregional approaches for neuromodulation incorporating the detection of short—duration beta and gamma band power elevations.

Disclosures: E. Ergit: None. **B.C. Lega:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

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Title: Establishing optogenetic approaches to test hippocampal-prefrontal causal relationships underlying the temporal organization of memories

Authors: *P. L. ASSAKURA MIYAZAKI[1], K. COOPER[2], G. A. ELIAS[3], N. J. FORTIN[2]; [1]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [3]Ctr. for Mol. and Behavioral Neurosci., Rutgers, The State Univ. of New Jersey - Newark, Newark, NJ

Abstract: Our ability to temporally organize memories is essential to daily life and is particularly vulnerable to neurological and psychiatric disorders. Although considerable research indicates that the hippocampus (HC) and prefrontal cortex (PFC) are critical for this function, their respective neuronal mechanisms and the dynamics of their functional interactions remain poorly understood. To address this issue, our lab employs a unique combination of complex behavioral, electrophysiological, and analytical approaches. Our recent work has identified distinct series of neural computations in the HC and the PFC, which lends to the hypothesis that there is a specific flow of information between the two structures during trials. To directly test this hypothesis, our goal is to incorporate projection-specific optogenetics manipulations in our behavioral and electrophysiological experiments. Here we present our experimental approach and preliminary results validating our use of viral vectors to target HC → PFC and PFC → HC projections

Disclosures: P.L. Assakura Miyazaki: None. **K. Cooper:** None. **G.A. Elias:** None. **N.J. Fortin:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR250.23/W23

Topic: H.08. Learning and Memory

Support: NIH Grant R01NS129874
AARG 21-850571

Title: Exposure to noise bursts during non-REM sleep impairs hippocampal sharp-wave ripples and memory consolidation

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Abstract: Sleep is critical for long-term memory stabilization. In particular, hippocampal sharp-wave ripples (SWRs) occurring during non-rapid eye movement sleep (NREMs)- brief oscillatory bursts of hippocampal activity- define periods of hippocampal reactivation of recent experiences and facilitate bidirectional communication between the hippocampus and cortical regions, promoting the process of memory consolidation. Conventionally, sleep is considered a state in which the brain is disconnected from the environment. However, sounds heard during sleep evoke robust responses throughout the auditory pathway. Moreover, anatomical and functional studies have shown that sounds heard during sleep influence hippocampal activity. Still, whether and how incoming sounds during sleep impact hippocampal SWRs and their role in memory consolidation remains unclear. To address this question, in this study, we used a closed-loop system to detect SWRs from the dorsal CA1 region of the rat hippocampus during a sleep session following spontaneous behavior. Real-time SWRs detected were paired with brief, non-waking broad-band noise (BBN) bursts. Interestingly, exposure to BBN bursts during sleep suppressed the ripple power and reduced the SWRs rate. Furthermore, BBN triggered during SWRs (On-SWR-BBN) suppressed ripple power significantly more than BBN triggered 2 seconds after SWRs detection (Off-SWR-BBN). Next, we test the influence of SWRs-BBN pairing paradigms during sleep on memory consolidation. To this end, SWR-triggered BBN bursts were presented
during sleep sessions after learning a place-reward association in a conditioned place preference paradigm. The effect of sound presentation during sleep on memory was quantified by comparing memory retention performance following the SWR-BBN paradigms to those with no BBN exposure. Interestingly, we found that On-SWR-BBN pairing during sleep dramatically reduced memory retention 24h after learning, evidencing a clear abolishment of memory consolidation. In contrast, Off-SWR-BBN pairing also reduced memory retention at 24h, but to a lesser extent, suggesting a partial effect on memory consolidation. Lastly, we tested whether the effect of the SWR-BBN stimulation during sleep on memory would change depending on the behavioral paradigm. In this sense, SWR-triggered BBN sounds were presented during sleep sessions after a contextual fear conditioning paradigm. Surprisingly, none of the SWR-BBN pairing paradigms affected the aversive memory retention. Together, these findings suggest that sounds heard during sleep interfere with SWR-facilitated memory consolidation in a time and task-specific manner.

Disclosures: K.G. Salgado-Puga: None. **G. Rothschild:** None.

Poster

PSTR250: Learning and Memory: Hippocampal-Cortical Interactions III

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Topic: H.08. Learning and Memory

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NIH/NIBIB (R01-EB026439)
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McDonnell Center for Systems Neuroscience
Fondazione Neurone
Brain & Behavior Research Foundation

Title: Entrainment of Intracranial Oscillations Through Theta-Burst Stimulation of the Basolateral Amygdala in Humans

Authors: *N. K. BRYSON[1], T. XIE[1], K. L. WAHLSTROM[2], J. CAMPBELL[3], J. R. MANNS[4], T. J. FOUTZ[5], C. S. INMAN[2], P. BRUNNER[1], J. T. WILLIE[1];
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Abstract: Memory is critical to maintaining quality of life, though nearly 10% of adults have self-reported memory loss and related cognitive decline. Prior work suggests that direct electrical brain stimulation, particularly theta-burst (8 Hz carrier waveform) stimulation to the amygdala, can enhance declarative memory in humans. A proposed neurophysiological mechanism links entrainment of theta-oscillations proportional to the strength of functional connectivity and theta and low gamma spectral power to improve memory performance. However, evidence for this mechanism is sparse and experimental observations have yet to be translated into meaningful clinical interventions. Thus, we aim to characterize memory network responses to theta-burst stimulation with the ultimate goal of optimizing therapeutic stimulation protocols. In this study, patients with intractable epilepsy and implanted with stereoelectroencephalography (SEEG) underwent theta-burst stimulation of the basolateral amygdala at various amplitudes, modulation frequencies, and pulse-widths. Responses from non-stimulated SEEG contacts were recorded simultaneously with scalp EEG and pupillometry. The entrainment of potentials at all electrode channels was quantified on the time series responses using the local and global variance of each stimulation trial, prior to, during, and after stimulation. Power spectral densities of these regions were also calculated to determine oscillatory and aperiodic power changes. Our results show that theta entrainment occurs primarily in hippocampal and thalamic nuclei, gamma entrainment occurs primarily in the ventral prefrontal cortex and insular cortex, and concurrent gamma-theta

oscillations are present in orbitofrontal cortex. These oscillations are sensitive to variation of stimulation charge density and precise stimulation site (i.e. lateral vs central amygdala, ipsilateral vs contralateral amygdala). Such entrainments may serve as biomarkers related to memory enhancement and facilitate development of personalized stimulation protocols for treatment of memory deficits.

Disclosures: N.K. Bryson: None. **T. Xie:** None. **K.L. Wahlstrom:** None. **J. Campbell:** None. **J.R. Manns:** None. **T.J. Foutz:** None. **C.S. Inman:** None. **P. Brunner:** None. **J.T. Willie:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.01/W25

Topic: H.09. Spatial Navigation

Support: NIH/NINDS intramunal research program

Title: Drifting activity fields in entorhinal cortex grid cells reveal that the population activity is attracted toward landmarks via two time-scale experience dependent plasticity

Authors: *L. CHEN[1], L. DONG[3], N.-W. TIEN[2], T. J. MALONE[6], S. CHANDRA[4], I. R. FIETE[5], Y. GU[7];
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Abstract: Medial entorhinal cortex (MEC) grid cells are widely known for their hexagonally patterned firing fields in space. Theoretical models have shown how grid cell activity is generated and updated based on velocity inputs, but little is known about how the responses are shaped by experience and develop across a multi-day spatial learning task. Studies have reported experience-dependent grid cell activity pattern change in response to environment alternation, but they have suffered from the limitation that the electrophysiological activity of the same MEC neurons could not be reliably tracked over a multi-day learning process. Here, we utilized *in vivo* two-photon calcium imaging to measure the activity of hundreds of grid cells over multiple days as mice learned a novel one-dimensional (1D) virtual reality (VR) environment. Using a novel algorithm to iteratively connect individual grid fields across consecutive runs on the track, we found that the run-by-run firing fields of the same grid cell displayed continuous shifts toward earlier locations on the track on each day (within-day backward drift) and across multiple days (cross-day backward drift). Cross-day backward-drifting grid fields were more likely to appear during the early stage of learning. Across learning, they slowly approached the closest environmental cue at an earlier location and stabilized around the cue. Also, between adjacent learning days, we observed discontinuities in the drifts, which could be evidence of a second

timescale of plasticity. Meanwhile, the fraction and slopes of within-day backward-drifting grid fields decreased across days, indicating that at late stage of learning, grid fields tend to represent environmental locations more stably and with higher precision. Lastly, even though individual grid fields on the same track might drift differentially (some backwards, others forwards, and some not at all), population phase analyses reveal that simultaneously imaged grid cells nevertheless maintain their cell-cell relationships even as individual fields drift. This apparent tension between differential changes in grid fields and a coherent population phase is resolved by analyses showing that in the vicinity of cues, the whole population activity collectively shifts backwards to the nearest cues. Together, our results show that grid cell population activity is gradually stabilized by and become anchored to salient environment cues. Our study provides valuable information of how intrinsically generated grid patterns become registered to world data to form cognitive maps of external spaces, while preserving their internal population geometry.

Disclosures: L. chen: None. **L. Dong:** None. **N. Tien:** None. **T.J. Malone:** None. **S. Chandra:** None. **I.R. Fiete:** None. **Y. Gu:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.02/W26

Topic: H.09. Spatial Navigation

Support: NIH/NINDS Intramural Research Program

Title: Neural dynamics of the medial entorhinal cortex underlying spatial memory deficits of PS19 tauopathy mice

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Abstract: While late-stage AD is characterized by significant neurodegeneration, early symptoms such as the impairment of spatial memory precede this loss. The medial entorhinal cortex (MEC) is one of the first brain regions affected by tau pathology, a hallmark of AD. In addition, MEC dysfunction leads to spatial learning and memory deficits in animals and humans, suggesting a connection between the MEC and the spatial memory loss of early AD. However, whether and how altered MEC activity underlies spatial memory deficits in AD remain unexplored. To address whether impaired MEC neuronal activity contributes to spatial memory deficits in AD, we compared the MEC neuronal activity in a mouse model of tauopathy (PS19) to that in wild-type (WT) mice during the encoding and maintenance of their spatial memory. Specifically, we used *in vivo* two-photon microscopy to track calcium dynamics of hundreds of MEC neurons over multiple days while mice learned to navigate virtual reality environments.

We first investigated the relationship between MEC neural activity and navigation behavior while mice learned a novel environment (NE) for ten days (memory encoding). Compared to WT mice, PS19 mice demonstrated impaired spatial learning that was associated with an overall reduction in spatial consistency of both between-day and within-day neuronal activity. Interestingly, activity patterns of PS19 mice better anchored to visual cues during the initial days in the NE but failed to form a spatially consistent map of the full environment during learning. PS19 and WT mice show different patterns in their activity distribution with respect to track position. In particular, the response amplitude and field distributions of WT mice in early learning show differences between successful and unsuccessful runs, demonstrating encoding of behavioral outcomes that potentially reinforces the successful experiences and facilitates learning. In contrast, the activity distributions of PS19 mice show little difference between successful and unsuccessful runs. When re-exposed to a familiar environment after eleven days, PS19 mice showed greater behavioral decay, suggesting they have poorly maintained spatial memory. This behavioral decay was again associated with less consistent MEC neuronal activity relative to the baseline day (between-day consistency). Together, these results demonstrate that altered MEC neuronal activity is associated with impaired spatial memory encoding and maintenance in a mouse model of tauopathy. This work could further our understanding of the mechanisms of early AD and potentially assist in the development of treatments specifically targeting MEC circuits.

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Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Topic: H.09. Spatial Navigation

Support: National Science and Technology Innovation 2030 Major Project of China (2022ZD0205000)
National Natural Science Foundation of China (T2322021)

Title: Disrupted firing of entorhinal cortex neurons in App^{NL-G-F} rats during allocentric navigation

Authors: *C. ZHENG;
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Abstract: Disrupted firing of entorhinal cortex neurons in App^{NL-G-F} rats during allocentric navigation
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Keywords: entorhinal cortex, grid cells, allocentric navigation, Alzheimer's disease
Alzheimer's disease (AD) is a multi-factorial neurodegenerative disorder, with spatial navigation impairment as one of the most typical symptoms, including allocentric navigation dysfunction. The medial entorhinal cortex (MEC) is thought to essentially support spatial memory and navigation, since it contains not only grid cells but also neurons that carry various types of spatial information. However, the relevance of MEC neurons firing patterns to allocentric strategy and how it would be interrupted in AD remains unclear. In this study, we used an allocentric and egocentric navigation task on cheese board to test the impaired coding patterns of MEC cells in spatial navigation dysfunction under AD pathology. Grid cell and non-grid cell ensemble activity and local field potentials were recorded in MEC in 4 App^{NL-G-F} rats and 4 WT rats. Behavioral trials consisted of a free exploration phase in which no reward was delivered, a familiarization phase in which rats explored rewards delivered at random locations and a test phase including 3 sessions in which rats were required to start from different directions and find the same pre-assigned reward location by either allocentric or egocentric strategy. We found that App rats preferred to use egocentric, rather than allocentric strategy to find reward location, compared with WT rats exhibiting quick switch from egocentric to allocentric strategy. During allocentric navigation task, the spatial representation of hexagonal-modulated grid cells in App rats were disrupted, and the response of the grid cells to the reward location was weakened. Furthermore, we also observed decreased power spectrum of theta and gamma rhythms, and attenuated theta-gamma coupling strength in MEC of APP rats. The phase locking of grid cells spiking to theta and gamma
was also significantly weakened. These results suggest that the spatially coding patterns of grid cells support allocentric spatial navigation function, and raise the possibility that oscillational interference to grid cells coding may relate to impaired performance of allocentric navigation in AD.
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Disclosures: C. Zheng: None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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DFG AL 1730/4-1

Title: Spatial coding but not object coding is impaired at early stages in mouse models of tau and amyloid pathology

Authors: *B. THROM, E. C. FUCHS, J. PENG, K. ALLEN, H. MONYER;
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Abstract: One of the brain regions that is affected very early in Alzheimer's disease is the medial entorhinal cortex (MEC), which harbors distinct cell types involved in spatial coding (such as grid cells, head direction cells, and border cells) and object coding (such as object-vector cells and object-tuned fast-spiking cells). In this study, we analyzed spatial and object coding in two mouse models with distinct pathologies at an incipient phase of the neurodegenerative process. To this end, we used silicon probes to record from the superficial layers of the MEC in freely moving mice in two widely used mouse models with Alzheimer's pathology, namely 5xFAD (amyloid-beta plaques) and TauP301S (neurofibrillary tangles) mice. Recordings were performed at 5 or 8 months (m) of age (7-14 mice per subgroup). Notably, in both models grid cell function was impaired, albeit with differential manifestations. Thus, in 5xFAD mice there was a remarkable decrease in the proportion of grid cells, whereas in TauP301S mice grid cells appeared normal but exhibited reduced map stability. The proportion of head direction and border cells were similar across groups, but in 8 m old TauP301S mice they displayed reduced map stability, rotating in concert with the grid cell orientation. In contrast to the impairments in grid cells in the two mouse models, the proportion of object-vector cells and object-tuned fast-spiking cells was similar between mutant and control mice. Also, object-tuning scores were similar across genotypes. Finally, theta oscillations, which provide a temporal matrix for spatial coding, displayed a reduced frequency and increased amplitude in both mouse models at 8 m of age. In conclusion, we found that spatially selective neurons were more susceptible to dysfunction than object-coding cells in spite of the differential pathology in these two mouse models. Furthermore, the dysfunction at the electrophysiological level is congruent with the behavioral phenotype. Thus, both 5xFAD and TauP301S mice displayed impairments in an object-place-context task, but not in an object recognition task. These findings warrant future efforts to develop spatial navigation tasks as a screening tool for early Alzheimer's disease.

Disclosures: B. Throm: None. **E.C. Fuchs:** None. **J. Peng:** None. **K. Allen:** None. **H. Monyer:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Program #/Poster #: PSTR251.05/W29

Topic: H.09. Spatial Navigation

Support: NIH Grant NS053907
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Title: Generating the head direction signal: Two types of head direction cells in the lateral mammillary nuclei

Authors: *J. S. TAUBE[1], J. R. DUMONT[2], J. A. GRAHAM[3], J. L. MARCROFT[1], M. E. SHINDER[4], R. W. STACKMAN, Jr.[5], R. M. YODER[6];
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Abstract: Head direction (HD) cells discharge as a function of the animal's perceived directional heading in the horizonal plane independent of its location and on-going behavior. They are believed to form the basis for one's sense of direction and have been identified in many brain areas throughout the brain - both cortically and subcortically. The generation of the HD signal is thought to occur across the connections between the lateral mammillary (LMN) and dorsal tegmental nuclei (DTN). Computational models have suggested that a ring attractor network underlies the connections and mechanisms that generate and update the signal. Ring attractors usually contain cells that are sensitive to angular head velocity (AHV), which are important for updating the HD signal during head turns. To date, both HD and AHV cells have been identified in both the LMN and DTN. However, attractor models also include cells that are sensitive to both parameters conjunctively (HD + AHV) and have been referred to as 'rotation' cells (e.g., Skaggs et al., 1995). Rotation cells are critical for the attractor network to function properly. Currently, no conjunctive cells have been identified in the LMN or DTN, which challenges the view that an attractor type network underlies the HD signal. Here we analyzed single-unit cell recordings from both LMN and DTN and show that there are two types of HD cells in these areas. One type of HD cell in the LMN is AHV independent and is only sensitive to the animal's HD (~65%), while a second type of HD cell is also sensitive to AHV (~35%). Among the conjunctive type of HD cell, examination of their AHV tuning curves found both symmetric and asymmetric cell types. Similar results were found for the HD cells in DTN, but with a higher percentage for the conjunctive cell type (59.5%) than the AHV independent cell type. Importantly, most AHV sensitive HD cells (LMN: 88.0%, DTN: 68.2%) were also sensitive to the animal's linear velocity (LV). In contrast, very few HD cells in the anterodorsal thalamus, a downstream area from the LMN and believed to convey the HD signal to cortical areas, were found to contain correlates for AHV (9.6%) or LV (16.8%). These findings demonstrate that the requisite rotation type of HD cell is present in brain areas thought to be responsible for generating the HD signal and is consistent with the view that an attractor style network may underlie the generation of the HD signal across the LMN and DTN.

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Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.06/W30

Topic: H.09. Spatial Navigation

Support: Canada Research Chairs
Canadian Institute of Health Research
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New Frontiers in Research Fund

Title: Stereo olfaction underlies stable head direction coding in mice following vision loss

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Abstract: Background: Many species rely on stereo olfaction, the ability to detect subtle differences in odor concentrations between the two nostrils, to estimate the location of smells to help them navigate through the world. However, a direct link between stereo olfaction and the brain's spatial awareness systems is missing. We previously showed that head direction (HD) cells in blind mice rely on olfactory inputs for their tuning, as ablating olfactory sensory neurons resulted in the degradation of head direction cell tuning. Here, we provide evidence showing that our earlier report of stable head direction tuning in blind animals depends on stereo odor sensing.Methods: Using 32-channel silicon probes, we recorded from HD cells in the anterodorsal thalamic nucleus (ADn) of freely moving congenitally blind (Gnat1/2^{mut}) and later-onset blind mice (rd1) after inhibiting stereo olfaction – either by blocking olfactory processing in a single nostril or by merging the airflow into both nostrils.Results: By inhibiting stereo olfaction in blind mice, we found that head direction cells lose their stable unimodal directional tuning. Furthermore, we designed a novel head orientation task that utilizes closed-loop medial forebrain bundle (MFB) stimulation to show that disrupting stereo olfaction significantly impairs a blind mouse's awareness of its heading direction.Conclusions: We thus provide a direct link between stereo olfaction and the brain's HD system, and highlight the contribution of HD cells to spatial navigation.

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Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

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Program #/Poster #: PSTR251.07/W31

Topic: H.09. Spatial Navigation

Title: Boundary Reconstruction from Egocentric Boundary Cells in Rat Retrosplenial Cortex across Diverse Environmental Sizes

Authors: *H.-L. WANG[1], T.-Y. LU[1], B. CHEN[1], Y. KUO[1], Y. LIN[2], Y.-Y. CHEN[1,3];
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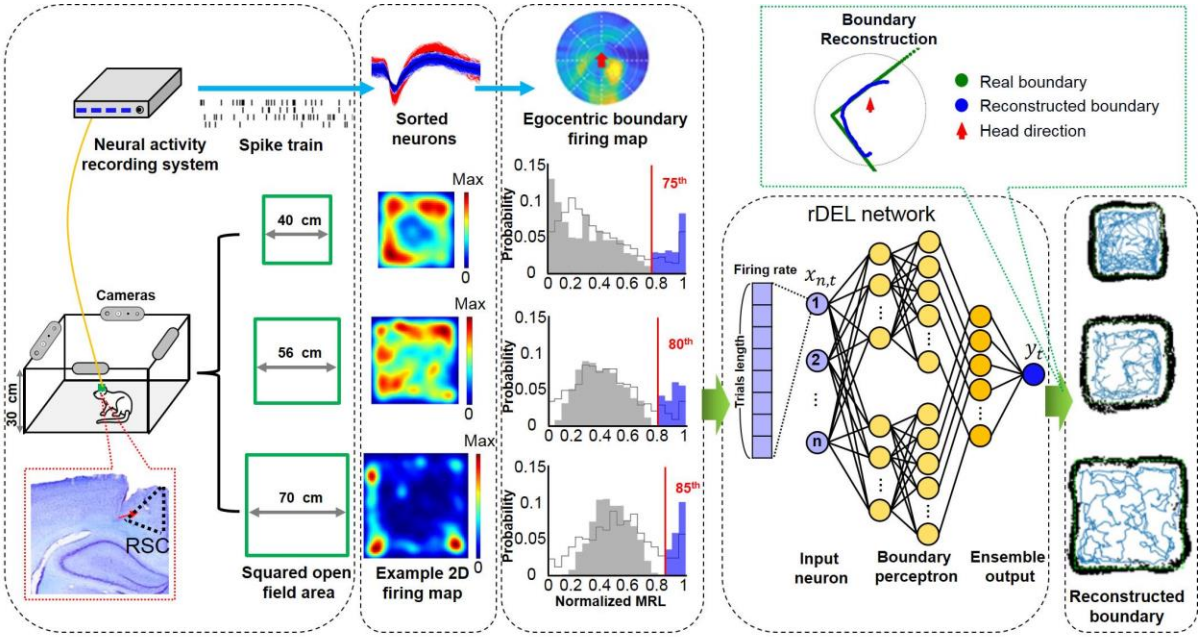
Abstract: Abstract
The egocentric boundary cell (EBC) in the retrosplenial cortex (RSC) has been extensively studied for its pivotal roles in spatial navigation and localization, exhibiting responses to specific distances and angles of boundaries. However, despite the comprehensive understanding of EBCs' characteristics and functions, it remains unknown whether this information encoded by EBCs can be effectively used to reconstruct the overall environmental boundaries. In this study, we proposed a regression dynamic ensemble learning (rDEL) network to predict boundary distances from the neural activity of EBCs in rat RSC under different head orientations. The complete environmental boundaries were then reconstructed using the simultaneous localization and mapping method. We achieved complete boundary reconstruction in varying environments sizes using neural activities from EBCs. Additionally, we observed the requirement for applying different mean resultant length (MRL) thresholds to extract EBCs in environments of varying sizes. Our study highlights the potential of EBCs in reconstructing environmental boundaries and emphasizes the importance of environmental size in neural decoding. By demonstrating the feasibility of using EBC neural activity for boundary reconstruction, our study contributes to understanding neural mechanisms in spatial navigation. Future research may focus on refining our methodology and exploring EBCs' roles in more complex spatial environments.

Graphical abstract
We achieved the reconstruction of environmental boundaries using spatial information encoded in EBCs through the regression rDEL network. Furthermore, we observed that varying environmental sizes influence the criteria for EBC extraction using MRL.



Disclosures: H. Wang: None. **T. Lu:** None. **B. chen:** None. **Y. Kuo:** None. **Y. Lin:** None. **Y. Chen:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.08/W32

Topic: H.09. Spatial Navigation

Support: U01NS111695

Title: Inputs to the head direction network contain eye movement signals

Authors: *J. STOTT[1], M. A. VAN DER MEER[2];
[1]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [2]Psychological & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Models of the head direction (HD) ring attractor network assume that angular head velocity (AHV) signals are provided as input to 'rotation cells' in the dorsal tegmental nucleus (DTN). One candidate region to provide this signal is the nucleus prepositus hypoglossi (NPH). However, NPH is argued to be an oculomotor nucleus in foveate animals. Previous rodent NPH recordings have lacked eye tracking (Lannou et al., 1984; Graham et al., 2023; rat) or recorded from an extended area that included medial vestibular nucleus (MVN) and gigantocellular nucleus (Gi) (Kaufman et al., 2000; gerbil). Could what looks like AHV input into the rodent HD system actually be eye movement related?
We performed acute silicon probe recordings in NPH in head-fixed male and female mice. A mounted camera tracked eye-movements elicited by the vestibular-ocular reflex (VOR) during manual, sinusoidal rotations of the recording platform. A subset of transgenic Ai32 mice received prior DTN injections of a Cre-containing retrograde virus. This resulted in ChR expression in DTN afferents. When successful, this allowed for 'optotagging' of DTN-projecting NPH cells. After a recording session was complete, current was passed through the probe to make an electrolytic mark.
More than half of NPH cells showed significant modulation just before the time of saccade onset. A majority of these cells showed a characteristic firing increase just before saccades in one direction, and a suppression of firing before saccades in the opposite direction. This profile was reminiscent of oculomotor neurons; however, some cells showed increases for both nasal and temporal saccades. A large majority of cells also showed significant, linear tuning to the angular velocity of the platform during the slow phase of the VOR, as required by the canonical ring attractor model. Because AHV and eye velocity were anticorrelated, we cannot rule out the possibility that this tuning represents an eye movement signal. A general linear model analysis confirmed that both AHV and eye movement correlates were encoded in NPH neurons. AHV cell tuning curves were almost entirely asymmetric, in contrast to Graham et al., 2023, who reported equal numbers of symmetric-type cells. Importantly, recordings from DTN-projecting cells likewise showed eye movement related signals.
These findings pose interesting questions. Do DTN rotation cells also encode eye movement signals? Do eye movement responses cancel out at a population level, or are eye movement responses informative in generating the HD signal? Further modeling work may be needed to understand what effect eye movement signaling would have on the ring attractor output.

Disclosures: J. Stott: None. **M.A. van der Meer:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Topic: H.09. Spatial Navigation

Support: NIH/NINDS grant R34NS123819 to Jan Drugowitsch

Title: A causal inference perspective on learning in the head direction system

Authors: *Z. AJABI, J. DRUGOWITSCH;
Neurobio., Harvard Univ., Boston, MA

Abstract: Successful spatial navigation requires the ability to continuously keep track of one's head direction in space. In the brain, this is achieved by populations of head direction (HD) neurons that allow an animal to form an internal representation of its orientation in space. Recent studies have identified plasticity in the process of forming an internal HD representation that allows the HD system not only to form stable associations between HD angles and the corresponding visual scenes, but also to flexibly relearn these associations when a change in the environment is detected. A learning mechanism is, therefore, essential to maintain a consistent HD representation with respect to a global reference frame, across different environments. Nonetheless, it remains unclear which sensorimotor features ought to modulate learning, and in particular trigger re-learning upon changes in the environment. In fruit flies, a rotation-dependent dopamine release promotes learning through a "when-to-learn" signal to update the synaptic weight matrix linking HD neurons to neurons carrying visual information. Triggering learning when rotating makes intuitive sense if the visual information is noise-free and unambiguous and the environment changes frequently, such that any rotation can be expected to yield novel information. Yet, in natural environments visual inputs can be noisy, ambiguous and/or irrelevant, making rotation-modulated learning less appropriate. Here, we ask how learning in the HD network depends on the statistical properties of the visual world, and explore, from a normative perspective, the implications on optimal navigational strategies that maximize stability of the internal HD representation. To do so, we formulate the problem of maintaining a stable and consistent HD representation through causal inference, whereby an ideal (Bayesian) observer is continuously arbitrating between maintaining and relearning associations between HDs and visual scenes as the visual information keeps changing due to observation noise, movement and/or changes in the environment. Our model shows that an increase in rotational speed supports learning only when the noise in the environment is low (i.e. lab conditions). Indeed, high rotation speeds impede proper learning as the observation noise increases. Our work thus provides a more nuanced view on learning in the HD system which allows us to determine environment-dependent optimal navigational strategies and derive testable and biologically interpretable learning rules.

Disclosures: Z. Ajabi: None. **J. Drugowitsch:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.10/

Topic: H.09. Spatial Navigation

Support: National Institutes of Health's National Institute on Aging, grant 5R01AG076198-02

Title: Comparing memory and prediction in grid cells on the scale of seconds

Authors: *S. ZOMORODI[1], E. L. NEWMAN[2], Z. TIGANJ[1,3];
[1]Dept. of Computer Sci., Indiana Univ. Bloomington, Bloomington, IN; [2]Dept. of Psych. and Brain Sci., Indiana Univ. Bloomington, Bloomington, IN; [3]Dept. of Psych. and Brain Sciences, Indiana University Bloomington, Bloomington, IN

Abstract: While navigating in space, we are typically not just aware of the current location but also the trajectory that we took to get there, as well as the trajectory that we plan to take in the near future. Plenty is known about the neural firing that encodes the current location, however less is understood about the neural code for past and future locations. Here we attempt to decode past and future locations from simultaneously recorded grid cells in rat MEC during open field exploration. To isolate the memory and prediction in grid cells from the temporal autocorrelation in the visual inputs and animals' motion, we focused on comparing the decoded information about the past and the future. We found that information about the past location can be decoded more accurately than information about the future location for up to *several seconds*. This finding suggests that the neural representation of past trajectories may be more stable or readily accessible than that of anticipated future paths.

Disclosures: S. Zomorodi: None. **E.L. Newman:** None. **Z. Tiganj:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.11/W34

Topic: H.09. Spatial Navigation

Title: The Entorhinal Cortex's Phase Code for Navigation

Authors: O. E. M. DESPARD[1], A. V. M. HERZ[2], ***M. STEMMLER**[2];
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Abstract: Grid cells in medial entorhinal cortex not only show spatially organized firing but also systematic phase advances and delays with respect to the population's multi-unit activity (MUA), which exhibits regular peaks every 120 milliseconds (theta-rhythm). Based on an analysis of 483 grid cells from Gardner et al. (2022), cells' phase- and firing-patterns fall into different classes. Conjunctive grid cells that are tuned both to head direction and spatial location tend to be phase-locked to the MUA. Other grid cells fire 180 degrees out of phase with the MUA. Most non-conjunctive grid cells, though, harbor a hidden head-direction signal in the temporal phase relative to the MUA. These cells often fire rapid bursts of spikes whose timing is a function of both head direction and spatial position, thereby multiplexing body-centered and world-centered information. The ensemble activity, measured in terms of the temporal phases, spans a 3-torus composed of two loops for the spatial coordinates and one loop for the head direction. By exploiting dihedral symmetries, we show how an ideal observer can decode these timing signals.

Disclosures: O.E.M. Despard: None. **A.V.M. Herz:** None. **M. Stemmler:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

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Program #/Poster #: PSTR251.12/W35

Topic: H.09. Spatial Navigation

Support: Horizon 2020 ICT (https://cordis.europa.eu/project/id/101016787, DEEPER)
Chan Zuckerberg Advancing Imaging through Collaborative Projects, 2023-2024

Title: High-resolution two-photon imaging of astrocytic calcium signals in freely moving animals during naturalistic spatial navigation

Authors: *S. CURRELI, A. SATTIN, L. MADDALENA, T. FELLIN;
Optical Approaches to Brain Function Lab., Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Astrocytes exhibit complex changes in their intracellular calcium concentration. These calcium signals span multiple spatial and temporal scales[1,2] and form the foundation of the functional interplay between astrocytic and neuronal networks[3,4]. Recent findings indicate that in the hippocampus of awake mice, astrocytic calcium signals encode information about high-level cognitive variables[5,6] and that this information is complementary to that encoded by nearby neurons[5]. However, these findings were obtained in head-restrained animals navigating in virtual reality, an experimental configuration with lower complexity of sensory stimuli compared to

naturalistic spatial navigation. To overcome this limitation, here we investigated whether hippocampal astrocytes encode spatial information in freely moving animals navigating in a real bi-dimensional arena. To reach this goal, we used miniaturized high-resolution two-photon microscopes (MINI2P[7]) to record calcium signals from hippocampal astrocytes expressing the genetically encoded calcium indicator GCaMP6f. We monitored subcellular calcium signals originating from astrocytic somata, proximal processes, and gliapil during complex animal behavior, including naturalistic spatial navigation. Analysis of this novel high-dimensional dataset will be performed using information theoretical methods and machine learning approaches to identify emergent encoding dynamics of astrocytic networks under freely moving experimental conditions.

Bibliorgaphy:
1. Bazargani, N. & Attwell, D. Astrocyte calcium signaling: the third wave. Nat Neurosci 19, 182-189 (2016).2. Semyanov, A., Henneberger, C. & Agarwal, A. Making sense of astrocytic calcium signals - from acquisition to interpretation. Nat Rev Neurosci 21, 551-564 (2020).3. Araque, A., et al. Gliotransmitters travel in time and space. Neuron 81, 728-739 (2014).4. Volterra, A., Liaudet, N. & Savtchouk, I. Astrocyte Ca(2)(+) signalling: an unexpected complexity. Nat Rev Neurosci 15, 327-335 (2014).5. Curreli, S., Bonato, J., Romanzi, S., Panzeri, S. & Fellin, T. Complementary encoding of spatial information in hippocampal astrocytes. PLoS Biol 20, e3001530 (2022).6. Doron, A., et al. Hippocampal astrocytes encode reward location. Nature 609, 772-778 (2022).7. Zong, W., et al. Large-scale two-photon calcium imaging in freely moving mice. Cell 185, 1240-1256 e1230 (2022).

Disclosures: S. Curreli: None. **A. Sattin:** None. **L. Maddalena:** None. **T. Fellin:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.13/W36

Topic: H.09. Spatial Navigation

Support: New Faculty Startup Fund Seoul National University(Korea)
NRF-2022M3E5E801772313(Korea)
NRF-2019M3E5D2A01058328 (Korea)

Title: Egocentric neural representation of geometric vertex in the retrosplenial cortex

Authors: K. PARK[1], Y. YEO[1], K. SHIN[2], ***J. KWAG**[1];
[1]Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Neural representation of the environmental features in an egocentric coordinate system is important for constructing an egocentric cognitive map. To create a geometrically detailed egocentric cognitive map, neural representations of the edges and vertices of

environmental features are necessary. While egocentric neural representations of edges, such as egocentric border cells and egocentric boundary vector cells exist, those of vertices are currently unknown. Using in vivo calcium imaging of granular retrosplenial cortex (RSC) neurons in mice freely exploring various environmental geometries, we discovered neurons that generated spatial receptive fields exclusively near the vertices of environmental geometries, which we termed vertex cells. Each spatial receptive field of vertex cells occurred at a specific orientation and distance relative to the animal, tuned by head direction, indicating an egocentric vector coding of the vertex. Moreover, the goal directed navigation task selectively strengthened the egocentric vector coding of the vertex near the goal location. Together, these results suggest that the egocentric vector coding of vertices by RSC neurons may help delineate a geometrically detailed egocentric cognitive map that could guide goal-directed navigation.

Disclosures: K. Park: None. **Y. Yeo:** None. **K. Shin:** None. **J. Kwag:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.15/W38

Topic: H.09. Spatial Navigation

Support: New Faculty Startup Fund Seoul National University
NRF-2022M3E5E801772313
NRF-2019M3E5D2A01058328
MOTIE-20012355

Title: Egocentric neural representation of environmental geometry by retrosplenial parvalbumin-positive interneurons

Authors: *J. YANG, J. KWAG;
Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Neurons representing egocentric boundaries/borders and vertices have been identified in the retrosplenial cortex (RSC). While most studies have shown that excitatory neurons in the RSC are involved in representing space, it remains unclear whether GABAergic interneurons in the RSC can perform the same function. In this study, we conducted *in vivo* Ca^{2+} imaging on GCaMP6s-expressing PV interneurons in PV-Cre mice freely exploring a square open chamber. Our preliminary results suggest that subpopulations of PV interneurons exhibit egocentric spatial selectivity to environmental geometry such as boundaries/borders and vertices. These observations indicate that both excitatory neurons and GABAergic interneurons in the RSC may partake in constructing an egocentric cognitive map for spatial navigation.

Disclosures: J. Yang: None. **J. Kwag:** None.

Poster

PSTR251: Grid Cells and Spatially Modulated Cells II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR251.16/X1

Topic: H.09. Spatial Navigation

Support: New Faculty Startup Fund Seoul National University
MOTIE-20012355
NRF-2022M3E5E801772313
HU20C0233
NRF-2019M3E5D2A01058328

Title: Egocentric Neural Representation of Environmental Geometry in the Retrosplenial Cortex is Impaired in the 5XFAD Mouse Model of Alzheimer's Disease

Authors: *Y. YEO[1], J. KWAG[2];
[1]Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Brain and Cognitive Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Neurons that represent egocentric space have been found in the retrosplenial cortex (RSC), which are important in constructing an egocentric cognitive map. Interestingly, the RSC has also been reported to be one of the earliest brain regions affected by amyloid beta accumulation in Alzheimer's disease (AD), characterized by deficits in episodic memory and spatial navigation. However, it is yet unknown how amyloidosis in AD affects egocentric neural representation. To address this question, we performed *in vivo* Ca^{2+} imaging of GCaMP-expressing excitatory neurons in the RSC while the control mice and 5XFAD mice freely explored a square open chamber. Our preliminary result suggest that egocentric neural representation of environmental geometry are impaired in the 5XFAD mice when compared to control mice, indicating that amyloidosis disrupts the egocentric neural representation of space.

Disclosures: Y. Yeo: None. **J. Kwag:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.01/X2

Topic: H.12. Aging and Development

Support: National Natural Science Foundation of China 82101252

Title: Odor enrichment mitigates postoperative cognitive dysfunction in elderly patients

Authors: X. MEI[1,2], *Z. XIE[3], Y. SHEN[1,2];
[1]Mental Hlth. Ctr. affiliated to Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [2]Anesthesia and Brain Research Institute, Tongji University School of Medicine, Shanghai, China; [3]Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Postoperative cognitive dysfunction (POCD) is a prevalent complication that affects geriatric patients following surgery, which has short or long-term adverse effects, but its targeted intervention remains elusive. Odor enrichment could reduce postoperative cognitive impairment in animal models. However, there is limited research in geriatric patients. To address this gap, we conducted a clinical study to investigate the relationship between olfactory function and POCD in geriatric patients and to evaluate whether odor enrichment could attenuate POCD. This prospective cohort clinical trial was conducted in Shanghai 10th People's Hospital. Eligible subjects aged at least 65 years old, with American Society of Anesthesiologist (ASA) class I to III and normal cognition, who had been referred for a total knee replacement (TKR) or total hip replacement (THR) under general anesthesia, were screened and enrolled. Enrolled participants were randomized into odor enrichment group or sham group. The neuropsychological test battery was used to assess cognitive function preoperatively and postoperatively. Odor identification ability was assessed by five-odor-olfactory detection arrays. Odor-enriched participants were exposed to different odors from 3-day before to 5-day after anesthesia and surgery. A total of 435 patients were screened. The final data analysis included 131 participants [72.5% female, median age 72 (IQR: 68, 76) years old]. 33 of the 131 participants (25.2%) developed POCD. The participants with decreased odor identification scores (N = 32) had a higher incidence of POCD than those with increased or unchanged odor identification scores (N = 99) (40.6% vs 20.2%, *P = 0.030*). Participants exposed to odor enrichment (N = 62) did not show improvement of olfaction compared to the participants in sham group (N = 69). Consistently, odor enrichment did not decrease the incidence of POCD as compared to the participants in control condition. However, among the participants who developed POCD (N = 33), participants exposed to odor enrichment (N = 19) had a milder decrease in Verbal Fluency Test (VFT) scores compared to the sham group (N = 14) [-7 (-3, -15) vs. -14 (-9.75, -22.5), *P = 0.031*]. The results indicated that patients with a rapid decline in olfactory function were more likely to develop POCD. Nevertheless, our findings suggested that odor enrichment might have a positive impact on specific cognitive domains, such as verbal fluency, potentially mitigating some cognitive impairments caused by anesthesia and surgery. This observation opens the door to further research into using odor enrichment as a possible strategy for preventing or treating POCD.

Disclosures: X. Mei: None. **Z. Xie:** F. Consulting Fees (e.g., advisory boards); Baxter, NanoMosaic, Shanghai 4th People's Hospital, Shanghai 9th People's Hospital, Shanghai 10th People's Hospital, Mental Health Center affiliated to Shanghai Jiao Tong University School of Medicine, Anesthesiology and Perioperative Science. **Y. Shen:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.02/X3

Topic: H.12. Aging and Development

Support: PRIN 2022 PNRR - P2022XJ3WH
EBRAINS-Italy

Title: Symbolic and non-symbolic numerical representations in old and young subjects. An exploratory EEG study

Authors: B. MERCANTE[1], C. MELONI[2], R. FANARI[2], S. BESHARATI[3], S. M. SOLINAS[4], *P. ENRICO[5];
[1]Inst. of Biophysics, Natl. Res. Council (CNR), Palermo, Italy; [2]Dept. of Pedagogy, Psychology, Philosophy, Univ. of Cagliari, Cagliari, Italy; [3]Dept. of Psychology, Univ. of the Witwatersrand, Johannesburg, South Africa; [4]Dept. of Biomed. Sci., Univ. of Sassari, Sassari, Italy; [5]Univ. of Sassari, Sassari, Italy

Abstract: Numeracy has significant implications throughout the lifecourse. Its age-induced decline represents a vulnerability factor in elderly populations when it comes to solving everyday tasks. However, the reasons behind age-induced decline in numeracy are still unclear. In this study, we used a parity judgment task to study variations in number cognition between old and young participants. Since numeracy is associated with both symbolic and non-symbolic representations, three numerical formats were used: arabic digits (N), finger representations (F), and dots (D). Fifty-three healthy subjects joined the study: 31 young (24.1±4.73 y.o.) and 22 old (68.8±4.38 y.o.). EEG (64 channels) was recorded during the task. Mixed Effects Models analysis showed that performance accuracy for D was significantly lower than for N and F ($p = .02$). There were significant main effects for reaction time for Group (old, young), Condition (N, F, D) and Gender. Notably, old adults responded slower than young adults ($p < .001$) and both groups performed significantly faster in the task for N compared to F and D ($p < .001$). Significant interaction effects showed that performance between males and females were comparable for arabic digits ($p = .14$), with females showing significantly slower response for non-symbolic stimuli than males (F, $p = 0.04$; D, $p = .007$). In sensor space, multivariate pattern analysis shows that the neural correlates supporting the different number representations can be classified as categorically separate, with an early dissociation (~200 ms) between N, F, and D stimuli when compared to each other. This provides provisional evidence that symbolic and non-symbolic representations recruit functionally distinct neuronal processes. Subjects in the old group showed a slight reduction in decoding and an increase in its latency. Numerical cognition involves a highly-integrated network, with a key role of the intraparietal sulcus and fusiform gyrus. In source space, we found reduced activation levels and increased latency of response in the primary cortical areas involved in numerical cognition in the old group. In the same group, all stimuli also induced a wider cortical response with respect to young subjects. We hypothesize that the reduced activity of the main cortical areas may contribute to the age-induced decay in numeracy and that the recruitment of other cortical areas may serve as a compensatory mechanism. The results of this exploratory study provide further evidence for the multifaceted processes involved in numerical reasoning and its age-related decline, with a potentially distinct neural network for symbolic and non-symbolic numerical representations.

Disclosures: B. Mercante: None. **C. Meloni:** None. **R. Fanari:** None. **S. Besharati:** None. **S.M. Solinas:** None. **P. Enrico:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.03/X4

Topic: H.12. Aging and Development

Support: JSPS-KAKENHI #23KK0046
JSPS-KAKENHI #23K22372

Title: The impact of arm-crossing and simultaneity on tactile temporal perception in young and older adults

Authors: *C. GOTO[1], Y. YOTSUMOTO[2], N. TACHIBANA[3];
[1]Dept. of Integrated Sci., The Univ. of Tokyo, Meguro-ku, Japan; [2]Life Sci., The Univ. of Tokyo, Tokyo, Japan; [3]The Univ. of Tokyo, Tokyo, Japan

Abstract: Prior research has established that alterations in spatial configurations, such as crossing arms, impact cognitive tasks and the temporal processing of tactile stimuli (Yamamoto & Kitazawa, 2001; Buchholz et al., 2012). Specifically, studies have shown that participants' ability to discern the vibration frequency of stimuli delivered to each hand deteriorates when their arms are crossed. However, research on age-related changes in tactile perception, particularly among older populations, remains sparse. While existing studies have identified increased vibration detection thresholds (Goble et al., 1995) and diminished performance in temporal order judgment tasks among older adults (Craig et al., 2010), the effects of aging on vibration frequency discrimination and the specific impact of arm-crossing are not well-understood. To address this gap, we conducted a vibration frequency discrimination task with 10 young adults (average age 19.5 ± 1.5 years) and 30 older individuals (average age 74 ± 9 years). We used a device equipped with solenoids and Raspberry Pi 4B (OS: Raspbian 10) to administer tactile stimuli to the index fingers of both hands under two conditions: simultaneous (simultaneous stimuli to both hands) and sequential (consecutive stimuli). Our findings reveal that both age groups experienced significant arm-crossing effects in the simultaneous condition, with no increase in effect magnitude with age. This suggests that the transformation of skin-based sensory information to an external reference frame might be relatively unaffected by aging. Additionally, the older participants demonstrated a general decline in task performance, particularly pronounced in the simultaneous condition. According to Kuroki et al. (2017), difficulties in discriminating temporally proximal tactile stimuli arise not from low-level neural interactions, but from impaired integration at higher levels of central processing. This indicates that higher brain functions, critical for the temporal accuracy of signal integration, are likely to degrade significantly with age.

Disclosures: C. Goto: None. **Y. Yotsumoto:** None. **N. Tachibana:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.04/X5

Topic: H.12. Aging and Development

Support: Sekisho Corporation
TOYOTA Foundation
JSPS KAKENHI 24K18207

Title: Regular offline esports play enhances exercise effects on executive function in association with oxytocin dynamics in elderly individuals

Authors: *S. DOBASHI, S. TAKAHASHI, H. MATSUOKA, D. FUNABASHI, S. YOSHITAKE, T. MATSUI;
Univ. of Tsukuba, Tsukuba, Japan

Abstract: Loneliness/isolation is a significant global risk factor for dementia among the elderly, and innovative solutions are required to foster social bonding. Potential neuroendocrine factors of cognitive dysfunction associated with loneliness/isolation are the dynamics of oxytocin (OT) and cortisol (C), which are social bonding neuropeptides. Physical sports play must be a way for health and well-being in the body and mind via not only physical activity but also reliving loneliness/isolation through OT dynamics. However, for the elderly, physical fitness levels can limit participation. Offline esports, which require less physical activity but enhance social bonding through OT production unlike online play in young people, offer an alternative for the elderly. We thus hypothesized that regular offline esports play boosts habitual exercise effects on executive function by fostering a sense of cohesion in the community among the elderly. To test this hypothesis, thirty-one elder people (15 males, 16 females, 80.7 ± 8.6 years old, non-depression) who routinely attend a welfare facility for the elderly were assigned to two groups: exercise with cognitive training (E+c) and with esports (E+e). Both groups participated in an exercise program provided by the facility at least once a week for three months. After the exercise session, the E+c group watched cognitive training videos, while the E+e group played esports (competitive drum-type music and racing games) for approximately 20 min. Physical fitness (body size, timed up and go test, 30-sec. chair-stand test, one-leg standing test, and tapping test), psychological indices (bonding index, verbal memory, and executive function), and neuroendocrine indices (salivary OT and C levels and their ratio) were assessed before and after the intervention. All physical fitness indices were maintained with both interventions. The average of bonding index, as evaluated by the inclusion of others in the self (IOS), remained unchanged by the intervention. Verbal memory was also unaffected, but executive function, assessed by the Stroop task, significantly improved with esports intervention. The average of OT and C resting levels and their ratio did not show dynamic change by the interventions, but the

McNemar test revealed that improvements in executive function were associated with the IOS scores, OT levels, and OT/C ratio. Our findings demonstrate that regular offline esports play enhances habitual exercise effects on executive function among elderly individuals in association with community cohesion and OT levels. Esports can be universal sports to promote social bonding for well-being, regardless of age or gender.

Disclosures: S. Dobashi: None. **S. Takahashi:** None. **H. Matsuoka:** None. **D. Funabashi:** None. **S. Yoshitake:** None. **T. Matsui:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.05/X6

Topic: H.12. Aging and Development

Title: Blood CDKN2A Gene Expression in Aging and Neurodegenerative Diseases

Authors: *H. MORI[1,2], Y. FUNAHASHI[1], Y. YOSHINO[1], K. YAMAZAKI[1], S. OCHI[1], J. IGA[1], S.-I. UENO[1];
[1]Ehime Univ., toon city, ehime prefecture, Japan; [2]Neuropsychiatry, Shokokai Imabari Hosp, Imabari, Japan

Abstract: Background: *Cyclin-dependent kinase inhibitor 2A* (*CDKN2A*) is an important gene in cellular senescence and aging.**Objective:** This study assessed the utility of blood *CDKN2A* mRNA expression levels and methylation status as a potential biomarker for aging and the pathogenesis of Alzheimer's disease (AD).**Methods:** The correlation between *CDKN2A* mRNA expression levels and age was examined in 45 healthy subjects, after which mRNA expression levels were compared among 46 AD patients, 20 mild cognitive impairment due to AD patients, 21 Parkinson's disease patients, 21 dementia with Lewy bodies patients, and 55 older healthy controls. The methylation rates of the second exon of the *CDKN2A* gene, known to influence its expression levels, was also examined.**Results:** A significant correlation between *CDKN2A* mRNA expression levels and age was found (Spearman's rank correlation coefficient: $r = 0.407$, $p = 0.005$). *CDKN2A* mRNA expression levels in blood were significantly decreased in AD patients, although those of healthy controls were significantly increased with age. Further, only in AD patients were *CDKN2A* mRNA expression levels significantly and positively correlated with methylation rates.**Conclusion:** Although further research with a larger sample size is needed to elucidate the relationships between *CDKN2A* gene expression in blood and the development of other neurodegenerative diseases, *CDKN2A* mRNA expression in blood may be a biomarker for differentiating AD from normal aging and other neurodegenerative diseases.

Disclosures: H. Mori: None. **Y. Funahashi:** None. **Y. Yoshino:** None. **K. Yamazaki:** None. **S. Ochi:** None. **J. Iga:** None. **S. Ueno:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.06/X7

Topic: H.12. Aging and Development

Title: The metabolic effects of estradiol in perimenopause

Authors: *A. TESTO[1], J. A. DUMAS[2];
[1]Univ. of Vermont Neurosci. Grad. Program, Burlington, VT; [2]Psychiatry, Univ. of Vermont Larner Col. of Med., Burlington, VT

Abstract: Menopause is defined as the cessation of reproduction and involves hormonal changes relevant to both normal and pathological brain aging. The menopause transition, known as perimenopause, typically occurs between 40 and 58 years of age. The transition is often accompanied by neurological symptoms such as disruptions in sleep, anxiety and depression, and changes in cognitive performance. After menopause, the lower levels of estradiol combine with existing cardiovascular and metabolic risk factors to influence brain aging. Decreasing estradiol may affect endothelial functioning, body fat composition and distribution, changes in lipids and lipoproteins, and inflammation. C-reactive protein (CRP), an acute-phase inflammatory protein, has a complex relationship with estradiol across an individual's lifetime. Before menopause serum CRP levels are inversely related to serum estrogen levels; however, this relationship does not persist post-menopause. The relationship between estradiol and CRP during the menopause transition is still an active area of investigation and was the focus of the current study. We utilized data from the Human Connectome Project-Aging (HCP-A) 2.0 release. From these data, we identified female individuals between 480 months (40 years) and 671 months (55 years and 11 months) of age to capture the typical age range of the menopause transition in the United States who did not have PCOS. This left an N of 153, however, 25 additional participants were excluded do to missing blood draw data resulting in a final N of 128, with a mean age of 575.53 months (*SD*=59). Blood draw data included measures of estradiol and high-sensitivity CRP. Participants had a mean estradiol level of 82.69pg/ml (*SD*=103.9) and a mean CRP level of 2.94mg/L(SD=4.46). In order to reduce skewness, we performed a log transformation on the CRP data (*M*=0.52, *SD*=0.14). Statistical tests included a GLM to investigate the relationship between estradiol and CRP followed by a Pearson's correlation to determine directionality. Estradiol explained a significant proportion of variance in log transformed CRP (R^2 = .04, $F(1,125) = 5.435$, $p = .02$). Estradiol level was negatively correlated with log transformed CRP ($r(125) = -.204$, $p = .02$. These findings suggested that higher estradiol levels and lower CRP levels were related to one other during the menopause transition. This relationship may have long-term implications on brain aging as both ovarian hormones and cardiovascular health have been linked to both normal and pathological brain aging. In the next phase of our analysis, we will examine the relationship between brain connectivity and estradiol and CRP levels.

Disclosures: A. Testo: None. **J.A. Dumas:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.07/X8

Topic: H.12. Aging and Development

Support: NIA P01 AG014449

Title: Clinical framework to study the neurobiology of high cognitive performance/successful aging in the elderly

Authors: *M. MALEK-AHMADI[1], E. J. ROGALSKI[2], S. E. PEREZ[3], E. J. MUFSON[4]; [1]Banner Alzheimer's Inst., Phoenix, AZ; [2]1.Cognitive Neurol. and Alzheimer's Dis. Ctr., CNADC, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [3]Dept Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ; [4]Translational Neurosci., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The factors that protect against cognitive decline in people termed successful agers remain under investigated. To assist in the investigation of the neurobiology of successful agers, we present a neuropsychological criteria that defined higher functioning older adults (age 80+) as those with an episodic memory score that exceeded an adult at age sixty. The last WMS-R Logical Memory Delayed Recall (LM-DR) scores from 152 cognitively unimpaired autopsy cases 80 years or older (87.40±4.37, range 80-99) from the Rush Religious Orders Study were compared to normative episodic memory performance at age 60. Based upon normative data from the National Alzheimer's Coordinating Center Unified Dataset (UDS) a LM-DR score of 14 corresponds to normal performance (z = 0.06) for a 60 year-old. Individuals with a LM-DR score of 14 or higher were classified as High Cognitive Performers (HCP) (n = 68, 57% female), and those scoring below 14 were classified as Normal Aged Controls (NAC) (n = 84, 56% female). For comparison, 19 mild cognitive impairment (MCI) cases whose age at death was 80 or higher (88.59±4.11, 81-96, 68% female) were also evaluated. The sum of counts for neuritic plaques (NP), diffuse plaques (DP), and neurofibrillary tangle load from the entorhinal cortex, CA1, midtemporal cortex, inferior parietal cortex, and midfrontal cortex were evaluated separately across clinical groups. LM-DR score for HCP was 16.85±2.55 (range 14-24) and 9.79±2.36 (range 4-13) for the NAC group. The MCI group's mean LM-DR was 9.32±3.43 (range 3-17) and was significantly lower than the HCP group ($p<0.001$), but not the NAC group ($p = 0.48$). Braak stage ($p = 0.31$), CERAD classification ($p = 0.92$), and NIA-Reagan neuropathological diagnosis ($p = 0.65$) did not differ between the HCP, NAC, and MCI groups. Statistical analysis revealed that neuritic plaque load ($p = 0.44$), diffuse plaque load ($p = 0.27$), and neurofibrillary tangle load ($p = 0.28$) were similar between the HCP, NAC and MCI cases. LM-DR scores did not correlate with NP load ($r = -0.08$, $p = 0.32$), DP load ($r = -0.14$, $p = 0.07$), or NFT load ($r = -0.12$, $p = 0.13$). These preliminary findings indicate that successful cognitive aging beyond age 80 appears not to be associated with the standard AD neuropathology even in those with a clinical diagnosis of MCI. Further studies will investigate the cellular and molecular mechanisms that underlie successful cognitive aging.

Disclosures: M. Malek-Ahmadi: F. Consulting Fees (e.g., advisory boards); Biomedical Research Alliance of New York. **E.J. Rogalski:** None. **S.E. Perez:** None. **E.J. Mufson:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.08/X9

Topic: H.12. Aging and Development

Title: Prospective study on the role of chronic pain in post-operative cognitive dysfunction

Authors: *R. WU[1], J. WANG[2], L. DOAN[3], M. ROCKHOLT[3];
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Abstract: Post-operative cognitive dysfunction (POCD) affects 10-18% of adults after surgery, but has been reportedly found in as many as 75% of older adults. Recent evidence suggests that inflammation triggered by surgery causes these cognitive changes. Chronic pain often accompanies inflammation, and thus chronic pain before surgery could be a potential risk factor for POCD development. The older adult population also carries a high burden of chronic pain; reportedly between 25-80% of older adults worldwide experience chronic pain. Despite extensive investigation into chronic pain and surgery as separate risk factors contributing to cognitive decline among the elderly, a critical knowledge gap persists in understanding the presurgical risk factors for POCD. Our study specifically investigates the relationship between chronic pain preceding surgery and the development of POCD in patients 65 years old and older. We combine pain questionnaires with neuropsychological test batteries, electroencephalography (EEG), and serum biomarkers to better understand the mechanisms underlying this potential association. We use the T-cog Neuropsychological Battery Form C2T as a cognitive assessment at baseline before hip or knee arthroplasties, then follow-up with assessments at 1 week, 1 month, and 3 months post-operatively to assess POCD development. We administer the McGill Pain Questionnaire, PROMIS Pain Intensity Form 3A, PROMIS Neuropathic Pain Quality Form 5A, and PROMIS Pain Interference Form 6A scales at baseline and at 3 months to assess chronic pain. Using the standard deviation (SD) method with impairment defined as 1 SD below baseline normative measures, we found that ~20% of patients had cognitive decline at 3 months. In conclusion, the exploration of the interplay between chronic pain and POCD could help to potentially optimize practice patterns to prevent development of POCD in patients with pre-existing chronic pain.

Disclosures: R. Wu: None. **J. Wang:** None. **L. Doan:** None. **M. Rockholt:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

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Program #/Poster #: PSTR252.09/X10

Topic: H.12. Aging and Development

Title: Cognitive evaluation with the Montreal Cognitive Assessment in patients with Systemic Lupus Erythematosus in a Mexican hospital.

Authors: *G. NOLASCO-ROSALES[1], I. E. JUAREZ-ROJOP[3], F. OLAN[4], D. RUIZ[1], A. GENIS-MENDOZA[5], J. J. MARTÍNEZ-MAGAÑA[6], T. B. GONZÁLEZ-CASTRO[2], J. L. MONTEALEGRE-PÉREZ[1], B. JIMÉNEZ-BAYONA[1];
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Abstract: Neurocognitive manifestations are frequent in adults with systemic lupus erythematosus. Nonetheless, there is scarce information about the clinical assessment and management of cognitive impairment in patients with lupus. This study aimed to evaluate cognitive status in patients with systemic lupus erythematosus who attended a consultation at a hospital in Tabasco, Mexico. We recruited 36 patients from the outpatient clinic of rheumatology in a hospital from Southeast Mexico. The subjects were interviewed to collect clinical and demographics data. Montreal Cognitive Assessment was employed to evaluate mild cognitive impairment (score ≥26). Our patients were 35 females and one male, 40.94 ±12.23 years old, with 11.17 ± 4.02 years of schooling. The most frequent manifestations in our population were arthritis (83.3%, n=30), alopecia (72.2%, n=26), and headache (72.2%, n=26). Comorbidities in the sample were hypertension (50%, n=18), psychiatric disorders (22.2%, n=8) and obesity (19.4%, n=7). Also, we observed a high frequency (75%, n=27) of cognitive impairment by use Montreal Cognitive Assessment in Mexican patients with SLE. The azathioprine medication was associated with mild cognitive impairment (p=0.036). These findings suggest that patients with systemic lupus need a multidisciplinary approach to identify early neurocognitive manifestations, as cognitive changes can develop in young patients and worse the quality of life.

Disclosures: G. Nolasco-Rosales: None. **I.E. Juarez-Rojop:** None. **F. Olan:** None. **D. Ruiz:** None. **A. Genis-Mendoza:** None. **J.J. Martínez-Magaña:** None. **T.B. González-Castro:** None. **J.L. Montealegre-Pérez:** None. **B. Jiménez-Bayona:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

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Topic: H.12. Aging and Development

Support: RS-2023-00208884
RS-2023-00265824

Title: Exploring the interplay between motor and cognitive functions in the elderly: the role of cognitive subdomain functioning in motor skills

Authors: *W. HAN, H. KIM, J. LEE;
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Abstract: Motor and cognitive functions gradually decline over the course of aging. Existing studies have reported that both function levels are related to each other. This study aims to investigate the additional relationships between motor and cognitive functions, including specific motor skills and cognitive subdomains, in the elderly. Furthermore, we aim to investigate how specific cognitive subdomains are related to various motor skills with different movements and complexity. One hundred elderly persons participated in this study. The Korean Mini-Mental State Exam (K-MMSE) and the Seoul Neuropsychological Screening Battery were assessed to examine cognitive global and subdomain functions, including attention, language, visuospatial, memory, and executive functions. The 9-Hole Pegboard Test (9HPT), the 10 Meter Walking Test (10MWT) at comfortable and maximum speeds, the 6 Minute Walking Test (6MWT), the Time Up and Go Test (TUG), and the Four Square Step Test (FSST) were assessed to examine specific motor skills. Correlation analysis was used to investigate the relationship between cognitive and motor functions, respectively. Furthermore, multiple linear regression was utilized to investigate the role of cognitive subdomain functioning in each motor skill. K-MMSE and all motor skills were correlated with each other. All cognitive subdomains were correlated with all motor skills. The level of correlation with K-MMSE was highest in the order of FSST, TUG, 10MWT at maximum speed, 9HPT, 10MWT at comfortable speed, and 6MWT. Furthermore, in multiple linear regression analysis, different cognitive domains contributed according to different motor skills. 9HPT was significantly associated with the memory and executive domains, while the 10MWT at comfortable speed was associated with the executive domain. The 10MWT at maximum speed showed associations with the executive and visuospatial domains. Moreover, the TUG, FSST, and 6MWT were associated with the language domain. These results demonstrated that motor and cognitive function levels are generally related to each other. The importance of cognitive contribution was confirmed by examining that the contribution of cognitive function increased as motor skill complexity increased, such as speed and balance. Lastly, cognitive subdomains differently contributed according to upper and lower motor skills, and lower motor skills with different speed or balance. By examining the relationship between cognition and motor function in detail, this study could contribute to establishing strategies to prevent cognitive and motor function decline in the elderly effectively.

Disclosures: W. Han: None. **H. Kim:** None. **J. Lee:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.11/X12

Topic: H.12. Aging and Development

Title: The ocular front: A gateway to locate Alzheimer's disease

Authors: *A. DAS, S. H. BODAKHE;
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Abstract: The ocular front: A gateway to locate Alzheimer's disease Ashmita Das; Prof. (Dr.) Surendra H. Bodakhe
Neurodegenerative illnesses are known to advance without manifesting sufficient signs in the body. Nevertheless, research conducted throughout time has established a remarkable link between the ocular front part and the brain. A recent study sheds light on this correlation by investigating the observation that visual alterations typically manifest approximately 12 years prior to the advancement of dementia. The purpose of our work is to identify biomarkers originating from the eye that can be used to detect dementia-related alterations in the brains of experimental rats. We have concentrated on studying the impact of a diet high in cholesterol on the evolution of Alzheimer's disease. We have examined many factors such as lenticular opacification, intraocular pressure, central and peripheral vision fields, and retinal vasculature to determine if they can serve as potential indicators for the onset of neurodegeneration. In order to identify the precise pharmacological pathway responsible for these physiological changes, we have evaluated the concentrations of oxysterols, ABCA1, Claudin-5, TGFβ1, and MMP-9 in the plasma, brain, retinal, and lens extracts. Upon administration of a high cholesterol diet (HCD), we observed a significant increase in the levels of oxysterols. This led to a disruption in the levels of ABCA1 and a decrease in the claudin protein, which are responsible for maintaining the integrity of the blood brain barrier and blood retinal barrier. As a result, there was a leakage in these barriers. These alterations caused an increase in the production of reactive oxygen species (ROS), which in turn sped up the TGFβ1 signalling pathway and triggered the accumulation of fibrogenic substances in the retina, lens, and brain. Eventually, this process resulted in the deposition of Aβ in the plasma, retina, lens, and brain. The physiological alterations mentioned resulted in the creation of pocket-like capsules in the lenses. Additionally, there was an accumulation of Aβ in the retina, which caused changes in intraocular pressure (IOP) and ultimately led to cognitive impairment. It is noteworthy that the physiological alterations were observed more rapidly in the ocular region than in the brain.
This study presents strong evidence that regular examination of the ocular front can be used to detect the evolution of dementia and can be considered a reliable biomarker for quickly and easily identifying neurodegenerative alterations in the central nervous system.

Disclosures: A. Das: None. **S.H. Bodakhe:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

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Program #/Poster #: PSTR252.12/X13

Topic: H.12. Aging and Development

Support: NIH Grant R01-AG052419
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NIH Grant UL1-TR001420

Title: Elevated beta-amyloid deposition in older adults is associated with worse search efficiency in a grocery shopping task

Authors: *A. BRAINARD[1], S. A. BRINKERHOFF[2], S. N. LOCKHART[3], L. A. ZUKOWSKI[4]; [1]Dept. of Biol., High Point Univ., High Point, NC; [2]Dept. of Neurol., Univ. of Alabama at Birmingham, Birmingham, AL; [3]Intrnl. Med., Wake Forest Sch. of Med., Winston Salem, NC; [4]Dept. of Physical Therapy, High Point Univ., High Point, NC

Abstract: Alzheimer's disease (AD), characterized by declines in cognition and function, cannot be diagnosed until significant cognitive decline and neuropathological changes (e.g., beta-amyloid [Aβ] deposition) have occurred, limiting treatment options. Identifying subtle cognitive deficits present in those at risk of developing AD, assessed by Aβ positivity (Aβ+), during the performance of a cognitively demanding everyday task, such as grocery shopping, may enable earlier intervention. This study aims to: 1) identify differences between older adults (OA) and young adults (YA) in grocery shopping inefficiency and changes in inefficiency after successive grocery searches, and 2) explore the influence of Aβ on grocery shopping inefficiency metrics in OA. **METHODS:** 56 OA without cognitive impairment (78±5y, 25 females, including 22 Aβ+) and 28 YA (31±3y, 17 females) participated. Participants performed 2 trials for 2 different grocery item types (4 trials total). Grocery shopping inefficiency was calculated as speed/accuracy, where grocery item correctness (0-100%) reflected accuracy and elapsed time to select the grocery item reflected speed. The change in inefficiency from the first to second trial for each grocery item type was also calculated. Linear mixed effect regressions (LMERs) compared the two grocery search inefficiency metrics by age group (OA vs YA). Additional LMERs estimated the effect of continuous Aβ SUVR (on PET) on the two grocery search inefficiency metrics in OA, with trial order as a covariate. **RESULTS:** OA exhibited worse grocery search efficiency than YA ($p<0.001$). However, OA and YA did not differ in the change in search inefficiency from the first to second trial ($p>0.05$). There was an interaction between Aβ and trial order ($p=0.043$) on search inefficiency, such that OA with lower Aβ improved their efficiency from the first to the second trial, while OA with greater Aβ reduced their efficiency. Consequently, in OA with greater Aβ, change in search efficiency worsened from the first to second trial ($p=0.034$), relative to OA with lower Aβ. **CONCLUSIONS:** Relative to YA, OA exhibited age-related declines in grocery search efficiency. Within OA, those at elevated risk of developing AD (i.e., greater Aβ) exhibited better search efficiency during the first trial than those at low risk, who may have spent longer searching for the prespecified grocery item to maximize accuracy. However, those at elevated risk exhibited worsening efficiency during the second trial, while those at low risk improved. Those at elevated risk may have spent longer ruminating about the previous grocery search and failed to consolidate what was previously learned.

Disclosures: A. Brainard: None. **S.A. Brinkerhoff:** None. **S.N. Lockhart:** None. **L.A. Zukowski:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

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Program #/Poster #: PSTR252.13/X14

Topic: H.12. Aging and Development

Title: Diagnostic predictability of qualitative errors on the Clock Drawing Test in dementia

Authors: *J. KAMINSKI[1], K. SALMON[1], B. JOHNSON[2], J. NICHOLSON[1], D. TRANEL[3]; [1]Univ. of Iowa, Iowa City, IA; [2]Univ. of Iowa, Iowa City, IA, IA; [3]Dept Neurol, Univ. of Iowa, Iowa City, IA

Abstract: The Clock Drawing Test (CDT) is a well-known neuropsychological measure used to distinguish abnormal from generally normal cognitive functioning. The CDT is widely used in neurological populations including stroke and dementia. Though seemingly simple, drawing a clock and setting the time requires the intact functioning of numerous cognitive skills and the recruitment of several neuroanatomical regions and pathways. The multifaceted nature of the CDT offers many advantages but is also a major limitation in that the test is nonspecific, especially when a traditional 4-point scoring system (1=intact, 4=severely impaired) is employed. However, qualitative analysis of CDT errors has demonstrated some diagnostic predictability in dementia populations. Prior research has shown that stimulus-bound errors are more common in patients with Alzheimer's dementia (AD) whereas spatial errors are more common in patients with vascular (VD) and Parkinson's dementia (PD). Though these category-based predictions are general, analysis of individual errors is possible and has the potential to provide greater diagnostic predictability. The current study aims to identify the frequency of 26 specific errors across dementia populations. Patients with AD were expected to more frequently make errors on itmes that would indicate a stimulus-bound approach, and VD and PD patients were expected to make more errors on items that required intact visuospatial skills. Blinded transcription of retrospective data from over 1,500 patients is underway. The current study evaluates a random pilot sample of 50 primarily White patients (58% female) with a mean age of 72 and education of 12.3 years who completed a neuropsychological evaluation and were diagnosed with suspected AD, VD, PD, frontal temporal dementia (FDT), or mixed etiology. While significant group differences for individual errors were not found, trends were observed. Patients with AD more frequently demonstrated a spatial error in which numbers were at varied distance from the circle edge, followed by patients with PD, VD, mixed, and FTD ($\chi 2$ (4, N=50)=8.800, p=0.066). Additionally, patients with mixed dementia most commonly had a top left quadrant error, followed by PD, VD, AD, and FTD $\chi 2$ (4, N=50)=11.755, p=0.019. No participants replaced Arabic or roman numerals with any other symbol, making this error the least specific. These pilot data are drastically limited by an underpowered sample but suggest the need for analysis of more minute details to distinguish etiologies. More data are expected to

unveil more prevalent error trends, and subsequently improve diagnostic accuracy to inform clinical care in dementia populations.

Disclosures: J. Kaminski: None. **K. Salmon:** None. **B. Johnson:** None. **J. Nicholson:** None. **D. Tranel:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.14/

Topic: H.12. Aging and Development

Title: Using Electrophysiology to Identify Differences in Verbal and Semantic Processing in Mild Cognitive Impairment Relative to Healthy Aging

Authors: *S. JANI[1,2], C. ALAIN[3];
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Abstract: Mild cognitive impairment (MCI) is an intermediate stage between healthy cognitive aging and dementia, in which individuals display more pronounced cognitive decline than is expected for their age, but can still maintain functional independence. Despite individuals with MCI having a much higher risk of developing dementia than their neurologically healthy (NH) peers, diagnosis is hindered by high inconsistency in how MCI is assessed. To address this gap, we aimed to find differences in evoked oscillatory activity (EOA) and event-related potentials (ERPs) between *n*=30 MCI participants and *n*=30 NH participants aged 60-85 years old when performing tasks involving language processing. Each participant completed the Toronto Cognitive Assessment (TorCA) to assess performance in verbal recall, visuospatial function, working memory, executive control, and attention. They then performed a phoneme discrimination task in which they heard a sequence of the nonsense syllables /ba/, /pa/, and /ta/, and were asked to respond when they heard /ta/. Next, they performed a semantic priming paradigm in which they were presented with pairs of mono- or bi-syllabic, and asked to judge their semantic relatedness. High-density scalp EEG recording was used during both tasks. We expect to find significant differences in EOA and ERPs in MCI relative to NH participants, and that these differences will be consistent across the MCI group. As previous research has shown deficits in verbal and semantic fluency and sustained attention in MCI, we predict that MCI participants will display higher error rates and slower response times than their NH peers. Finally, we expect error rates and response time to be correlated with TorCA scores, such that a lower TorCA score will be predictive of an increased number of errors and a slower response time. This study is significant, as it aims to identify significant differences in neural activity between MCI and NH adults, and to potentially determine the utility of EEG in distinguishing individuals with MCI from their NH counterparts. We hope that we can lay a foundation for

facilitating equitable access to MCI assessment and diagnosis using electrophysiology, enabling individuals with MCI to get the support they need to ensure the best possible prognosis.

Disclosures: S. Jani: None. **C. Alain:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

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Topic: H.12. Aging and Development

Support: R01AG017917
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P30AG010161
P30AG072975

Title: Sex differences in the association between decline in blood pressure and change in cognition

Authors: *N. W. BAUMGARTNER, A. W. CAPUANO, Z. ARVANITAKIS;
RUSH Alzheimer's Dis. Ctr., RUSH Univ. Med. Ctr., Chicago, IL

Abstract: Hypertension, which is among the most common vascular risk factors, is associated with cognitive impairment and Alzheimer's dementia. While both men and women over age 60 have similar rates of hypertension (~75%), women comprise nearly two-thirds of Alzheimer's disease cases and experience faster cognitive decline. Importantly, blood pressure is modifiable through lifestyle and medical intervention, presenting a prime target to reduce cognitive decline and dementia risk. The objective of this study was to test for sex differences in the relationship between decline in blood pressure and change in cognitive function in late life. Data from 4,721 older adults (female = 3,502, 74%) with a mean baseline age =76.7 years (SD=7.7) were enrolled in the one of five community-based cohort studies at the Rush Alzheimer's Disease Center (ROS/MAP/MARS/AA-Core/LAT-C) and followed for up to 30 years. Clinical evaluations documented baseline and annual systolic and diastolic blood pressure (mean of two sitting and one standing measurement using a sphygmomanometer), and 19 neuropsychological tests summarized into a composite global cognition score. Bivariate mixed-effects models were conducted jointly modeling both global cognition and blood pressure. Results revealed decline in systolic pressure was related to decline in global cognitive function over time for female ($Z = 6.09$, $p < 0.01$) but not male subjects ($Z = 0.07$, $p = 0.94$). Change in diastolic pressure was not related to change in cognition for either sex ($Zs < -1.69$, $ps > 0.09$). These findings suggest that declining systolic pressure during aging is related to cognitive decline in women but not in men. Prior studies have linked elevated blood pressure with impaired cognitive function. However, our current research highlights that declining blood pressure over time in older women is also

associated with cognitive decline. Given that blood pressure is a modifiable risk factor, these results point to the need for interventions to alter blood pressure, in addition to future research on sex differences, vascular risk factors, and brain health in aging.

Disclosures: N.W. Baumgartner: None. **A.W. Capuano:** None. **Z. Arvanitakis:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

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Program #/Poster #: PSTR252.16/X16

Topic: H.12. Aging and Development

Title: Effect of human umbilical cord blood plasma derived from prenatal yoga practitioners on neurocognition in aged mice.

Authors: *R. SHARMA[1], P. KUMAR SAHA[2], T. SINGH[3], K. KUMAR[4], A. ANAND[5]; [2]Obstetrics and gynaecology, [3]Radiodiagnosis and imaging, [4]Psychiatry, [1]PGIMER, Chandigarh, India; [5]Neurol., P.G.I.M.E.R, Chandigarh, India

Abstract: Abstract: Effect of human umbilical cord blood plasma derived from prenatal yoga practitioners on neurocognition in aged mice.
Rajat Sharma[1], Pradip Kumar Saha[2], Tulika Singh[3], Krishan Kumar[4], Akshay Anand [1]
[1]Neuroscience Research Lab, Department of Neurology, Post Graduate Institute of Medical Education and Research, Chandigarh, India [2]Department of Obstetrics and Gynaecology, Post Graduate Institute of Medical Education and Research, Chandigarh, India [3]Department of Radiodiagnosis and imaging, Post Graduate Institute of Medical Education and Research, Chandigarh, India [4]Department of Psychiatry, Post Graduate Institute of Medical Education and Research, Chandigarh, India
Purpose: Aging is the inevitable biological process and is one of the primary risk factor for various neurodegenerative disorders. In this study we will investigate the effect of prenatal yoga practitioner derived umbilical cord blood plasma on neurocognition in aged mice. **Methods**: A total of 26 participants with uncomplicated pregnancy aging 18-35 years will be recruited at 17th -20th week. Yoga intervention will be given till delivery and umbilical cord blood collected and plasma will be separated. Doppler will be done at level 2 ultrasound at baseline and at 8th month follow-up. Systolic /diastolic (S/D), Pulsatility index (PI), Resistance index (RI) and Diastolic notch (DN) readings of right uterine, left uterine and umbilical artery will be compared at base line and at 8th month. After that we will use enzyme linked immunosorbent assay to analyse the expression of neurotrophic, inflammatory, anti-aging and stress markers in Umbilical Cord Blood plasma (UCB-plasma) and further UCB-plasma will be administered intravenously in aged mice. Neurobehavioral assessment will be done using behavioural tests like MWM , NOR and OFT and molecular assessment will be done using q-PCR and IHC for neurotrophic, neurogenesis, apoptotic and memory associated markers. Data will be analysed using SPSS statistical software. T-test, chi-square and ANOVA test will be done to compare different groups.

<0.05 will be considered as a statistical significance. **Expected Outcome**: A total of 8 patients recruited with baseline doppler reading of right uterine artery (S/D 2.87 ± 0.71, PI 1.22 ±0.28, RI 0.85 ±0.58), left uterine (S/D 2.7 ±0.71, PI 1.22 ±0.26, RI 0.54 ±0.13 and umbilical artery (S/D 2.98 ±0.54, PI 1.07 ±0.31, RI 0.64 ± 0.067) further yoga intervention is ongoing and expected to see changes due to prenatal yoga and further analysing the effect of UCB-plasma in aged mice.**Keywords: Aging, human umbilical cord blood, yoga, aged mice.**

Disclosures: R. Sharma: None. **P. Kumar Saha:** None. **T. Singh:** None. **K. Kumar:** None. **A. Anand:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

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Topic: H.12. Aging and Development

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Title: Impairment of the motor cortical plasticity reflects neurophysiological features associated with Alzheimer's disease pathology

Authors: *T. MURAKAMI[1], R. HANAJIMA[1], Y. UGAWA[2];
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Abstract: Recently, a diagnostic strategy of Alzheimer’s disease (AD) has been shifted to the biomarker-based classification focusing amyloid-beta (Aβ) plaques, phosphorylated tau, and neurodegeneration. Animal experiments showed that both Aβ and tau accumulations impair long-term potentiation (LTP) induction. We investigated relationships between the human motor cortical plasticity and established biomarkers for AD diagnosis in subjects with cognitive decline.
Twenty-six consecutive subjects who complained of memory problems participated in this study. We first conducted neuropsychological tests and AD-related biomarkers measurements (cerebrospinal fluid (CSF) sampling and amyloid positron-emission tomography (PET) scan), then subjects were classified into three groups according to the biomarker-based AT(N) classification system: AD group; A+T+(N)+ or A+T+(N)-, Alzheimer’s pathologic change group; A+T-(N)-, and AD biomarkers negative group; A-T-(N)-. We applied transcranial quadripuse stimulation with an interstimulus interval of 5ms (QPS5) to induce LTP-like cortical

plasticity. Motor evoked potentials were recorded from the right first-dorsal interosseous muscle before and after QPS5. QPS5-induced LTP-like plasticity was compared among the groups. We also studied correlations of QPS5-induced plasticity with cognitive functions or AD-related biomarkers.
Nine patients were classified to AD group, 9 to Alzheimer's pathologic change group, and 8 to AD biomarkers negative group. Normal motor cortical LTP-like plasticity was induced by QPS5 in AD biomarkers negative group but not in AD and Alzheimer's pathologic change groups. QPS5-induced LTP-like plasticity positively correlated with cognitive scores. The degree of LTP-like plasticity negatively correlated with levels of CSF-tau, and the amount of amyloid PET accumulation at the precuneus, and positively correlated with the CSF-Aβ42 level. In the amyloid PET positive subjects, non-responder rate of QPS5 was higher than the CSF-tau positive rate.
Impairment of QPS5-induced LTP-like plasticity could be associated with Aβ and tau accumulations. QPS5 could detect abnormality at earlier stages than CSF-tau in the amyloid-PET positive subjects. Assessing motor cortical plasticity using QPS5 could be a useful neurophysiological biomarker for AD pathology.

Disclosures: T. Murakami: None. **R. Hanajima:** None. **Y. Ugawa:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.18/X18

Topic: H.12. Aging and Development

Support: This research was supported in part by the Intramural Research Program of the NIH, National Institute on Aging.

Title: STARRRS (Successful Trajectories of Aging: Reserve and Resilience in RatS): a novel longitudinal open-data resource

Authors: *C. P. COOPER[1], H. LU[2], E. A. RADAKOVIC[1], E. W. MATIFAS[3], J. N. VERGEL DE DIOS[1], P. W. ZUNDEL[1], K. W. FISHBEIN[3], W. W. QIAN[3], A. T. SHAFER[1], E. A. STEIN[2], L. H. CHENG[1], C. BANUELOS[1], J. M. LONG[1], R. A. MCDEVITT[4], S. R. ESSIG[1], J. LEE[1], E. J. PEREZ[1], P. R. RAPP[1];
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Abstract: A prevailing question in the cognitive aging field is what mechanisms explain how some people remain cognitively intact as they age, while others are not so fortunate. Preclinical rodent models are an important resource in tackling the topic, but a significant translational

challenge is that most animal studies have been cross-sectional. In response, an NIA collaboration across multiple laboratories partnered to launch STARRRS (Successful Trajectories of Aging: Reserve and Resilience in RatS). STARRRS is a first-in-kind, open science longitudinal phenotypic data and biospecimen resource from a well-defined rodent model of neurocognitive aging. Each month a new cohort of male and female Long Evans rats enters the program and subsequently undergoes an array of behavioral characterization, neuroimaging, and biospecimen collections at 5-7 months, 14-16 months, ~22 months, followed by endpoint cognitive assessment and necropsy at ~24-25 months. The goal of this longitudinal approach is to provide a resource for pinpointing when successful versus at-risk aging begin to diverge and to identify behavioral and biological measures that predict different aging trajectories. By 2028, STARRRS is projected to have generated the largest repository of openly accessible longitudinal preclinical data of its kind, with behavior, neuroimaging, and biospecimen data from over 800 rats, encompassing over 2,000 MRI scans. STARRRS yield to date includes an unprecedented collection of resting state functional magnetic resonance imaging (rsfMRI) scans employing a state-of-the-art consensus protocol for obtaining reliable rsfMRI data from rats. Using baseline neuroimaging data from a subset of 138 subjects (n = 68 males, n = 70 females) acquired on a program-dedicated 9.4T MRI scanner, we performed group independent component analysis to identify large-scale brain networks and dual regression analysis to evaluate sex differences. The results 1) validate previously characterized large-scale brain networks that are homologous to humans, such as the default mode network (DMN), 2) describe other networks with more spatial precision, for example the cerebellum network, and 3) reveal networks previously understudied in rats, namely a connectivity pattern that resembles the human dorsal attention network (DAN). We found sex differences in connectivity patterns for several networks, including the presumptive DAN. These results only begin to hint at the potential when excavating large preclinical datasets, and we encourage researchers to plan for mining the wealth of data the STARRRS initiative aims to make available.

Disclosures: C.P. Cooper: None. **H. Lu:** None. **E.A. Radakovic:** None. **E.W. Matifas:** None. **J.N. Vergel de Dios:** None. **P.W. Zundel:** None. **K.W. Fishbein:** None. **W.W. Qian:** None. **A.T. Shafer:** None. **E.A. Stein:** None. **L.H. Cheng:** None. **C. Banuelos:** None. **J.M. Long:** None. **R.A. McDevitt:** None. **S.R. Essig:** None. **J. Lee:** None. **E.J. Perez:** None. **P.R. Rapp:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.19/X20

Topic: H.12. Aging and Development

Support: K H-B (771428)
CM P- P (775528)
VM R-R (626561)

AO C-S (800824)
CA G-M (828884)

Title: Effect of obesity and aging on cognitive and motor functions in mice

Authors: *A. BRAUN DE PEDRO[1], V. M. RUIZ-RODRÍGUEZ[2], A. O. CUELLAR SANTOYO[2], C. GALINDO[2], K. HERNANDEZ-BALDERAS[2], C. PAREDES-POPOCA[2], O. A. PATRÓN SOBERANO[2], A. M. ESTRADA SANCHEZ[2];
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Abstract: Excess body fat during obesity can cause chronic changes that affect the functioning of vital organs such as the brain. Obesity-related changes have been linked to cognitive impairment and contribute to the development of neurodegenerative diseases such as Alzheimer's disease. Another risk factor related to neurodegenerative disease is aging, where brain areas related to cognitive processes, such as the hippocampus and cerebral cortex, are particularly affected. In addition, aging causes a reduction in motor skills along with slower reflexes and less muscle tone. To better understand the relationship between obesity and aging to the hippocampus and cerebral cortex vulnerability, we evaluated the effect of twelve-week consumption of a high-fat diet (HFD) in young (3 months old) and old (12-16 months old) C57BL/6J female and male mice; the control group was fed with a standard diet. To determine the effect of the HFD on the function of the mentioned brain areas, we evaluated mice’s performance on the novel object recognition test at the end of the HFD and the nest-building behavior during the twelve weeks; this test is sensitive to motor and cognitive changes. Our results showed that young HFD-fed mice gained more weight than old mice. However, both groups showed altered performance in the novel object recognition test, indicating that HFD consumption similarly impaired short-term memory in young and old mice. Impaired nest-building behavior also occurs in young and old mice fed with HFD. However, HFD impacted the motor and cognitive component of nest-building in young mice earlier. Our results suggest that HFD has adverse effects on behaviors related to the hippocampus and cerebral cortex, and the age factor apparently does not increase mice's vulnerability to HFD. Interestingly, young mice are more susceptible to obesity-induced motor and cognitive function changes than older mice.

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Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.20/X21

Topic: H.12. Aging and Development

Title: Anxiety-like behaviors in mitoNEET knockout mice

Authors: *G. N. WILSON, K. HERNANDEZ, K. SMITH, K. MONAGHAN, J. HUBER, W. J. GELDENHUYS, PhD;
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Abstract: For decades, the scientific community has observed a connection between neuropsychiatric disorders and cognitive impairment. Recently, anxiety has received increased attention as a possible prodromal symptom of Alzheimer's and mild cognitive impairment (MCI), not just an associated comorbidity. The variable and incomplete response to customary treatments demonstrates a gap in our understanding of mechanisms driving anxiety. Previous research has implicated mitochondrial defects as a common, underlying feature of both cognitive impairment and some psychiatric disorders, yet specific mechanisms are poorly characterized. Identifying common targets that may impact both psychiatric and cognitive symptoms of neurodegenerative disease would provide a major advancement in therapeutic options.
A decrease in the mitochondrial protein, mitoNEET (CISD1), has been implicated in diseases like Alzheimer's and Parkinson's, suggesting it plays a role in disease pathogenesis. MitoNEET is an iron-sulfur [2Fe-2S] cluster-containing protein on the outer mitochondrial membrane. This cluster is redox-active and thought to act as a sensor for mitochondria, playing a role in bioenergetic regulation and cell survival. Our previous work has indicated that transgenic mice lacking mitoNEET have impaired learning and memory, with a specific deficit in novel object recognition memory at ages 9-12 months. Using this same mouse model, our current study investigated various exploratory behaviors associated with anxiety in rodents. Based on preliminary observations, we hypothesized that mice lacking mitoNEET would also show pro-anxiety behaviors at 9-12 months of age.
Using 22 mixed-sex mitoNEET knockout mice and wildtype littermates, we found that mitoNEET knockout mice show decreased exploratory behavior within the Y-maze, compared with controls. These mice also exhibited decreased time in open arms within both the elevated zero-maze and plus-maze, suggestive of increased anxiety. We also assessed c-fos in the amygdala of these animals, following exposure to novelty, to understand underlying neural activity. To our knowledge, this is the first study demonstrating a link between mitoNEET loss, anxiety, and decreased cognition. Ongoing work continues to establish a timeline to demonstrate if anxiety appears before cognitive decline in this mitochondrial mouse model. We hope to not only establish a clear relationship between anxiety and memory outcomes but also to therapeutically target these symptoms by using mitoNEET agonists at early stages of neuropsychiatric and/or cognitive impairment.

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Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.21/X22

Topic: H.12. Aging and Development

Support: NIH Grant AG062509
NIH Grant AG070761

Title: Exacerbation of postoperative delirium-like behavior in aged mice by elevated levels of pulmonary Tau-PT217 induced by hyperoxia, infection, and microplastic pollution

Authors: *X. LI[1], W. YANG[2], J. SONG[2], L. ZHANG[2], Y. DONG[2], Y. ZHANG[2], Z. XIE[2]; [1]Massachusetts Gen. Hospital/Harvard Med. Sch., Charlestown, MA; [2]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hospital/Harvard Med. Sch., Charlestown, MA

Abstract: Introduction: Our previous research has revealed that Tau-PT217 can be detected in lungs shortly after anesthesia/surgery, which may enter the brain through the bloodstream, leading to delirium-like behavior in aged mice. The objective of this study is to further explore the impact of hyperoxia, infection, and microplastic pollution on the levels of Tau-PT217 and behavioral changes. We hypothesize that these pathogens can lead to increases in pulmonary Tau-PT217 levels, consequently exacerbating postoperative delirium-like behavior in aged mice. Methods: We established an anesthesia/surgery model in 18-month-old wild-type C57BL/6J female mice following the previous methods. Postoperative delirium (POD)-like behavior in mice was evaluated using the Confusion Assessment Method (CAM) in mice[2]. Before the anesthesia/surgery procedure, mice underwent 100% oxygen inhalation for 4 hours, received intratracheal injections of 0.5 mg/kg lipopolysaccharide (LPS, 0.5 mg/kg) or accepted microplastic particles (2 mg/kg). Changes in Tau-PT217 levels in the lungs, blood, and brain tissues of mice were assessed using techniques such as Western blot, Immunofluorescence and Nanoneedle. Results: We found that mice subjected to hyperoxia, infection, and microplastic pollution prior to anesthesia/surgery displayed elevated baseline levels of Tau-PT217 in lungs. Subsequently, the aged mice received the anesthesia/surgery plus hyperoxia, infection or microplastic pollution developed severer postoperative delirium-like behavior and greater elevation of Tau-PT217 in lungs, blood and train tissues than the aged mice only received the anesthesia/surgery. Conclusions: Our findings revealed that hyperoxia, infection, and microplastic pollution could significantly potentiated the postoperative delirium-like behavior in aged mice. Such potentiation effects could result from the damage of the lungs, as evidenced of elevated Tau-PT217 levels in the lungs, by hyperoxia, infection, and microplastic pollution. Future research should focus on identifying the underlying mechanism by which hyperoxia, infection, and microplastic pollution increased the Tau-PT217 levels in lungs, blood and brain tissues, and developing interventions targeting the pulmonary Tau-PT217 to prevent postoperative delirium.

Disclosures: X. Li: None. **W. yang:** None. **J. Song:** None. **L. Zhang:** None. **Y. Dong:** None. **Y. Zhang:** None. **Z. Xie:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.22/X23

Topic: H.12. Aging and Development

Support: Janell and Joe Marek '57 Alzheimer's Disease Research Fund
WoodNext Foundation

Title: Shift Work Schedules Alter Immune Cell Regulation and Accelerate Cognitive Impairment during Aging

Authors: *K. DE SOUZA[1], G. C. ALLEN[1], D. J. EARNEST[2], M. NEWELL ROGERS[3]; [1]Texas A&M Univ., Bryan, TX; [2]Neurosci & Exp. Therapeut., Texas A&M Hlth. Sci. Ctr. Col. of Medicin, Bryan, TX; [3]Surgery, Texas A&M Hlth. Sci. Ctr. Col. of Medicin, Temple, TX

Abstract: Disturbances of the sleep-wake cycle and other circadian rhythms typically precede the age-related deficits in learning and memory, suggesting that these alterations in circadian timekeeping may contribute to the progressive cognitive decline during aging. Because both age-related dementia and altered regulation of circadian rhythms have been associated with immune cell activation and inflammation, we tested the long-term effects of circadian rhythm dysregulation in C57Bl/6J mice exposed to shifted light-dark (LD) cycles during early adulthood (4-6mo) on cognitive performance and immune cell regulation at middle age (13-14mo). Entrainment of the activity rhythm was consistently stable in all mice maintained on a fixed LD 12:12 cycle but was fully compromised during exposure to shifted LD cycles (12hr advance/5d). Following exposure to experimental lighting conditions, the circadian rhythm of activity in shifted LD mice was marked by a delayed phase angle of entrainment and increased day-to-day variability in activity onset times that persisted into middle-age. These alterations in the pattern of light-dark entrainment were closely associated with dramatic impairment in the Barnes maze test for the entire group of shifted LD mice at middle age, well before cognitive decline was first observed in aged (18-22mo) animals maintained on fixed LD cycles. In conjunction with the effects of circadian dysregulation on cognition, shifted LD mice were characterized by significant expansion of B cells, including CLIP+ B cells, in the spleen and a decrease in the population of splenic T cells at middle age. In mice exposed to shifted LD cycles, the expansion in splenic B cells was negatively correlated with cognitive performance such that when the number of B cells was higher, the cognitive Index scores were lower and performance was worse in the Barnes maze. Collectively, these results indicate that early exposure to shift work-like schedules alone accelerates cognitive decline later in life and alters the regulation of adaptive immune cell types that may play a role in mediating this long-term effect of circadian rhythm dysregulation.

Disclosures: K. De Souza: None. **G.C. Allen:** None. **D.J. Earnest:** None. **M. Newell Rogers:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.23/X24

Topic: H.12. Aging and Development

Title: Reference data ranges of behaviors displayed by C57BL/6J mice in common neurobehavioral assays.

Authors: *Z. BICHLER, J. WOTTON, T. LASTER, N. STROUD, V. D. KNICKERBOCKER, K. PERRON, J. SUCHOVIC, M. G. BAUER, S. A. MADDOX, C. WISE, J. OSGOOD, J. WHITE;
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Abstract: C57BL/6J is the most widely used inbred mouse strain and the first to have its genome sequenced. Used in a wide variety of research areas, it is one of the most well-characterized models available with more than 15,000 studies published to date. Many of these studies are referenced within the Mouse Phenome Database (https://phenome.jax.org/), where papers are listed, and individual data transcribed. This rich dataset is a valuable resource but as there are many differences in experimental procedures and design it can be difficult to generalize. Studies are also often under-powered, which decreases the reliability and reproducibility of the results, not in favor of the 3Rs. We decided to use the advantage of being a core facility to generate typical data ranges for specific behavioral phenotypes observed uniquely in C57BL/6J mice, based on the accumulation of a huge number of animals tested in the same assays with standardized protocols and controlled environmental conditions. Data were collected at the NeuroBehavior Phenotyping core at The Jackson Laboratory since 2017 following strict validation procedures established for the assays as well as for the experimenters. Phenotypic data obtained on C57BL/6J male and female mice tested at different ages from 6 to 96 weeks were combined from our data base (Platform for Science, Thermo-Fisher) or from archived raw data, all derived from outputs coming from specific software (Ethovision, Noldus; Fusion, Omnitech; FreezeFrame, Actimetrics) or manually entered. We choose to focus on specific assays grouped into categories of behavioral data assessing well-being (frailty score, grooming, and nest building assays), motor performance (open field test, rotarod, and grip strength), memory abilities (Y-maze tests, fear conditioning tests) and mood disorders (light/dark box, elevated 0-maze). Combined group sizes were up to 200 mice providing excellent statistical power to determine typical score ranges depending on the sex, age, and specific treatments such as control drugs or Ethanol injections. The effect of the experimenter was also evaluated and changes in standard procedure with time was considered. Expected typical data ranges generated from this meta-analysis may become a useful reference in the future for any scientist in search for a comparison with the C57BL/6J mouse strain. Acknowledgement: The authors would like to extend the co-authorship to all former staff members of the Neurobehavioral Phenotyping core at the Center for Biometric Analysis at The Jackson Laboratory as they have generated or help generate essential data needed for this work.

Disclosures: Z. Bichler: None. **J. Wotton:** None. **T. Laster:** None. **N. Stroud:** None. **V.D. Knickerbocker:** None. **K. Perron:** None. **J. Suchovic:** None. **M.G. Bauer:** None. **S.A. Maddox:** None. **C. Wise:** None. **J. Osgood:** None. **J. White:** None.

Poster

PSTR252: Natural Brain Aging: Behavior and Cognitive Disorders

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR252.24/X25

Topic: H.12. Aging and Development

Support: NIH Grant AG062509
NIH Grant AG070761

Title: Contribution of serum amyloid A to the Tau-PT217 mediated postoperative delirium-like behavior in aged mice

Authors: *M. XU[1], Y. WANG[2], C. WANG[2], W. ZHANG[1], S. FU[1], Y. DONG[1], Y. ZHANG[1], F. LIANG[1], Z. XIE[1];
[1]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA; [2]Radiology, Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: Introduction: Postoperative delirium (POD), a prevalent complication in senior patients, is linked to elevated mortality, morbidity, and an increased risk of Alzheimer's disease and related dementias [1]. The pathogenesis of POD remains largely elusive. Notably, anesthesia and surgery induce POD-like behavior in aged mice via elevated blood levels of Tau-PT217 [2]. We, therefore, set out to seek intervention(s) of POD aiming at reducing blood Tau-PT217 levels. **Methods**: 18-months-old wild-type (WT) C57BL/6J female mice were used. Mice in the anesthesia/surgery group underwent a simple laparotomy under 1.4% isoflurane anesthesia plus 40% oxygen [2]. The assessment of POD-like behavior was conducted using the Confusion Assessment Method (CAM) in mice [3]. Thermotherapy was administered by elevating the body temperature of aged mice from 37 °C to 39 °C for 1 hour, repeated four times with 30 minutes break in between following the anesthesia/surgery. Proteomics, ELISA, nanoneedle [2], whole body and single-cell PET imaging, nanobeam technology, co-immunoprecipitation, liver cellular Tau trafficking assay, and others were used in the studies. **Results**: We observed a significant rise in blood levels of serum amyloid A (SAA), an acute-phase inflammation-related protein primarily synthesized by liver, following anesthesia/surgery. Subsequently, the thermotherapy intervention demonstrated its efficacy in mitigating the anesthesia/surgery-induced elevations in both SAA and Tau-PT217 levels in the blood of aged mice. The thermotherapy alleviated the POD-like behavior in the aged mice. Mechanistically, SAA interacted with Tau-PT217, as evidenced by co-immunoprecipitation at the protein level and nanobeam analysis at the single molecular level. Moreover, SAA prevented the entry of Tau-PT217 into liver cells, as determined by cellular PET imaging and a single cellular model. The therapeutic impact of thermotherapy on POD-like behavior in aged mice was attributed to the reduction in blood levels of SAA. This reduction facilitated the release of Tau-PT217 previously bound to SAA, enabling increased entry of Tau-PT217 into liver cells for metabolism and degradation. Consequently, this process led to decreased blood levels of Tau-PT217 and the inhibition of POD-like behavior in the aged mice. **Conclusions**: These data provide a foundation for understanding how thermotherapy can modulate the levels of Tau-PT217 via SAA, ultimately mitigating the POD-

like behavior in aged mice. **References**: **1.** Fong TG, Inouye SK. Nat Rev Neurol, 2022. **2.** Lu J, et al., Alzheimers Dement, 2023. **3.** Peng M, et al., Sci Rep 2016.

Disclosures: M. Xu: None. **Y. Wang:** None. **C. Wang:** None. **W. Zhang:** None. **S. fu:** None. **Y. Dong:** None. **Y. Zhang:** None. **F. liang:** None. **Z. Xie:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.01/X26

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: R01NS120832
NSF 1707352
U01NS099709

Title: Interluminescence: synaptic transmission by bioluminescence

Authors: *A. SLAVIERO[1], M. PRAKASH[2], D. LIPSCOMBE[3], N. C. SHANER[4], C. I. MOORE[5], U. HOCHGESCHWENDER[2], E. EMAMGHOLI ZADEH[2], M. TREE[2];
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Abstract: Interluminescence is a BioLuminescent-OptoGenetic (BL-OG) approach where synaptic transmission is achieved by bioluminescent light, generated by a luciferase oxidizing a luciferin, from a presynaptic neuron activating optogenetic ion channels in the postsynaptic neuron. We use two strategies to position the luciferase in the synapse. In our ‘Act-Int’ approach, luciferase is released if the presynaptic neuron is active. This activity-dependence is achieved by translocating the luciferase into vesicles whose release from the presynaptic terminal is contingent on neural activity, similar to neurotransmitters. In our ‘Persist-Int’ approach, the luciferase is tethered to the presynaptic membrane. In this strategy, sustained modulation can be provided for as long as luciferin is present. To advance our understanding of the Interluminescence approach we used patch clamp recordings in primary neurons to compare the structure of interluminescence-induced (IL) and neurotransmitter-induced (NT) action potentials (APs). IL APs are significantly different from NT APs regarding half width, antipeak amplitude, rise tau, and action potential area. Further, postsynaptic IL APs for a given opsin have the same features regardless of the modality used (Act-Int or Persist-Int) or between different opsins within a modality. Specifically regarding Act-Int, we addressed two important questions: 1) How many presynaptic action potentials are required to induce one postsynaptic IL AP? and 2) Does light from presynaptically released luciferases exclusively activate opsins expressed by synaptically connected neurons? To answer the first question, we generated input-output curves relating presynaptic activity to IL responses in postsynaptic cells. We found that approximately

four presynaptic APs result in one postsynaptic IL AP. For the second question, we recorded from opsin-expressing neurons in proximity to but NOT synaptically connected to luciferase-expressing neurons and observed minimal-to-no response from surrounding bioluminescence, indicating the Act-Int effect is restricted to synaptically connected partners. Lastly, to assess Persist-Int activation of postsynaptic neurons at the population level *in vivo*, we applied 'Targeted Recombination in Active Populations' (TRAP) in TRAP2:: lox-stop-lox-EYFP mice to determine activation of opsin-expressing locus coeruleus neurons by Persist-Int-expressing neurons of the lateral hypothalamus.

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Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.02/X27

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Elsa U. Pardee Foundation
Central Michigan University Office of Research and Graduate Studies

Title: Developing and optimizing bioluminescent neurotransmitter sensors and neurotransmitter dependent neuromodulators

Authors: *K. A. TAYLOR[1], H. GALVIN[2], E. D. PETERSEN[3];
[1]Col. of Med., Central Michigan Univ., Mt. Pleasant, MI; [2]CMU Dept. of Biol., Central Michigan Univ., Mt. Pleasant, MI; [3]Col. of Med., Central Michigan Univ., Mt Pleasant, MI

Abstract: Many neurological diseases such as Alzheimer's Disease, Parkinson's Disease, and autism spectrum disorder have been shown to be associated with neurotransmitter disfunction or imbalance. Expanding on the types of neurotransmitters and methods that can be used to study them is important for revealing disease mechanisms and creating new treatments. In this study, we focus on gamma-aminobutyric acid (GABA) and acetylcholine (ACh), which are neurotransmitters found throughout the brain and are involved in many neurological disorders. We developed a variety of genetically encoded bioluminescent GABA and ACh sensors that are an attractive alternative to using fluorescent sensors because they do not require an excitation light source, allowing deeper areas of the brain to be recorded without damaging tissue and improving signal-to-noise ratio due to the lack of autofluorescence. We created a library of bioluminescent GABA and ACh sensor variants based on our prior glutamate sensors and tested them for responses to GABA and ACh respectively. Taking bioluminescence readings on a plate reader, we found that the sensors with a mutated GABA or ACh binding domain and optimized linkers have higher responses to saturating amounts of neurotransmitter than the ones with the

native GABA or ACh binding protein. To further improve the response of the sensors to neurotransmitters with the goal of using them to image brain activity in rodents, we are using rational design and further linker optimization with the goal of improving response amplitude and signal-to-noise ratio. We have also paired these light emitting sensors with light sensitive optogenetic channels to excite or inhibit neurons based on the presence of a specific neurotransmitter. We have used our glutamate sensor to hyperpolarize cells in the presence of glutamate and luciferin and will use this to diminish excitatory neurotransmission. We have also used the ACh sensor paired with an excitatory channel to depolarize cells in the presence of ACh and luciferin and will use this as a cholinergic synaptic amplifier.

Disclosures: K.A. Taylor: None. **H. Galvin:** None. **E.D. Petersen:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.03/X28

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH R21 Grant 1R21NS133935-01
NIH K01 Grant NS114191-01A1

Title: A Neuromodulation and Neuroimaging Method to Study Dynamics of Cell-Class Specific Circuits across Widespread Neural Networks

Authors: *E. MURPHY[1], D. WILLIAMS[2], M. DOYLEY[2], M. GOMEZ-RAMIREZ[1]; [1]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY; [2]Electrical and Computer Engin., Univ. of Rochester, Rochester, NY

Abstract: BioLuminescent OptoGenetics (“BL-OG”) is a hybrid chemo- and opto-genetic neuromodulation method that utilizes internally-generated bioluminescence to cause optogenetic reactions without the use of an external light device. In BL-OG, an enzyme (e.g., slow-burn Gaussia Luciferase; *sbGLuc*) is linked to an optogenetic element (e.g., Volvox-Channelrhodopsin-1; *VCHR1*) that is activated via bioluminescent light. Bioluminescence is produced when an injected chemical substrate (luciferin, e.g., h-Coelenterazine; h-*CTZ*) is catalyzed by the luciferase adjacent to the opsin. We previously showed that peripheral injections of the luciferin produce reliable and proportional BL-OG effects in neocortical areas of mice. Unlike other chemogenetic methods (e.g., DREADDs), we observed that BL-OG effects onset rapidly in the brain, with the duration of effects controlled by different parameters of the luciferin injection (e.g., dosage concentration, injection rate, etc.). In all chemogenetic methods, a single peripheral injection of the substrate leads to modulations in all neural areas that express the genetically-engineered molecule, limiting the ability to determine the contribution(s) of an individual node (or nodes) within a network to a particular behavioral function. Here, we combine focused ultrasound (FUS) and BL-OG to provide a noninvasive method that enables

spatially-specific modulation of selective ensembles distributed across a widespread network. The approach is to use FUS to disrupt the blood brain barrier (BBB), and inject a diluted dosage of the luciferin so that bioavailability of h-CTZ is only provided to the area with the disrupted BBB. Our data show increased BL-OG effects, produced by tail vein injections of h-CTZ, in brain areas targeted with FUS as compared to sham or no-FUS conditions. These data demonstrate that our combined FUS and BL-OG method can non-invasively generate site-specific neuromodulation within large-scale brain networks.

Disclosures: E. Murphy: None. **D. Williams:** None. **M. Doyley:** None. **M. Gomez-Ramirez:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.04/X29

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NIH Grant R01GM121944
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Title: Single-cell imaging in vivo using a new bright bioluminescent genetically encoded calcium indicator

Authors: *J. W. MURPHY[1], E. L. CRESPO[2], G. G. LAMBERT[3], U. HOCHGESCHWENDER[4], C. I. MOORE[1], N. C. SHANER[3];
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Abstract: Fluorescent genetically encoded calcium indicators (GECIs) have revolutionized our ability to probe the circuit dynamics of the brain. However, these fluorescence-based indicators have drawbacks, most importantly, the need to excite fluorophores with external light. Excitation light can be toxic and cause photodamage, produce misleading autofluorescence, and contribute to signal degradation due to light scattering and photobleaching of the fluorophore. Bioluminescence largely overcomes these pitfalls because light is emitted without the need for an external excitation source. As such, bioluminescent sensors can not only enhance imaging, but also reduce complexity e.g., in implanted microscopes (see Celinkis et al., 2020). We recently engineered a new, bright bioluminescent calcium indicator, ‘CaBLAM,’ utilizing a novel variant of the Oplophorus gracilirostris luciferase. This GECI provides single-cell and subcellular

resolution with high dynamic range in vitro. Here, we tested CaBLAM in vivo in Neuron-Derived Neurotrophic Factor (NDNF) interneurons in Primary Somatosensory Neocortex (SI). Using one-photon imaging, we found that CaBLAM generates signals bright enough to image at relatively high speeds (≥10 Hz) with a large dynamic range and single-cell spatial resolution. This step forward provides a new option for in vivo imaging, with distinct benefits for many experimental conditions.

Disclosures: J.W. Murphy: None. **E.L. Crespo:** None. **G.G. Lambert:** None. **U. Hochgeschwender:** None. **C.I. Moore:** None. **N.C. Shaner:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant RF1MH130391
NIH Grant U01NS128537
NIH Grant R01GM139850
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Title: Machine Learning Directed Engineering of Genetically Encoded Calcium Indicators

Authors: *S. J. WAIT[1], J. H. LEE[2], S. LIN[3], L. TORP[1], Y. WANG[4], C. K. KIM[3], A. BERNDT[1];
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Abstract: Genetically encoded calcium indicators (GECIs) provide a means to detect and monitor neuron activation in vivo spatiotemporally. Their fluorescent output is modulated by the presence of calcium in neuronal circuits. To meet experimental requirements, the proteins that comprise GECIs can be mutated to alter the biophysical characteristics of the sensor, such as dynamic range, ligand sensitivity, and kinetics. However, GECI engineering poses a significant challenge due to each GECI'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 alter sensor functionality. We previously demonstrated that our machine-learning pipeline could effectively learn from complex mutational datasets and promote efficient engineering of GECIs (Wait et al. Nature Computational Science, 2024). We used this platform to similarly engineer the biophysical properties of red-shifted calcium indicators (Ex./Em.: 580nm/605nm). Using point mutations identified by the machine learning pipeline, we have

discovered variants of the jRCaMP1b construct that display faster kinetics, higher baseline fluorescences, and greater sensitivities than the base construct. In addition, mutations obtained from the machine learning pipeline can be reflected onto the protein structure to highlight key interprotein interactions that govern each biophysical characteristic. We used these interactions as the basis for mutational libraries and high-throughput screening to identify beneficial combinatorial mutations at critical residues (Rappleye et al. ACS Sensors, 2023). In summary, we demonstrate the versatility of our machine-learning pipeline and its ability to complement high-throughput screening methodologies. These applications hold significant promise for improving the efficiency of GECI engineering, offering a new and powerful tool for the neuroscience, genetics, and bioengineering communities.

Disclosures: S.J. Wait: None. **J.H. Lee:** None. **S. Lin:** None. **L. Torp:** None. **Y. Wang:** None. **C.K. Kim:** None. **A. Berndt:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.06/X31

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BWF CASI to JEM
David & Lucille Packard Foundation
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McCamish Foundation

Title: An improved red calcium indicator with increased photostability and reduced blue light photoactivation

Authors: *X. ZHANG[1,2], E. ULUTAS[3], B. ADDISON[3], J. E. MARKOWITZ[3], D. KOVEAL[3]; [1]Biomed. Engin., Emory Univ. Sch. of Med. Neurosci. Grad. Program, Atlanta, GA; [2]Biomedical Engineering, Georgia Tech and Emory, Atlanta, GA; [3]Biomed. Engin., Georgia Technol. and Emory, Atlanta, GA

Abstract: Genetically encoded calcium indicators (GECIs) are widely used tools for studying neuronal activity. In order to extend these tools to measure multiple, genetically identified populations of cells at the same time, recent work has expanded the GECI toolbox to include indicators that fluoresce at different wavelengths - most prominently red, as it has a number of desirable optical properties. However, while most green GECIs have been highly optimized for brightness, response size, and cellular expression, their red counterparts still suffer from certain drawbacks including susceptibility to photobleaching and complex photophysics. Blue light induced artifacts (Akerboom et al. 2013; Dana et al. 2016) are particularly challenging to account for when performing simultaneous red and green imaging, or optogenetic experiments. A bright, photostable red GECI is therefore desirable to enable straightforward artifact-free interpretation

of calcium dynamics. To address this need, we engineered a novel GECI utilizing near optimal red fluorescent proteins. We performed library screening to maximize calcium responses and brightness and confirmed photostability and blue light responses in vitro. We also deployed our top sensor candidates in vivo and measured responses using fiber photometry. In summary, we developed a novel red fluorescent protein-based calcium indicator with enhanced stability and reduced artifacts, facilitating multicolor imaging and concurrent optogenetic applications, with ongoing efforts to optimize sensor performance in vivo and in vitro.

Disclosures: X. Zhang: None. **E. Ulutas:** None. **B. Addison:** None. **J.E. Markowitz:** None. **D. Koveal:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.07/X32

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH/NCI 5P30CA045508-36
DOD W81XWH2210045

Title: Development and Characterization of BioID2-GCaMP8, a novel calcium-indicator and biotin ligase to link neuronal activity to protein expression

Authors: *A. BATTISON[1], J. C. BORNIGER[2];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Neuroscience/Cancer Ctr., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Genetically encoded calcium indicators (GECIs), particularly the suite of GCaMP calcium indicators have paved the way for understanding mesoscale dynamics of nerve firings, however tying the functional imaging to a molecular explanation has remained elusive. Proximity labeling proteomics has also emerged as a powerful biochemical technique used to identify and study proteins that interact in the vicinity of a target protein or within a subcellular compartment. Here we develop and characterize a novel fusion protein which will contain a GCaMP calcium indicator for functional imaging and a proximity labeling enzyme (BioID2 and APEX) for spatial proteomics. By combining these two tools we show that we can record calcium signals from cells using two photon microscopy and then successfully extract local proteomes near the calcium sensor. Here we characterize the biophysical properties of this novel fusion protein and demonstrate its feasibility for extracting both proteomes and calcium dynamics both in vitro and in vivo.

Disclosures: A. Battison: None. **J.C. Borniger:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.08/X33

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Elsa U. Pardee Foundation
CMU Neuroscience Program

Title: Bioluminescent Kinase Sensors for detection of growth factor and inflammation signaling

Authors: *M. P. CHATTERTON[1], B. ST. ONGE[2], J. BAKKE[1], J. ROSSIGNOL[1], E. D. PETERSEN[3];
[1]Col. of Med., Central Michigan Univ., Mount Pleasant, MI; [2]Neurosci. Program, Central Michigan Univ., Mount Pleasant, MI; [3]Col. of Med., Central Michigan Univ., Mt Pleasant, MI

Abstract: Growth factor signaling is an important component of a large variety of cellular processes including metabolism, differentiation, proliferation, and migration. When growth factor signaling is altered it can lead to pathologies like cancer cells forming and proliferating within the body such as glioblastoma multiforme (GB). In this study, we focus on investigating and proposing novel therapeutic approaches utilizing genetically encoded Bioluminescent Kinase Sensors (BlinKS) to respond to growth factor signaling via kinases in the epidermal growth factor receptor (EGFR) signaling pathway. Specifically, this study is targeting the kinases within the MEK, RAS, and RAF signaling pathways. We developed a rational library of BlinKS variants with altered phospho-amino acid binding domains (PAABD) as well as varying kinase substrate peptides and permutations of the linker regions, either flexible or rigid at the interfaces of the protein fusion sites. We tested our BlinKS constructs in U87 glioblastoma cells expressing our candidate sensor variants, treated the cells with epidermal growth factor (EGF) and measured the response of the BlinKS sensors for light emitted by the sensor and by measuring an optogenetic transcriptional readout via a fluorescent reporter protein. Bioluminescence readings were conducted on a plate reader, and it was found that the cells treated with EGF produced more luminescence than those not treated with EGF. We also found our sensors targeting this signaling cascade to be able to control an optogenetic transcription system, reporting EGFR activation with GFP expression. We have also also developed versions of BlinKS that respond to inflammatory signaling via the JAK/STAT pathway downstream of IL-6 receptor, reporting the presence of an inflammatory compound (LPS) with an increase in light emission. We aim to test these sensors in a variety of neuroscience applications. This includes reporting glioblastoma tumor growth factor signaling and using the sensors to control transcription of therapeutic protein that will reign in unregulated growth. We will also be testing the inflammation sensing BlinKS to report and control neuroinflammation with the ultimate goal of limiting neurodegeneration.

Disclosures: M.P. Chatterton: None. **B. St. Onge:** None. **J. bakke:** None. **J. Rossignol:** None. **E.D. Petersen:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.09/X34

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Equanta: advancing opioid detection beyond physical presence

Authors: *S. EMAM[1], J. E. NYLAND[2];
[1]Northeastern Univ., Mechanicsburg, PA; [2]Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA

Abstract: We are reporting a state-of art device to detect fentanyl instantly and accuratelywithout having the physical matter present. This device encompasses cutting-edgecomponents that seamlessly collaborate to fulfill its intended purpose. Central to itsfunctionality are a state-of-the-art electrochemical gas sensor, advanced artificial intelligencealgorithms, a meticulously optimized electronic platform, and a user-intuitive mobileapplication.The sensing technology is based on self-assembled chemistry. The binding process betweenthe sensing layer and fentanyl induces a modification in the Fermi level of graphene on thesensing layer, resulting in a reduction in surface conductivityOur technology stands out when compared to other novel technologies in the market,offering the following advantages: 1) super sensitive: detects as low as 1 pg/ml of analyte inliquid/saliva/urine and 1 part per quadrillion in air, 2) broad range of sensitivity: from 1 pg/mlto 1 mg/ml with quantitative measurement, 3) highly selective: detects only one specificmolecule, not heroin or blank medium 4) immediate detection: in ~10 seconds, 5) reusablewith fast recovery time: less than a few minutes. Commonly used technologies for opioiddetection include ion mobility spectrometry (IMS), mass spectrometry (MS), infraredspectroscopy (IR), Raman spectroscopy, nuclear magnetic resonance, and biosensors. Whilecolor-based tests have long been employed for field drug testing, they have limitations, suchas they are not always effective with newly synthesized drugs, because they do not identifythe chemical structure; These methods offer accurate results, but they need to have thephysical matter present.The device is capable of functioning as a "scanner" to detect the presence of fentanylin the air, demonstrating sensitivity at sub-parts per quadrillion levels. This scanningfunctionality holds practical applications for various stakeholders, including DEA agents, lawenforcement officers, US Customs and Border Protection officials, or even prison guards.Furthermore, our adaptable technology can be reconfigured to detect opioids from exhaledbreath samples, alerting emergency rooms or emergency medical technicians on potentialdrug overdose instances. This versatile device thus addresses multiple critical needs of theUnited States within the realm of opioid detection and safety. The team comprises membersfrom Pennsylvania State University, Brown University, Harvard University, and QuarksenLLC.

Disclosures: S. Emam: None. **J.E. Nyland:** None.

Poster

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Program #/Poster #: PSTR253.10/X35

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH RF1MH130391
NIH U01NS128537
NIH R01GM139850

Title: High-throughput engineering of optogenetic tools for detecting endogenous opioids, fentanyl, and GPCR-coupled neuromodulation in vivo

Authors: *L. TORP[1], Y. WANG[1], S. WAIT[2], M. E. SODEN[3], M. R. BRUCHAS[4], A. BERNDT[5];
[1]Univ. of Washington, Seattle, WA; [2]Mol. Engin., Univ. of Washington, Seattle, WA; [3]Pharmacol., Univ. of Washington, Seattle, WA; [4]Dept. of Anesthesiol. and Pain Med., Univ. of Washington, Seattle, WA; [5]Bioengineering, Univ. of Washington, Seattle, WA

Abstract: Genetically encoded fluorescent indicators (GEFIs) enable real-time monitoring of neural activity with a high degree of sensitivity, specificity, and spatiotemporal resolution, and can be targeted to specific cell types in the brain. Engineering GEFIs has become a primary goal for many seeking to understand neuromodulation from a cellular to behavioral scale, but this is often a time and resource intensive process limited by the vast mutational landscape inherent to protein engineering. Particularly, investigators seeking to understand opioid signaling and GPCR-based neuromodulation are lacking robust and reliable tools for studying these systems in vivo. The Optogenetic Microwell Array Screening System (Opto-MASS) is a high-throughput screening platform previously developed in the Berndt Lab to determine the functional characteristics of thousands of GEFI variants in a single day. Here, we applied Opto-MASS to identify an improved opioid sensor designated μMASS by screening over 20,000 cpGFP linker variants. μMASS displays an increased fluorescence response to endogenous (met-enkephalin) and exogenous (fentanyl) opioid ligands at saturating conditions (~6.9 fold and ~5.5 fold) while retaining sub-micromolar ligand affinity (Kd=200nM and 118nM). To improve the baseline fluorescence, signal-to-noise ratio, and opioid selectivity of μMASS we coupled the high-throughput capabilities of Opto-MASS to a structure-based protein engineering rationale. This resulted in μMASS-Fentanyl, a GEFI which displays fentanyl-specific fluorescence response (Kd=275nM, ~4.0 fold increase to saturating fentanyl) with substantially decreased affinity for endogenous opioid ligands. Additionally, Opto-MASS was applied to engineer color-tunable and Gq-coupled opsin-based actuators for regulating neuronal activity in vivo. The Bruchas lab has previously established the lamprey parapinopsin (PPO) as a bistable actuator for neuron silencing through Gi-coupled inhibition when activated with blue (488nm) light. To shift the spectral activation of PPO towards violet or red absorption, we identified key residues within the retinal binding pocket to generate a 160,000-member mutagenesis library screened on the Opto-MASS

pipeline. An additional library targeted the intracellular loop 3 (ICL3) region of PPO to shift G-protein coupling from Gi to Gq. Following Opto-MASS screening we identified the color and Gq shifted variants PPO-Violet and PPO-Gq, respectively. Taken togther, we leveraged the high-throughput capabilities of Opto-MASS to engineer novel and robust tools for studying opioid signaling dynamics and controlling neuromodulation.

Disclosures: L. Torp: None. **Y. Wang:** None. **S. Wait:** None. **M.E. Soden:** None. **M.R. Bruchas:** None. **A. Berndt:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.11/Y1

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NSF CHE-2235835
NSF GRFP DGE 1841052

Title: Temporally gated integrator reporter for protein-protein interaction detection with immediate enzymatic readout

Authors: *J. SESCIL[1], G. ZHOU[2], W. WANG[3];
[1]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Chem. Biol., Univ. of Michigan, Ann Arbor, MI; [3]Life Sci. Inst., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Protein-protein interactions (PPIs) play crucial roles in many biological processes, and their dysregulation is implicated in numerous diseases. Therefore, a variety of tools have been developed to monitor PPIs, such as real-time sensors. These sensors produce a transient signal that can elucidate dynamic information about an event, but the signal's temporary nature limits further study of involved cells. Another class of reporters, integrating reporters, permanently mark cells involved in PPIs, allowing further investigation of labeled cells, such as RNA sequencing or proteomic analysis. For example, a PPI can reconstitute a split fluorophore, producing a direct fluorescent readout that leaves a fluorescent mark on involved cells. However, lack of signal amplification in many reporters leads to a decreased detection limit. Furthermore, many split protein reporters lack light or chemical-based gating, which limits the temporal resolution of the sensor. Transcription-based light-gated integrator sensors have also been reported, but can only detect cytosolic PPIs. Additionally, while these reporters amplify signal through transcription, they lose subcellular resolution and require over eight hours for signal readout, making them unsuitable for studies that require immediate readout, such as end-of-life animal experiments. A light-gated reporter based on enzyme reconstitution has also been developed but is challenging to use in live animals due to the need for peroxide and cofactor. My work focuses on developing a light-gated PPI detector with a bright, amplified signal resulting

from activity of a reconstituted biotin ligase, TurboID. I will show the design and optimization of this motif, which will have increased functionality for broader applications.

Disclosures: J. Sescil: None. **G. Zhou:** None. **W. Wang:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.12/Y2

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH DP2AG067666

Title: Cationic peptides erase memories by removing synaptic AMPA receptors through endophilin-mediated endocytosis

Authors: *K. T. BEIER;
Univ. of California Irvine, Irvine, CA

Abstract: Administration of the Zeta Inhibitory Peptide (ZIP) interferes with memory maintenance and long-termpotentiation (LTP). However, mice lacking its putative target, the protein kinase PKMz, exhibit normal learning and memory as well as LTP, making ZIP's mechanism unclear. Here, we show that ZIP disrupts LTP by removing surface AMPA receptors through its cationic charge alone. This effect was fully blocked by drugs that block macropinocytosis and is dependent on endophilin A2 (endoA2)-mediated endocytosis. ZIP and other cationic peptides selectively removed newly inserted AMPAR nanoclusters, providing a mechanism by which these peptides erase memories without effects on basal synaptic function. Furthermore, cationic peptides can be administered locally and/or systemically and can be combined with local microinjection of macropinocytosis inhibitors to modulate memories on local and brain-wide scales. Lastly, we leveraged these mechanisms to prevent amnesia induced by traumatic brain injury selectively at targeted brain sites. Our findings have critical implications for an entire field of memory mechanisms and highlight a previously unappreciated mechanism by which memories can be lost.

Disclosures: K.T. Beier: None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.13/Y3

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH New Innovator Award DP2MH129956
NIH BRAIN R01NS133755

Title: Development of a red-shifted chemigenetic voltage indicator

Authors: *E. W. SALTER[1], F. GALEAZZI[1], D. SHEINBERG[1], M. TEST[1], K. AMIN[2], B. PARADISO[1], A. S. ABDELFATTAH[1];
[1]Neurosci., Brown Univ., Providence, RI; [2]Brown Univ. Neurosci. Grad. Program, Providence, RI.

Abstract: Fluctuations in cellular transmembrane voltage are a primary mode of communication in the brain. Traditional measurements of transmembrane voltage using electrodes are invasive, low throughput and technically demanding. Conversely, optical readout of transmembrane voltage using genetically-encoded voltage indicators (GEVIs) enables non-invasive voltage measurement from multiple cells and/or subcellular compartments simultaneously. A current major limitation of GEVIs is that performance is optimal in the green/yellow spectral window. Imaging biological tissue at these wavelengths is hampered due to scattering, autofluorescence and absorption-induced tissue heating. All these non-ideal tissue properties decline with redder wavelengths. In addition, a red-shifted GEVI will provide spectral separation for coupling with channelrhodopsins for all-optical electrophysiology. To address this, we rationally engineer the GEVI Voltron2 to improve performance in the red/far-red spectral window. Voltron2 is a rhodopsin-based chemigenetic GEVI which functions by coupling voltage-dependent changes in the rhodopsin absorption to changes in fluorescence of HaloTag bound dyes. In this work, we focus on modifying the voltage-sensing rhodopsin domain to shift the absorption maximum to red/far-red wavelengths. We develop an assay to directly measure the red-shift of Voltron2 variants in primary neurons by co-staining with a yellow dye, Janelia Fluor $(JF)_{525,}$ and a red dye, JF_{608}. We then use electrical field stimulation of co-stained neurons to elicit action potentials while measuring fluorescence changes in both the yellow (JF_{525}) and red (JF_{608}) spectral windows from the same cells. Using this assay, we identify mutations which enhance the ratio of JF_{608} to JF_{525} voltage sensitivity, indicative of a red-shifted rhodopsin absorption. Current efforts are underway to further shift the peak voltage sensitivity to the far-red spectral window as well as improve overall voltage sensitivity. These far-red optimized GEVIs will improve the ability to optically record voltage deep within brain tissue. This will enhance *in vivo* measurement of both circuit function and subcellular voltage dynamics to reveal new brain-behavior relationships.

Disclosures: E.W. Salter: A. Employment/Salary (full or part-time):; Brown University. **A.S. Abdelfattah:** A. Employment/Salary (full or part-time):; Brown University.

Poster

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.14/Y4

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BBSRC BB/S003894 to K.T. and T.C.

Title: A novel ultrafast iGluSnFR3 variant, iGlu3Fast

Authors: O. TRAN[1], S. BERTELLI[2], H. HUGHES[1], A. J. R. PLESTED[2], ***K. TÖRÖK**[3]; [1]Neurosci. and Cell Biol., St George's, Univ. of London, London, United Kingdom; [2]Biol., Humboldt Univ. Berlin & Cluster of Excellence NeuroCure, Berlin, Germany; [3]Neurosci. and Cell Biol., SGUL, London, United Kingdom

Abstract: The first genetically encoded single fluorophore-based glutamate sensor, iGluSnFR opened a new era of imaging neuronal activity at the level of circuits as well as at synapses. However, with slow *off*-kinetics, iGluSnFR could only resolve low frequency glutamate release. A binding site variant of iGluSnFR, $iGlu_u$ with significantly faster *off*-kinetics, enabled the resolution of high (100 Hz) frequency glutamate release in hippocampal slices at individual CA3-CA1 synapses. Moreover, $iGlu_u$ revealed impairment of glutamate retrieval in HD mice models pointing to defective glutamate transport. Recently, the iGluSnFR3 generation (SF-Venus-iGluSnFR. v857) has been developed with increased dynamic range, making it attractive for *in vivo* imaging. However, similarly to iGluSnFR, glutamate *off*-kinetics of iGluSnFR3 limit it to resolving low frequency release only. We undertook to improve the kinetic properties of iGluSnFR3 for resolving high frequency glutamate release. We generated and characterized an ultrafast variant of iGluSnFR3 (iGlu3fast) while also increasing its fluorescence dynamic range. iGlu3Fast superseded iGluSnFR3 with a 42-fold glutamate-induced fluorescence increase compared to 26 for iGluSnFR3. For *off*-kinetics, measured using excess GluBP to trap the released glutamate, we obtained rate constants of 71 ± 3 and 340 ± 48 s^{-1} for iGluSnFR3 and iGlu3Fast, respectively, at 20°C. Glutamate concentration dependence of the *on*-kinetics of iGluSnFR3 and the S72T variant was fitted to a hyperbolic function that describes a two-step reaction in which rapid binding is followed by a conformational change driving fluorescence enhancement. The rate limiting *on*-rate was best fit to 1399 ± 43 and 832 ± 148 s^{-1}, for iGluSnFR3 and iGlu3Fast, respectively. Dissociation constants (K_d) measured by equilibrium titration were 373 ± 3 μM for iGluSnFR3 and 3.18 ± 0.05 mM for iGlu3Fast. Thus, the iGlu3Fast variant has a greater dynamic range, 5-fold faster *off*-rate and an 8.5-fold higher K_d for glutamate than iGluSnFR3, in solution. Expressed on the surface of HEK293T cells and assayed in a fast perfusion system, iGluSnFR3 and iGlu3Fast both displayed lower affinity with K_d values of 16.3 ± 4.1 μM and 162 ± 50 μM, respectively. When challenged with a 100 ms glutamate stimulus, iGluSnFR, iGluSnFR3 and iGlu3Fast responded with $\Delta F/F_o$ values of 1.76 ± 0.60, 6.5 ± 2.7 and 7.7 ± 4.1 and decay half-times of 36.5 ± 4.0, 30.3 ± 4.3 and 9.2 ± 2.7 ms, respectively. Thus, iGlu3Fast, with as fast *off*-kinetics as $iGlu_u$ and a 6-fold greater dynamic range, is a promising new tool for imaging glutamate neurotransmission at synapses and beyond. Funded by BBSRC BB/S003894 grant to K.T. and T.C.

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Poster

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Program #/Poster #: PSTR253.15/Y5

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant T32EB019944
NSF Grant 2123781

Title: Accelerating Neuronal Differentiation through Cell Cycle Modulation

Authors: *J. X. WANG[1], Z. DOU[2], S. KANG[3], G. UPADHYAY[4], H. KONG[5];
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Abstract: A significant barrier in creating pluripotent stem cell (PSC) derived neuron cultures is the time necessary for the differentiation and maturation of the resulting tissues. The current focus has been optimizing the efficiency and functionality of the resulting differentiated neuronal tissues, while long differentiation time scales have been viewed as an intractable problem and are either ignored or overlooked. Depending on neuron type, the differentiation process can extend to several months. Not only does this timescale require significant resources to maintain, but it also limits the repeatability and overall throughput for studying neuron development and disease modeling. Differentiation time scales need to be viewed as a variable that can be engineered, and novel methodologies to accelerate the differentiation process need to be developed. We demonstrate such a methodology through control of the pluripotent stem cell cycle. Cell cycle, pluripotency, and differentiation are each intimately connected. To control the cell cycle, we screened a diverse set of cyclin-dependent kinase inhibitors (CDKi). We identified that PSC population cell cycle distributions can be tuned based on the concentration of the CDKi. PSCs with varying cell cycle distributions were then differentiated towards motor neurons. Using PSCs transfected with Hb9-GFP, we found that treated cells differentiated to be Hb9+ at a higher rate and efficiency than untreated. Untreated conditions required 13 days to reach comparable amounts of Hb9+ cells present in treated conditions at 11 days of differentiation. On day 11 of differentiation, treated populations had a 2.54 (±0.28) fold increase in the amount of Hb9+ cells. We found that with partial arrest towards the G2 cell cycle phase before differentiation, quantified through calcium flux imaging, treated cells could generate coordinated burst firing events by day 11 of differentiation compared to untreated control at day 16. When quantifying the total number of active neurons and their connectivity, connectivity is defined as the number of connections each neuron has to another neuron. By day 11 of differentiation, treated PSCs consistently demonstrated a 1.65 (±0.26) fold increase in the amount of actively firing neurons and a 1.99 (±0.86) fold increase in connectivity. These results demonstrate that cell cycle control is a methodology that can be used to accelerate neuronal differentiation and supports the idea that differentiation time scales can be engineered.

Disclosures: J.X. Wang: None. **Z. Dou:** None. **S. Kang:** None. **G. Upadhyay:** None. **H. Kong:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.16/Y6

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant GM148812
NIH Grant CA255979
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NIH Grant AG071978

Title: Analysis of weak nuclease activities by ratiometric FRET

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Abstract: We describe an original and sensitive ratiometric FRET assay for the detection and quantitative assessment of weak DNase activities. The assay measures single-stranded DNA breaks. It uses a clip-shaped FRET oligoprobe, which assumes a complex conformation positioning two fluorophores at a distance of 23.8 Å from each other. Such positioning corresponds to the efficiency of energy transfer from donor to acceptor: $E_{FRET} = 0.993$. This efficient intramolecular FRET permits using the clip-shaped probe as a real-time DNA cleavage sensor for the detection of minor and slow DNA cleavage. We present its application for the quantitative analysis of an ultra-weak acid DNase in different pH environments. By assessing DNase reactions occurring in small volume samples and analyzing low amounts of nucleases, the approach can be useful in liquid biopsy studies.

Disclosures: V. Didenko: None. **C. Minchew:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BICCN R01MH128861
BICCN R01MH128888

Title: Speckle: a versatile proteomic mapping platform for multiplexed three-dimensional molecular imaging and high throughput combinatorial protein barcoding

Authors: *Y. TIAN[1], C. SOHN[4], W. GUAN[2], J. PARK[5], D. YUN[6], C. SU[3], M. P. FROSCH[7], K. CHUNG[1];
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Abstract: To systematically study complex biological systems, it is essential to examine the cellular and molecular information from the context of their spatial microenvironment. Advancements in spatial proteomics have yielded valuable insights for biological research, yet due to limited scalability and multiplexing capacity, high-throughput 3D proteomic mapping remains challenging. Here we present SPECKLE (Spatial Proteomics Enabled by Controlled Kinetics and Large Equilibrium), a multi-modal platform for volumetric proteomic mapping with multiplexing scalability. Using theoretical analysis and computational modeling, we predicted that uniform labelling of proteins with controllable density can be achieved by implementing a regime where antibody binding kinetics are reduced while high equilibrium constant for the binding reaction is maintained. Our results demonstrated the implementation of a combinatorial barcoding strategy for proteins for the first time and its scalability to millimeter-scale tissues. A comprehensive comparative analysis of Alzheimer's Disease (AD) and healthy controls revealed potential new insights into immunological mechanisms induced by pathology.

Disclosures: Y. Tian: None. **C. Sohn:** None. **W. Guan:** None. **J. Park:** None. **D. Yun:** None. **C. Su:** None. **M.P. Frosch:** None. **K. Chung:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.18/Y8

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Aav-compatible optogenetic tools for activating endogenous calcium channels in vivo

Authors: *D. KANG[1], H. LEE[1], J. LEE[2], Y. JEONG[3], W. HEO[4], S. LEE[1];
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Abstract: Calcium ions (Ca2+) play pivotal roles in regulating diverse brain functions, including cognition, emotion, locomotion, and learning and memory. These functions are intricately regulated by a variety of Ca2+-dependent cellular processes, encompassing synaptic plasticity,

neuro/gliotransmitter release, and gene expression. In our previous work, we developed “monster OptoSTIM1” (monSTIM1), an improved OptoSTIM1 that selectively activates Ca2+-release-activated Ca2+ (CRAC) channels in the plasma membrane through blue light, allowing precise control over intracellular Ca2+ signaling and specific brain functions. However, the large size of the coding sequence of monSTIM1 poses a limitation for its widespread use, as it exceeds the packaging capacity of adeno-associated virus (AAV). To address this constraint, we have introduced monSTIM1 variants with reduced coding sequence sizes and established AAV-based systems for expressing them in neurons and glial cells in the mouse brain. Upon expression by AAVs, these monSTIM1 variants significantly increased the expression levels of c-Fos in neurons and astrocytes in the hippocampal CA1 region following non-invasive light illumination. The use of monSTIM1 variants offers a promising avenue for investigating the spatiotemporal roles of Ca2+-mediated cellular activities in various brain functions. Furthermore, this toolkit holds potential as a therapeutic strategy for addressing brain disorders associated with aberrant Ca2+ signaling.

Disclosures: D. Kang: None. **H. Lee:** None. **J. Lee:** None. **Y. Jeong:** None. **W. Heo:** None. **S. Lee:** None.

Poster

PSTR253: Biochemical and Molecular Technologies II

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Program #/Poster #: PSTR253.19/Y9

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: W. M. Keck Award KF-05617242

Title: Defining timing of expression of AAV cargos in cortical motoneurons using bioluminescent reporters

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Abstract: Viral vectors such as AAV are extensively used to deliver cargos to neurons and glia for genetic labeling and tests of therapeutic candidates. Because expression of cargos is typically assessed using post-mortem assays, timing of expression requires multiple studies with multiple animals. To overcome this limitation, we are developing paradigms to track AAV cargo expression in real-time in living rodents with bioluminescent reporters. Bioluminescence is generated by an enzyme (luciferase) acting on its substrate (luciferin). AAV-driven expression of luciferase in the brain can be detected through the skull and scalp in living mice using an in vivo

imaging system (IVIS). Previous studies defined parameters of bioluminescence from AAV-driven expression of firefly luciferase (AAV-fLuc) following injections into the cortex of adult mice, which transduce neurons and glia non-selectively. Here, we use intersectional genetics to selectively transduce cortical motoneurons (CMNs) that give rise to the corticospinal tract using a Cre-dependent luciferase in conjunction with retrogradely transported AAV/Cre (AAVrg/Cre). TdT reporter mice received injections of AAVrg/Cre into the cervical spinal cord and injections of AAV expressing Cre-dependent luciferase into the motor cortex. With this approach, transduction with Cre is confirmed by expression of tdT and Cre-dependent luciferase is expressed only in CMNs that are transduced with AAVrg/Cre. Expression was tracked over time by injecting luciferin intraperitoneally and imaging mice using IVIS. Luminescence induced by luciferin ramped over 9 days post injection, then declined by approximately an order of magnitude over 2 days. To determine if loss of signal is due to shut-down of expression of Cre-dependent luciferase, mice received intra-spinal AAVrg/Cre injections to pre-transduce neurons, then 4 weeks later received intracortical Cre-dependent luciferase injections. In this case, luminescence peaked 6 days after cortical injection of Cre-dependent luciferase, and declined by about an order of magnitude over 4 days. To access whether transient expression was related to Cre-dependent luciferase expression via AAVrg/Cre, we cortically injected Cre-dependent luciferase into CamK2 driver mice that express Cre constitutively in many neuron types. In this paradigm, luminescence was robust at 2 days post-injection, plateaued for 10 days, and then declined by an order of magnitude by 12 days. The ability of bioluminescence to track timing of AAV cargo expression in defined neuron populations in real-time in living rodents revealed unexpected time-limited expression of some Cre-dependent AAV cargos.

Disclosures: C. Callicoatte: None. **M. Metcalfe:** None. **A. Luptak:** None. **J. Prescher:** None. **O. Steward:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OS is a co-founder, scientific advisor, and has economic interests in the company Axonis Inc, whichis developing novel therapies for spinal cord injury and other neurological disorders..

Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.20/Y10

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: JSPS Kakenhi (24H01221, 22K06454, 23H04154, 22KK0140)
Novo Nordisk Foundation (NNFOC0058058)
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Nanken Kyoten Grant No. 2021-kokusai 11 at MRI TMDU
AMED Brain/MINDS (JP21dm0207111)

Title: A novel transgenic mouse model for functional tracing of circulation via albumin-tagged fluorescent probes

Authors: M. VITTANI[1], X. WANG[1], A. LEE[1], Y. HIRAOKA[2], A. KONNO[3], P. KNAK[1], P. KUSK[1], M. NAGAO[1], A. ASIMINAS[1], T. MISHIMA[1], M. TERUNUMA[4], H. HIRAI[5], M. NEDERGAARD[1,6], K. TANAKA[2], ***H. HIRASE**[1,6];
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Abstract: Albumin is the most abundant protein in blood plasma and cerebrospinal fluid. We have previously developed liver-targeting adeno-associated viral vectors that express fluorescent protein-tagged albumin to visualize blood plasma in adult mice (DOI: 10.1016/j.crmeth.2022.100302). We have also established a virally induced CRISPR/Cas9-based knock-in of green fluorescent albumin in neonatal mice (DOI: 10.1101/2023.07.10.548084). Here, we have generated a new transgenic mouse model by CRISPR/Cas9 in which the bright red fluorescent protein mScarlet is knocked into the albumin locus to produce mScarlet-tagged albumin (Alb-mSc). In adult heterozygous knock-in mice, plasma Alb-mSc enabled the visualization of the entire cortical vasculature (up to ~1 mm in depth) using two-photon microscopy. Through the thinned-skull preparation, the fine structure of the dura mater was visualized with Alb-mSc that infiltrated into the extracellular space. In the cortex, Alb-mSc present in the extracellular space provided a fluorescence background making cellular elements appear as shadows. The Alb-mSc construct additionally features SpyTag003 (ST3), a peptide that can irreversibly bind to SpyCatcher003 (SC3). We used this ST3-SC3 technology to monitor the blood plasma microenvironment by expressing green SC3-fluorescent biosensor fusion proteins that form a macromolecule with Alb-mSc-ST3. We also report that extracellular space-facing SC3 expressed in astrocytes captures Alb-mSc-ST3 after nearby vessels were subjected to laser irradiation, demonstrating its utility as a detector of blood-brain barrier disruption. This novel transgenic mouse model will provide a powerful means for imaging vasculature while monitoring its permeability and microenvironment.

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Poster

PSTR253: Biochemical and Molecular Technologies II

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR253.21/Y11

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Liquid Sample Reading Device Using Fluorescence and Chemiluminescence

Authors: *R. BELTRAN-RAMIREZ[1], J. MARTINEZ-MENDOZA[2], J. A. DOMINGUEZ-RAMIREZ[1], C. ROMAN[3], X. M. BECERRA-GONZÁLEZ[1];
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Abstract: The present invention relates to the technical field of mechanics, electronics, physicochemical analysis, laboratory samples, and more specifically to methods for analyzing samples, as it provides a device for reading liquid samples through fluorescence and chemiluminescence. BACKGROUND Devices for reading liquid samples through fluorescence and chemiluminescence represent a significant advancement in the fields of biochemistry and molecular biology. These devices are essential for detecting and quantifying specific molecules in various liquid samples, leveraging the unique properties of fluorescence and chemiluminescence. Fluorescence involves the absorption of light by molecules and subsequent emission of light at a different wavelength, while chemiluminescence is based on light emission as a result of a chemical reaction. Since their introduction, these devices have found applications in a wide range of areas, including biomedical research, clinical diagnosis, and quality control in the food and pharmaceutical industries. The evolution of these devices has been driven by the need for more sensitive, specific, and rapid methods for analyzing complex samples. Technological developments have allowed significant improvements in sensitivity and specificity, facilitating the detection of extremely low concentrations of biomolecules, which is crucial for early and accurate disease diagnosis, as well as advanced scientific research. Additionally, the automation and miniaturization of these devices have improved the efficiency and accessibility of tests, enabling high-throughput analysis and reducing the time and cost associated with traditional analyses. The integration of digital technology and advanced software has further allowed the collection and analysis of complex data, enhancing the interpretation of results and facilitating data-driven decision-making in research and clinical practice. In summary, devices for reading liquid samples through fluorescence and chemiluminescence are fundamental tools that continue to evolve, driving advancements in various scientific fields and industrial applications. The present invention relates to an apparatus for conducting fluorescence and/or chemiluminescence tests, with means allowing connection to a computer for result visualization, enabling the tests to be conducted either individually or simultaneously. Additionally, each test can be carried out with different reagents simultaneously, providing advantages in reading liquid samples.

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Poster

PSTR253: Biochemical and Molecular Technologies II

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 1-DP2-ES027992
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JPB Foundation PIIF
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Title: Significant regionalized reduction of parvalbumin-expressing interneurons in prefrontal cortex of healthy mice captured by universal strategy for volumetric processing of whole brains

Authors: *D. YUN[1], Y.-G. PARK[2], J. H. CHO[3], N. EVANS[4], K. XIE[4], S. CHOI[4], A. ALBANESE[4], Y. TIAN[5], C. SOHN[4], Q. ZHANG[6], J. SWANEY[4], W. GUAN[4], J. PARK[4], G. DRUMMOND[7], H. CHOI[4], G. FENG[7], K. CHUNG[4];
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Abstract: Parvalbumin is a calcium-binding protein and neurons expressing PV are known to play a key role in regulating brain functions, including maintaining excitatory/inhibitory balance in the cortex. The loss of PV-expressing neurons in the frontal cortex is implicated with disorders such as schizophrenia and autism spectrum disorders (ASD) in both mouse models and in humans; however, proper physiological baseline of PV neurons may not have been properly established before. We developed a volumetric immunolabeling technology called eFLASH (electrophoretic-Fast Labeling using Affinity Sweeping in Hydrogel) based on a novel concept of Continuous Redispersion of Volumetric Equilibrium (CuRVE). CuRVE describes a new paradigm for volumetric processing where the change in tissue chemical reaction environment occurs at a rate slow enough to allow the redispersion of unevenly distributed chemicals, continuously, thereby maintaining chemical equilibrium tissue-wide at any given moment. With eFLASH, an implementation of CuRVE in spatial immunohistochemistry that can achieve uniform and complete immunolabeling of samples as large as whole rat brains, we discovered highly individually variable regionalized reduction of PV-immunoreactive (PV-IR) neurons in healthy wildtype adult mice. This phenomenon was not captured by Cre/*loxP* binary transgenic reporter system of PV-IRES-Cre/loxP-tdTomato mice, suggesting dynamic silencing or mosaicism of PV-expression in adulthood. When examined at whole brain, the apparent loss of PV-IR neurons was observed mostly in frontal pole, prelimbic area, infralimibic area, and orbital area with high degree of lateral variability. The distribution and the total volume of these low PV regions were highly variable between individual animals. This finding demonstrates the importance and the utility of scalable volumetric immunolabeling tools such as eFLASH for establishing accurate baselines for interrogating disease-specific cellular and molecular changes.

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Poster

PSTR254: Genomic and Transcriptomic Techniques I

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Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U01 MH119690
CIHR
CIFAR IVADO
CFREF

Title: Characterizing the rare copy number variant architecture of the cortical organization of the human brain

Authors: *K. KUMAR[1], S. KAZEM[1], Z. LIAO[1], J. KOPAL[2], G. HUGUET[1], T. RENNE[1], M. JEAN-LOUIS[1], Z. XIE[1], W. ENGCHUAN[3], O. SHANTA[4], Z. SACI[1], L. ALMASY[5], D. C. GLAHN[6], G. DUMAS[1], J. SEBAT[7], C. E. BEARDEN[8], T. PAUS[1], P. M. THOMPSON[9], R. A. BETHLEHEM[10], V. WARRIER[11], S. JACQUEMONT[1];
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Abstract: The development and organization of the human cerebral cortex are highly heritable. Genome-wide association studies (GWAS) have identified common variants influencing global and regional phenotypes derived using magnetic resonance imaging (MRI). Studies focusing on a small set of rare copy-number-variants (CNVs) previously associated with psychiatric conditions have shown large effects on the cortex. However, genome-wide studies of rare protein-coding CNVs have yet to be conducted, and the rare CNV architecture of the human cortex remains unknown.
We analyzed MRI-derived phenotypes and all protein-coding CNVs > 50 kilobases from 40,000 UK Biobank (53% female, 2173 deletion and 3273 duplication carriers) and 8,000 Adolescent Brain Cognitive Development study (ABCD, 53% female, 552 deletion and 1463 duplication carriers) participants. Our analysis focused on seven macrostructural measures derived from T1-weighted MRI: cortical thickness (CT), surface-area (SA), volume, folding, and curvature indices, as well as five microstructural measures derived from diffusion MRI. Since the current sample size remains underpowered to detect variant- and gene-level associations for most rare variants, we used a functional burden association test to aggregate CNVs that disrupt genes involved in 1032 biological functions.
Of 24,768 burden tests (12 phenotypes x 1032 functional gene sets x deletions and duplications), 437 showed FDR-significant associations. Volume, SA, and CT showed the strongest association

with gene dosage. Associations were replicated in the ABCD cohort with concordant effect sizes (r=0.51, p=1e-4), and with consistent directionality in 68%. CNV burden genetic correlations between pairs of phenotypes were concordant with those previously published using common variants (r=0.84, p=1e-4), suggesting a convergence between the common and rare variant architectures of the human cortex. The effect sizes of deletions and duplications were negatively correlated for 7 out of 12 phenotypes, suggesting that MRI traits show relative sensitivity to either deletions or duplications depending on the biological function, which is disrupted by gene dosage. We also investigated the overlap between the contribution of CNVs to brain MRI, cognitive and behavioral traits, as well as risk for neurodevelopmental disorders (i.e., autism). Because gene dosage has large negative and positive effects on gene expression (respectively), our analysis provides insight into the impact of changes in transcription on the cerebral cortex.

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Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.02/Y14

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: Knut and Alice Wallenberg Foundation

Title: The regional and cellular transcriptome landscape of the adult human brain: an integrative approach

Authors: *N. MITSIOS[1], E. GERRITS[2], W. ZHONG[3], S. S. BARDE[2], E. SKARWAN[4], T. ZHENG[2], E. HUSÉN[2], P. OKSVOLD[5], K. VON FEILITZEN[5], E. SJOSTEDT[2], E. RENNER[6], M. PALKOVITS[6], T. HOKFELT[2], J. MULDER[2];
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Abstract: The Human Protein Atlas (HPA; www.proteinatlas.org) is an open-access online database that provides an integrated overview of protein expression and distribution in all major human tissue types, including the 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 in-house generated and publicly available transcriptomic data. Previously, we generated RNA sequencing data from 967 samples obtained from 193 micro-dissected regions

and areas of the human brain and subsequently classified all protein coding genes based on regional distribution and co-expression, thus providing lists of genes associated to brain regions, cell types and functions. This enabled us to analyse the distribution of more than 1800 transcription factors and generate a detailed map of transcription factor distribution throughout the adult human brain. Following recent advances in RNA-sequencing technologies, high-resolution spatial transcriptomics has emerged as a powerful technique for studying the molecular signatures of single-cells within their cellular environment, thus providing valuable tools for mapping the spatial distribution of mRNA transcripts in the brain. In the HPA version 24, to be released in October 2024, we integrate single-nucleus RNA sequencing and spatial transcriptomic data (stereo-seq) to reveal the spatial patterns of gene expression in various cell types from the human cerebral cortex. We have combined high-content single-nuclei gene expression data together with the spatial location of individual transcripts to characterise the molecular and cellular composition of cortical layers. This will provide a unique and detailed view of the molecular organisation, will link proteins to cell types and domains, and eventually uncover some of the basic organisational principles of the human brain.

Disclosures: N. Mitsios: None. **E. Gerrits:** None. **W. Zhong:** None. **S.S. Barde:** None. **E. Skarwan:** None. **T. Zheng:** None. **E. Husén:** None. **P. Oksvold:** None. **K. von Feilitzen:** None. **E. Sjostedt:** None. **E. Renner:** None. **M. Palkovits:** None. **T. Hokfelt:** None. **J. Mulder:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

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Program #/Poster #: PSTR254.03/

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Long-term continuous lineage decoding in human brain organoids and grafts

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Abstract: Human embryonic stem cell (hESC)-derived organoids mimic human organ development, providing an accessible and ethically justified model for studying human organogenesis in vitro. The continuous landscape of the long-term developmental lineage in human nervous system remains to be elucidated. Here, we develop a genetically defined progressive barcoding system, termed single-cell CRISPR lineage tracing (scCITING), to allow long-term lineage decoding in the hESC-derived organoids. Applying scCITING, we revealed an early regional patterning of human brain organoids. We further combined scCITING with the clonal splitting strategy and tracked the continuous maturation trajectories from progenitors to their terminal fates under different conditions by maturation in organoids or in grafts. Taking advantage of the long-term editing feature of scCITING, we unprecedently reconstructed the

phylogenic fate maps for single progenitors by building a single-cell level tree rooting from each individual transcriptome-defined progenitor cell. We found the progenitor fates were mostly conserved cross the environments, despite of the distinctive cell type compositions in vivo and in vitro. Specifically, we identified multiple origins for midbrain dopaminergic neurons in both environments. Furthermore, we incorporated spatial transcriptome and uncovered different lineage and distributions of two transcriptionally distinct astrocyte subtypes in the grafts. Our approach is a stable system for studying the continuous single-cell lineage in human organoids, offering insights for understanding the organ development in human.

Disclosures: X. Zhang: None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.04/Y15

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH SBIR Grant R44HG011875

Title: Multiomic sequencing technology reveals crosstalk between chromatin proteins and DNA methylation in neurological disease

Authors: *K. FEDDER-SEMMES, V. UDAYAKUMARAN NAIR SUNITHA KUMARY, B. J. VENTERS, M. KEOGH;
EpiCypher, Inc., Durham, NC

Abstract: Precise regulation of chromatin proteins and DNA methylation (DNAme) underlies many neurobiological gene expression programs. Multiple lines of evidence link chromatin dysregulation to neurological conditions, encompassing autism spectrum and neurodegenerative disorders, as well as stress, depression, anxiety, and substance use. Understanding the neuroepigenetic mechanisms that govern nervous system formation and function has the potential to yield valuable insights into novel biomarker and therapeutic targets. The advent of next-generation sequencing has enabled base-pair resolution analysis of DNAme (bisulfite sequencing) and histone post translational modifications/chromatin-associated proteins (ChIP-seq) across multiple neural cell types. However, establishing direct links between chromatin features and DNAme remains a major challenge due to the poor sensitivity of ChIP and bisulfite workflows, complicating multiomic analyses from low cell numbers. To enable such studies, we developed Cleavage Under Targets and Release Using Nuclease with enzymatic methyl-sequencing (CUT&RUN-EM). CUT&RUN-EM integrates CUTANA™ CUT&RUN with NEBNext ® Enzymatic Methyl-seq to deliver high-resolution DNAme profiles at defined chromatin features, enabling a direct examination of their molecular links. CUT&RUN is a highly sensitive assay where protein A/G-micrococcal nuclease is tethered to chromatin within intact cells or nuclei and activated to cleave nearby DNA. EM-seq then enzymatically converts

methylated cystine residues within CpG islands to generate single-base resolution, unbiased DNAme profiles from sub-nanogram input DNA at a fraction of the sequence depth. Together, these innovations enable the application of precise multiomics for neuroscience research, particularly for specific populations or clinical samples where cell numbers are limited. Here, we used CUT&RUN-EM to gain insight into chromatin regulatory mechanisms underlying Rett Syndrome (RTT), an X-linked neurodevelopmental disorder that results from variants in the methylated DNA-binding protein *MECP2*. To this end, we characterized the DNAme binding capabilities of GST-tagged MeCP2 with and without RTT-identified variants. RTT-MeCP2 variants showed variable and unpredictable binding at methylated DNA compared with wild type MeCP2, demonstrating strong proof-of-principle that CUT&RUN-EM can be applied to study direct links between chromatin protein and DNAme pathways. These studies provide the first multiomic view of MeCP2 engagement and set the stage for the integration of new epigenomic technologies to advance neuroscience research.

Disclosures: K. Fedder-Semmes: A. Employment/Salary (full or part-time):; EpiCypher. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); New England Biolabs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpiCypher. **V. Udayakumaran Nair Sunitha Kumary:** A. Employment/Salary (full or part-time):; EpiCypher. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); New England Biolabs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpiCypher. **B.J. Venters:** A. Employment/Salary (full or part-time):; EpiCypher. 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.; EpiCypher, New England Biolabs. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); New England Biolabs. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpiCypher. **M. Keogh:** A. Employment/Salary (full or part-time):; EpiCypher. 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.; EpiCypher. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EpiCypher.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.05/Y16

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH ES100221

Title: Comparison of spatial transcriptomics platforms for analysis of gene expression in hippocampal area CA2

Authors: B. KANDEMIR[1], E. P. HARRIS[2], T. WANG[3], K. GERRISH[3], *S. M. DUDEK[4]; [1]Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC; [2]Neurosci. Instititute, Georgia State Univ., Atlanta, GA; [3]Natl. Inst. of Envrn. Hlth. Sci., NIH, Research Triangle Park, NC; [4]Natl. Inst. of Envrn. Hlth. Sci., Researc, Research Triangle Park, NC

Abstract: Hippocampal area CA2 is now known to be an important node in the networks underlying behaviors such as social recognition and aggression and so assessing the molecular profile of CA2 is a critical part of understanding how these behaviors could modulated by hormones, for example. Accurately measuring gene expression in CA2, which is considerably smaller than its neighboring areas, CA1 and CA3, however, has presented as somewhat of a challenge; manual dissection of mouse CA2 is difficult and laser capture microscopy (LCM) is extremely labor-intensive. New methods in spatial transcriptomics have now been used successfully on brain tissue and maintain spatial information not possible with single cell/nuclei sequencing. Among the various spatial transcriptomics platforms, Nanostring's GeoMx Digital Spatial Profiling (DSP) and 10X Genomics' Visium have emerged as powerful tools with high data dimensionality and relatively high throughput. In this study, we aimed to characterize gene expression in area CA2 by comparing data from these two spatial transcriptomics platforms with each other and with RNA-seq data from LCM (Farris, et al., 2019). Here we compared differentially expressed genes (DEGs) in the CA2 (vs. CA1 and CA3) using 2 mice for GeoMx and 3 mice for Visium. We identified 118-209 genes with greater CA2 expression in the Visium data set, and 327-250 genes with greater CA2 expression in the GeoMx data ($p<0.05$ and $log2FC>0.58$) (out of thousands identified with LCM-RNA-seq). Notable was that only 66 of the differentially expressed genes in CA2 vs. CA1, and 59 genes in CA2 vs. CA3 were common across the two platforms, and only 51 genes were consistently enriched in CA2 compared to CA1 and CA3. These included known CA2 markers like *Amigo2* and *Pcp4*, but excluded others, particularly the low expressing genes like *Avpr1b*. In conclusion, although both platforms can be used to identify CA2-specific genes, both miss most of them. Although GeoMx DSP revealed more genes enriched in CA2, the overlap of common genes was less than 50% and was a fraction of the genes identified using LCM-RNA-seq. Thus, increasing the number of animals will be critical for studies looking at changes in gene expression across experimental groups.

Disclosures: B. kandemir: None. **E.P. Harris:** None. **T. Wang:** None. **K. Gerrish:** None. **S.M. Dudek:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.06/Y17

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

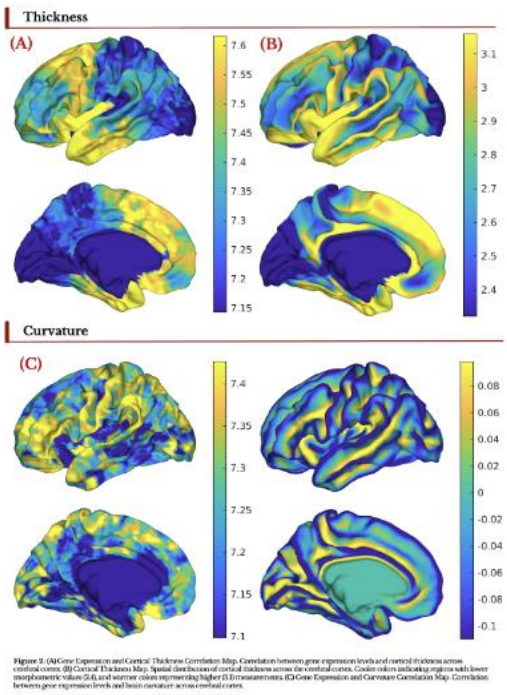
Title: Exploring the Genetic Basis of Cortical Thickness and Curvature Through Integrated Neuroimaging and Genomics

Authors: *T. JAFAR[1], N. CHOWDHURY[2];
[1]Gerontology, USC Keck Sch. of Med., LOS ANGELES, CA; [2]USC, LOS ANGELES, CA

Abstract: Cortical thickness and curvature are critical neuroanatomic features that vary with gene expression, potentially influencing cognitive abilities and susceptibility to neurodevelopmental disorders. This study utilizes high-resolution 3D T1-weighted MRI scans and gene expression data from the UK Biobank and Allen Brain Atlas to identify genetic markers associated with these cortical features. Our analysis included 43,031 participants, representing demographics of average age: 70.68 years; 52.7% female. We mapped gene expression data onto the cortical surface to facilitate a vertex-wise comparison with cortical thickness and curvature identifying specific genes whose expression significantly correlated with these measurements. Subsequent gene ontology (GO) analysis was performed with these significant genes, using Panther and ReviGO tools, to assess correlations between cortical morphometry and gene expression, with a focus on neuroimmune interactions and synaptic functions. GO analysis revealed significant correlations between cortical thickness and curvature and gene expression was enriched for genes related to immune responses and synaptic signaling. Notably, genes associated with the adaptive immune response (p=1.19E-10) and chemical synaptic transmission (p=8.17E-05) displayed strong correlations with cortical thickness, underscoring their potential impact on cortical structure. These findings suggest a profound genetic influence on the brain's structural and functional architecture. Furthermore, our findings highlight potential genetic targets for further research into the mechanisms underlying neurodevelopmental disorders and cognitive function. As we continue to refine our analysis with larger, diverse datasets, the goal is to enhance the predictive power of our models and improve their generalizability across populations. This research contributes to our understanding of the genetic architecture and opens up new avenues for targeted genetic studies that could lead to advancements in diagnosing and treating brain disorders.

Top 10 Thickness	
Gene Ontology	**p-value**
adaptive immune response	1.19E-10
carbohydrate derivative biosynthetic process	3.00E-05
cellular component assembly	2.21E-05
chemical synaptic transmission	1.60E-04
detection of chemical stimulus involved in sensory perception of smell	3.22E-18
detoxification	4.57E-05
homeostatic process	8.46E-05
immunoglobulin production	7.99E-07
modulation of chemical synaptic transmission	8.17E-05
negative regulation of cellular process	5.42E-05

Top 10 Curvature	
Gene Ontology	**p-value**
adiponectin-activated signaling pathway	1.39E-03
positive regulation of adipose tissue development	1.73E-05
ventricular compact myocardium morphogenesis	1.55E-04
myeloid progenitor cell differentiation	4.64E-04
RIG-I signaling pathway	4.64E-04
positive regulation of myeloid dendritic cell cytokine production	4.64E-04
nuclear mRNA surveillance	3.62E-04
Notch receptor processing	9.81E-04
response to oxygen-glucose deprivation	9.81E-04
netrin-activated signaling pathway	9.81E-04



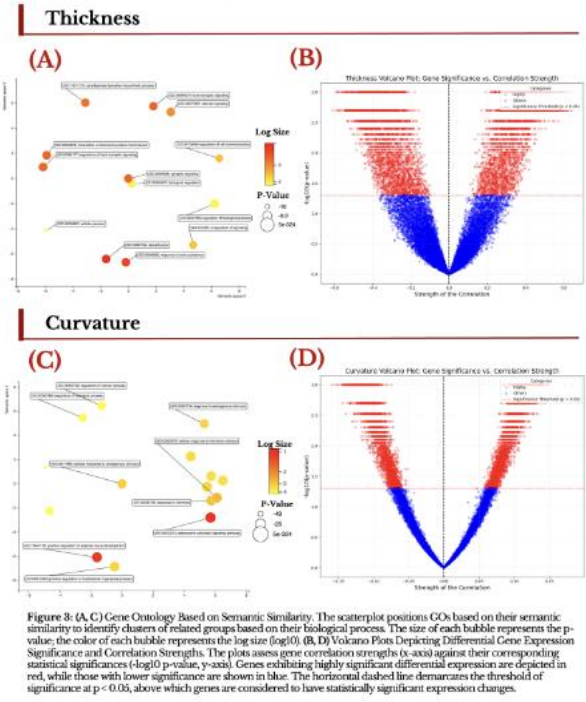


Figure 3: (A, C) Gene Ontology Based on Semantic Similarity. The scatterplot positions GOs based on their semantic similarity to identify clusters of related groups based on their biological process. The size of each bubble represents the p-value; the color of each bubble represents the log size (log10). (B, D) Volcano Plots Depicting Differential Gene Expression Significance and Correlation Strengths. The plots assess gene correlation strengths (x-axis) against their corresponding statistical significances (-log10 p-value, y-axis). Genes exhibiting highly significant differential expression are depicted in red, while those with lower significance are shown in blue. The horizontal dashed line demarcates the threshold of significance at $p < 0.05$, above which genes are considered to have statistically significant expression changes.

Disclosures: T. Jafar: None. **N. Chowdhury:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.07/Y18

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant 5R35GM131726

Title: Inter-individual genetic differences contribute to cell type-specific brain oxygen stress responses

Authors: *B. D. UMANS[1], O. ALLEN[1], Y. GILAD[2];
[1]Med., Univ. of Chicago, Chicago, IL; [2]Med., The Univ. of Chicago, Chicago, IL

Abstract: Noncoding genetic variation has been linked to many diseases of the central nervous system. Most noncoding, trait-associated variants are presumed to affect gene regulation, but understanding the target genes, cell types, developmental periods, and cellular processes they affect has proven a major challenge to the translation of human genetic studies into deeper biological insights. Large-scale surveys of gene expression variation in human tissues have begun to fill this gap, but human brain tissue can be sampled only opportunistically post-mortem, meaning that our understanding of gene regulatory contributions to brain phenotypes lack crucial dimensions including interactions with environmental perturbations and stress.
To map gene-environment interactions in developing human brain cells, we formed dorsal brain organoids from a panel of 21 genotyped human induced pluripotent stem cells. Organoids gave rise to a spectrum of cell types, including subtypes of radial glia, intermediate progenitors, excitatory and inhibitory neurons, and early glia, allowing us to examine responses in a variety of disease-relevant cell types. Because oxygen insufficiency, as occurs during apnea or infection, and elevated oxygen, as occurs after resolution of hypoxia and in some therapeutic contexts, challenge brain metabolism and antioxidant systems, we acutely exposed organoids to elevated or reduced oxygen for 24 hours before performing single-cell transcriptional profiling. We mapped expression quantitative trait loci (eQTLs) in each cell type to identify heritable gene regulatory differences exposed by oxygen manipulation. These cell type- and context-specific eQTLs are candidates for mediating the effects of genetic variation on central nervous system phenotypes, and we assessed their functional importance by comparing them to loci nominated by genome-wide association studies (GWAS). Thousands of regulatory effects emerged only after perturbation, including in over 1600 disease-relevant genes. Moreover, the combination of single-cell resolution and diverse sampling conditions allowed the discovery of novel associations, including 70 instances in which lead eQTL variants were themselves significant GWAS variants but implicated a different gene than identified by GWAS, highlighting the value of our experimental system for pinpointing the cell type, condition, and target gene on which regulatory variants act. Future studies will explore the impact of gene regulatory differences in the context of other perturbations and allow focused experimental interrogation of the contributions of genetic risk loci to diseases of the central nervous system.

Disclosures: B.D. Umans: None. **O. Allen:** None. **Y. Gilad:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.08/Y19

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: BICAN Developing Mouse: U01MH130962

Title: Technical Considerations for the Effective Analysis of Fresh Frozen, Postnatal Mouse Brain Samples in Spatial Transcriptomics

Authors: J. ARIZA TORRES[1], A. RUIZ[2], S. BARTA[3], A. OYAMA[4], A. AYALA[1], T. EGDORF[2], D. MCMILLEN[5], J. CAMPOS[5], N. MARTIN[1], J. NAGRA[1], P. OLSEN[1], N. VALERA CUEVAS[6], E. LIANG[5], M. KUNST[7], R. MATHIEU[1], M. MAJEED[1], X. CHEN[5], J. WATERS[1], H. ZENG[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Mol. Biol., Allen Inst., Seattle, WA; [3]Brain Sci., Allen Inst., Seattle, WA; [4]Mol. Biol. - Histology, Allen Inst. for Brain Sci., Seattle, WA; [5]Allen Inst., Seattle, WA; [6]Imaging, Allen Inst., Seattle, WA; [7]Imaging, Allen Inst. for Brain Sci., Seattle, WA

Abstract: Spatial transcriptomics has emerged as an indispensable tool in research, facilitating the analysis of gene transcription within precise spatial contexts. Over the past decade, numerous spatial technologies have emerged, each offering distinct features such as spatial resolution, sensitivity, multiplexing capability, throughput, and coverage. These technologies consistently enable the localization of hybridized RNA molecules in both fresh frozen and FFPE tissue samples. However, it's essential to acknowledge that a one-size-fits-all approach is untenable. Optimizing analysis conditions for fresh frozen tissue, particularly in postnatal mouse brain samples (P0-P20), requires distinct methodologies from those used in adult tissues. Critical considerations include the methodology employed to achieve optimal spatial analysis in such young tissue, encompassing factors like tissue dissection techniques, tissue quality affected by the freezing process, and permeabilization, all of which significantly influence performance and subsequent analysis. To address these challenges, we have developed a rapid freezing protocol tailored for postnatal brains and standardized the specific permeabilization, or digestion methods for each time point (P0-P20). These optimizations have demonstrated efficacy in spatial transcriptomics analysis of postnatal brain samples and are adaptable across various spatial technologies, whether custom or commercially available. As a proof-of-principle, we performed high-throughput in situ sequencing (BARSeq) on postnatal (P5) brain sections flash frozen using our rapid protocol. Compared to adult tissue protocols, rapid protocol sections showed 6-fold improvement in tissue integrity maintenance, similar gene/read counts, fewer errors, and consistent cell types. Similar findings were observed in P0, P4, and P20 mouse samples, where tissue prepared for MFISH using commercial platforms exhibited deterioration and loss of tissue integrity. This was attributed to freezing artifacts in the peripheral areas of the tissue and high vacuolization and tissue loss resulting from the aggressive permeabilization and digestion methods employed following the standard protocol from commercial spatial methodologies. These issues led to transcriptomic dropout when applying adult tissue preparation protocols. This

highlights the necessity of tailored methods to achieve reliable spatial transcriptomics analysis in diverse tissue types and developmental stages.

Disclosures: J. Ariza Torres: None. **A. Ruiz:** None. **S. Barta:** None. **A. Oyama:** None. **A. Ayala:** None. **T. Egdorf:** None. **D. McMillen:** None. **J. Campos:** None. **N. Martin:** None. **J. Nagra:** None. **P. Olsen:** None. **N. Valera Cuevas:** None. **E. Liang:** None. **M. Kunst:** None. **R. Mathieu:** None. **M. Majeed:** None. **X. Chen:** None. **J. Waters:** None. **H. Zeng:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.09/Y20

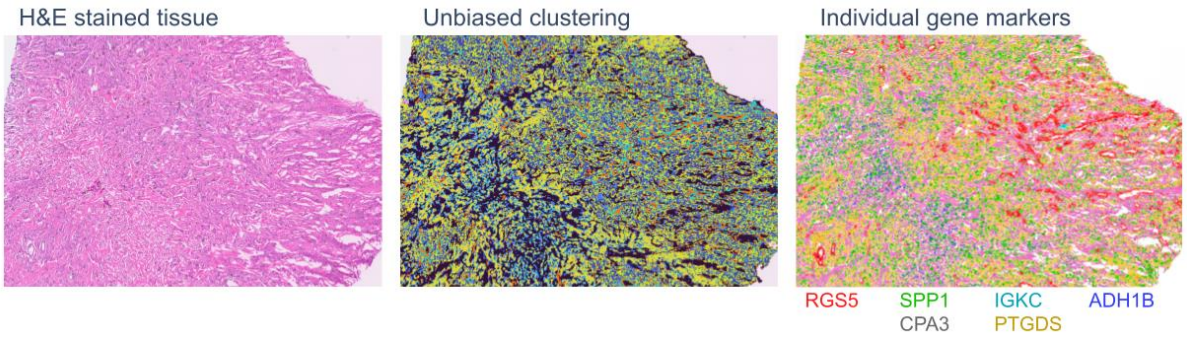
Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Visium HD enables spatially resolved, single-cell scale resolution whole transcriptome mapping of the mammalian brain

Authors: D. M. PATTERSON[1], *H. OH[2], J. COWEN[1], J. F. PERNA III[1], A. M. TENTORI[1]; [1]10x Genomics, Pleasanton, CA; [2]Mol. Biol., 10x Genomics, Pleasanton, CA

Abstract: Characterizing cellular diversity is critical to understanding how the CNS physiologically functions, develops, and organizes; as well as revealing how changes in gene expression within these populations drive pathogenesis. Recent advances in spatial transcriptomics technologies have improved our ability to map gene expression in tissue, but methods that provide single cell scale resolution of the whole transcriptome are required to properly characterize complex tissue biology. We developed the Visium HD Spatial Gene Expression assay which is a novel, high-resolution spatial technology that maps the whole transcriptome at single cell scale.
The Visium HD assay has been optimized to ensure high-resolution data, robust performance, and broad applicability. Sample preparation and tissue staining are performed with typical histology practices using FFPE or fresh frozen tissue sections on standard glass microscope slides. The assay is compatible with H&E or IF imaging to provide a multi-modal readout and enable validation of gene expression data. The spatial barcoding step happens within the controlled environment of the CytAssist instrument to ensure precise transcript localization onto the Visium HD slide.
To demonstrate the power of Visium HD in neuroscience research, we characterized gene expression of healthy mouse brain tissue and human meningioma tissue. The human meningioma sample highlights the utility of Visium HD to characterize gene expression of a clinically-relevant sample. We identified high-resolution expression patterns of key disease progression markers including NOTCH3 and THY1(tumorigenesis and cancer progression), RGS5 and ACTA2 (angiogenesis), and a range of immune markers such as IGKC and SPP1. By analyzing the spatial arrangement of cell types and these disease markers, we can explore how disease

impacts specific cell populations. This approach could reveal new understanding of disease and suggest innovative treatment strategies.



Disclosures: D.M. Patterson: A. Employment/Salary (full or part-time):; 10x Genomics. **H. Oh:** A. Employment/Salary (full or part-time):; 10x Genomics. **J. Cowen:** A. Employment/Salary (full or part-time):; 10x Genomics. **J.F. Perna:** A. Employment/Salary (full or part-time):; 10x Genomics. **A.M. Tentori:** A. Employment/Salary (full or part-time):; 10x Genomics.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.10/Y21

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: MIRA Grant

Title: Spatially resolved transcriptomic insights into whole honey bee brain with Multiplexed error-robust fluorescence in situ hybridization (MERFISH)

Authors: *Q. FENG;
Univ. of Illinois Urbana-Champaign Neurosci. Grad. Program, Champaign, IL

Abstract: Evolutionary biology and neuroscience have long studied the mechanisms of behavior variation within and between species. It is known that behavioral traits not only arise from inherited information, but social information can also affect neural wiring, leading to genomic modification and ultimately changes in brain and behavior. We used cutting-edge spatial transcriptomics technology, multiplexed error-robust fluorescence in-situ hybridization (MERFISH), to gain new insights into the molecular basis of aggressive behavior. We are using the honey bee (*Apis mellifera*) model, which has clearly distinguishable behavioral states with a combination of complex neuroanatomical architecture and a small enough overall brain size to permit whole brain imaging on single slides.Here we imaged 130 aggression-related transcripts in approximately 155,000 cells across an entire soldier brain and generated over 10

transcriptionally distinct cell clusters from 9.2 million transcripts with high spatial resolution. In addition, we showed an enrichment of dopaminergic neurons at various depths within the brain, characterized by the co-expression of three transcripts: TyHyd, VMAT, and Dat. We observed known distinctive clusters of dopaminergic neurons on both sides of the esophagus (C1) at 144 μm depth, under the lateral calyces of the MB (C3) at 352 and 448 μm, and in the lateral antennal lobes (S1) at 544 μm. Most strikingly, we discovered a novel dopaminergic neuron cluster above the central body at 448 μm, which has never been reported. This study illuminates the molecular and cellular architecture of the honeybee brain and helps us understand the cellular underpinnings of social behaviors.

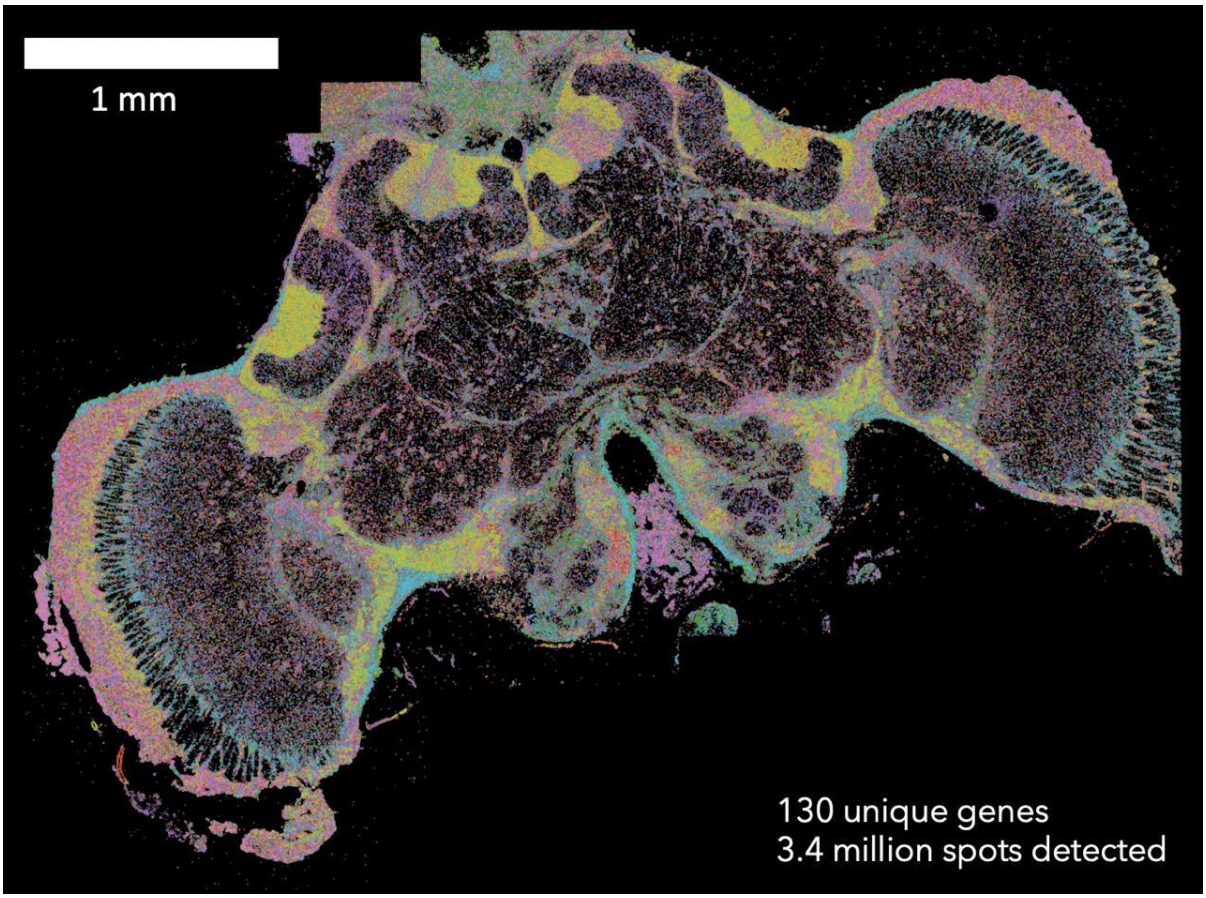


Figure 1. MERFISH data showing 130 decoded aggression genes on an expanded honey bee brain coronal section.

Disclosures: Q. Feng: None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.11/Y22

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH RF1MH128876

Title: Spatial tri-omic atlases of mouse and human brain development and neuroinflammation

Authors: *D. ZHANG[1], L. A. KIRBY[2], G. CASTELO-BRANCO[3];
[1]Yale Univ., New Haven, CT; [3]Med. Biochem. and Biophysics, [2]Karolinska Institutet, Stockholm, Sweden

Abstract: The development of the mammalian brain is a meticulously regulated process involving the genesis, differentiation, and maturation of various cellular lineages. These lineages are crucial for the complex structure and function of the brain. Spatial omics enabled the construction of spatially resolved cell atlases to better understand cellular function, interaction, physiology and pathophysiology in relation to the brain tissue architecture. Furthermore, the ability to interrogate multiple layers of the omics information allows for dissecting the underlying mechanism driving brain development, differentiation, arealization, and alterations in disease. Herein, we developed first-of-its-kind technologies for spatial tri-omics profiling of chromatin accessibility or histone modifications, mRNA expression, and ~200 proteins (spatial-ATAC-RNA-Pro-seq and spatial-CUT&Tag-RNA-Pro-seq) on the same brain tissue section at near single-cell resolution. We applied these technologies to construct the spatial tri-omic atlases of the mouse brain at different developmental stages ranging from early embryonic to junior (embryonic days 11, 12, 14, 16, 18, and postnatal days 0, 2, 5, 10, 21) in comparison with the human brains. We further dissected the spatial molecular and cellular atlases in the neuroinflammatory microenvironment of a lysolecithin mouse model (at peak and remission time points), and deciphered novel gene regulation mechanism that alter cell state, dynamics, and function in disease. We examined the spatiotemporal dynamics of mouse brains and revealed strong associations between lysolecithin mouse model and specific mouse brain cell types, and elucidated the mechanisms driving abnormal cell states that could not be readily discerned using single-modality methods. The datasets we generated could be used as a resource for understanding the developing brain function and disease for both mouse and human.

Disclosures: D. Zhang: None. **L.A. Kirby:** None. **G. Castelo-Branco:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.12/Y23

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH SBIR Grant R43-DA057749

Title: Development of an ultrasensitive transcriptomic mapping platform to study opioid-regulated circuits in neuronal tissue

Authors: J. T. ANDERSON[1], A. C. NOWLAN[2], M. OATLEY[1], M. A. CHEEK[1], M. J. MEINERS[1], Z. A. MCELLIGOTT[3], M. KEOGH[1], ***B. VENTERS**[1];
[1]EpiCypher, Durham, NC; [2]Univ. of North Carolina, Chapel Hill, NC; [3]Psychiatry; Bowles Ctr. for Alcohol Studies, Univ. of North Carolina, Chapel Hill, NC

Abstract: Acute transcriptional responses are essential for neuronal development, learning, and substance use disorders. However, current approaches to study transcription dynamics have significant limitations. mRNA-seq assays for stable/polyadenylated RNA measure total transcript abundance, but provide limited insight into early transcriptional responses. Assays for 'nascent RNAs' (*e.g.*, PRO-seq) measure early transcriptional changes but require complex workflows and high cell inputs, limiting their use in primary tissues and clinical research. To overcome these limitations, we have developed a CUT&Tag-based approach to directly and quantitatively profile chromatin-engaged RNA polymerase II (Pol II) as a proxy for nascent RNA in primary neuronal cells. In Pol II CUT&Tag, nuclei are extracted from fixed brain tissue and immobilized onto magnetic beads for streamlined handling. The addition of a phospho-specific Pol II C-Terminal Domain (CTD) antibody identifies transcriptionally-engaged polymerase and further recruits a Tn5 transposase fusion (pAG-Tn5). Localized tagmentation is followed by direct PCR amplification and library sequencing to identify genomic sites of Pol II engagement. Notably, the assay includes novel DNA-barcoded nucleosome spike-in controls bearing Pol II CTD epitopes (CTD-dNucs) for in-assay antibody validation, technical monitoring, and quantitative cross-sample comparisons. For assay development, we first screened nearly 40 commercial Pol II antibodies for specificity / efficiency via our CTD-dNuc standards. The best performing reagents were used to benchmark Pol II CUT&Tag against PRO-seq (the current gold-standard nascent RNA assay) in cultured human cells, yielding equivalent data measuring transcriptional dynamics in response to hormone treatment. Finally, optimized Poll II CUT&Tag was used to investigate transcriptional dynamics after opioid exposure. Cohorts of mice were treated with a fentanyl time-course; and brain tissue harvested, fixed and dissociated to isolate nuclei from striatal neurons. Subsequent Poll II CUT&Tag identified opioid-regulated genes and tracked their transcriptional dynamics over timed opioid exposure. Overall, Poll II CUT&Tag is a sensitive and scalable approach that will revolutionize the study of transcriptional dynamics in complex tissues, supporting substance use research and (pre)clinical development.

Disclosures: J.T. Anderson: None. **A.C. Nowlan:** None. **M. Oatley:** None. **M.A. Cheek:** None. **M.J. Meiners:** None. **Z.A. McElligott:** None. **M. Keogh:** None. **B. Venters:** A. Employment/Salary (full or part-time):; EpiCypher is a commercial developer and supplier of reagents (e.g., fully defined semi-synthetic nucleosomes) and platforms (e.g., Pol II CUT&Tag and dCypher-Luminex) used in this study. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This work was supported by NIH SBIR Grant R43-DA057749 for which AN and ZAM were subcontracted on this project. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JTA, MO, MAC, MJM, BJV and MCK own shares in EpiCypher and MCK is a board member of

same. BJV and MCK hold patent US20220042074A1, entitled “DNA-barcoded nucleosomes for chromatin mapping assays”.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

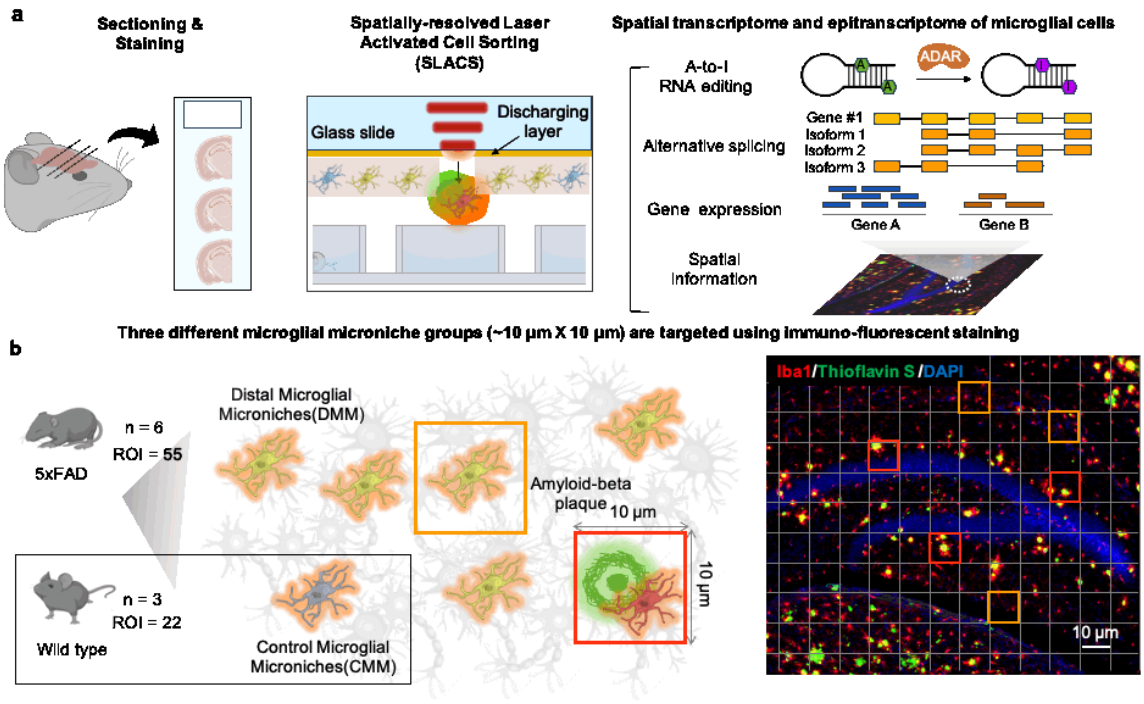
Program #/Poster #: PSTR254.13/Y24

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Title: Spatially resolved microglia interaction with amyloid beta plaque reveals different epitranscriptomic features in Alzheimer's disease

Authors: *G. KIM;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Alzheimer's disease (AD) is characterized by amyloid plaques in early pathogenesis. Early amyloid accumulation is notably associated with microglial dysfunction; these innate immune cells usually promote CNS homeostasis but exhibit impaired functions in AD. Recent research indicates that microglia adjust their gene expression in response to nearby amyloid plaques, a phenomenon involving plaque-induced genes (PIGs). These genes are thought to be critical in the microglial reaction to accumulating amyloid. However, while spatial transcriptomics has helped identify expression changes, it falls short in functional gene analysis. This gap in understanding impedes the full elucidation of AD pathogenesis and the creation of precise treatments. Our study focused on the dentate gyrus of 5xFAD mice, applying Spatially-resolved Laser Activating Cell Sorting (SLACS) to selectively dissociate this region from pathological specimens. Immunofluorescence staining differentiated amyloid plaques from microglia. We then isolated microglia near and far from plaques and conducted full-length transcriptome analysis to examine gene expression, Isoform changes, and epitranscriptomic profiles. This method enabled a detailed exploration of molecular alterations in microglia linked to the amyloid pathology within the AD-affected hippocampus. Analysis revealed that microglia proximal to plaques displayed distinct expression of known plaque-induced genes (PIGs) compared to those more distant. Furthermore, genes like plp1is differentially edited from adenosine-to-inosine (A-to-I), emphasizing the nuanced molecular variation driven by spatial plaque association. Using SELECT-seq on our mouse model, we discovered unique isoform changes and A-to-I editing in microglia-amyloid beta interactions, identifying potential biomarkers for RNA therapies like RNAi or CRISPR. These findings pave the way for applying these insights to human brain data, offering new avenues for therapeutic development against Alzheimer's disease.



Disclosures: G. Kim: None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.14/Y25

Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NINDS Grant R01NS093057

Title: Investigating cholesterol metabolism in post-stroke recovery via optogenetic neuronal stimulation

Authors: *N. RADIT[1], H. CHEN[1], H. UCHINO[2], T. C. CHIANG[3], A. KIM[4], A. G. LEE[5], M. Y. CHENG[3], G. K. STEINBERG[6];
[1]Neurosurg., Stanford Univ., Palo Alto, CA; [2]Stanford Univ., Stanford, CA; [3]Neurosurg., Stanford Univ., Stanford, CA; [4]Neurosurg., Stanford Univ., Redwood City, CA; [5]Stanford Univ., Palo Alto, CA; [6]Stanford Univ., Stanford, CA

Abstract: Post-stroke brain stimulation is a promising neurorestorative strategy, yet the underlying molecular mechanisms driving recovery remain unclear. Previously we have demonstrated that optogenetic neuronal stimulations in the ipsilesional primary motor cortex (iM1) promote functional recovery. To understand the mechanisms driving stimulation-induced

post-stroke recovery, we investigated the transcriptome of iM1 using RNA sequencing.
C57Bl6 male mice (12-14 weeks) underwent stereotaxic surgery to express Channelrhodopsin (AAV-CaMKIIa-ChR2-EYFP) in excitatory neurons in iM1, with optical fiber implanted in the same location. After 5-6 weeks, the mice underwent a transient middle cerebral artery occlusion (30 min) to induce stroke. Each mouse received one session of optogenetic stimulation daily from post-stroke days (PD)5-14. Non-stimulated mice went through identical procedures without stimulation. Rotating beam test was used to evaluate functional recovery after stroke. iM1 samples from stimulated, non-stimulated stroke mice at two timepoints (post-stroke day7 and 15) and sham mice (sham surgery without stroke) were processed for RNA sequencing (n=5 per group). Cholesterol staining dyes such as BODIPY and filipin were used to visualize cholesterol changes in the brain.
Repeated iM1 stimulations resulted in a robust recovery on the rotating beam test at PD14, with significant improvement in distance traveled and speed ($p<0.05$). RNA sequencing analysis (stimulated vs non-stimulated mice) revealed a robust difference in transcriptomic landscape between stimulated and non-stimulated stroke mice at post-stroke day 7 and 15. At PD7, a higher number of differentially expressed genes (DEGs) were detected in the iM1 of non-stimulated mice. By PD15, however, the stimulated mice exhibited a greater number of DEGs in iM1. Ingenuity pathway analysis revealed that iM1 stimulation altered multiple cholesterol biosynthesis and metabolism pathways. Interestingly, most of these cholesterol metabolism pathways were downregulated in iM1stimulated mice. Preliminary data from cholesterol staining with BODIPY showed noticeable changes in cholesterol levels in iM1 after stroke.
Our transcriptome data revealed important insights into the molecular signaling of optogenetic stimulation-induced recovery, particularly cholesterol metabolism. Ongoing studies include qPCR validation of cholesterol metabolism related genes, and co-staining of cholesterol levels with cell type specific markers (neurons, glia, microglia). These data suggest that reduction in cholesterol accumulation after stroke may be a key mechanism in post-stroke recovery.

Disclosures: N. Radit: None. **H. Chen:** None. **H. Uchino:** None. **T.C. Chiang:** None. **A. Kim:** None. **A.G. Lee:** None. **M.Y. Cheng:** None. **G.K. Steinberg:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.15/Y26

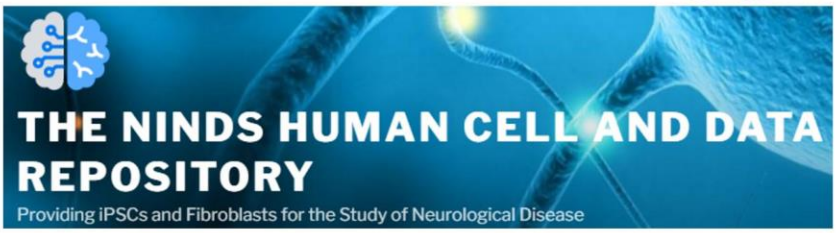
Topic: I.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant U24NS0955914
NIH Grant U24MH068457
NIH Grant 75N95023D0009
NIH Grant 75N95021F001

Title: Integrated Analytical Biobanking as a foundational component of NIH research in Neuroscience

Authors: *M. SHELDON[1], S. NAHAS[2];
[1]Scientific Affairs, SAMPLED, Piscataway, NJ; [2]SAMPLED, Piscataway, NJ

Abstract: Since 1999, SAMPLED, formerly known as RUCDR Infinite Biologics®/ Infinity BiologiX, has been awarded grants and contracts to operate the genetics resources for several agencies of the NIH, including the NIMH Repository & Genomics Resource, NIDA Center for Genetic Studies, NIDA Adolescent Brain Cognitive Development (ABCD) and HEALthy Brain and Child Development Study (HBCD) Studies and the NINDS Human Cell and Data Repository. These large-scale multinational efforts involve the collection, laboratory processing, analysis and storage of human biospecimens of many types, including blood, saliva, urine and feces, to name a few. The research projects funded by these institutions touch on numerous important areas of major public health concern, including psychiatric illnesses (e.g, Schizophrenia, Bipolar Disorder, Depression), addiction and substance abuse, neurological disorders (e.g., Parkinson's, ALS, FTD) and human neurodevelopment (ABCD and HBCD). It is therefore of utmost importance to adhere to the highest standards in the collection and processing of these precious samples that result from the participation of hundreds of thousands of affected individuals. The facilities, infrastructure, expertise and training required to support these genetic resources are considerable. To this end, over the years SAMPLED has extended the conventional model of the biorepository as a facility for the deposit, storage and withdrawal of biospecimens to establish the world's first Global Integrated Analytical Biorepository (GIAB). The GIAB offers services that support large scale research or clinical projects from end to end, from sample collection kit assembly and shipping, sample accessioning, processing, multiomic analysis, to storage and global scale distribution with fully compliant cold chain logistics. This presentation will focus on the services, biospecimens, clinical and genomic data that are available to the neuroscience community through their auspices, with particular emphasis on the NINDS and NIMH resources managed by SAMPLED.



Disclosures: M. Sheldon: None. **S. Nahas:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.16/Y27

Topic: A.08. Development of Neural Systems

Support: BICAN Developing Mouse Atlas #1U01MH130962-01

Title: Charting the Developing Mouse Brain: Towards a Comprehensive Spatial Transcriptomic Atlas

Authors: *R. MATHIEU[1], M. KUNST[1], L. CHING[1], J. QUON[1], D. MCMILLEN[1], J. CAMPOS[1], N. MARTIN[1], J. NAGRA[1], P. OLSEN[1], N. VALERA CUEVAS[1], M. VANNESS[1], A. RUIZ[2], J. ARIZA TORRES[2], S. BARTA[2], M. REDING[3], E. LIANG[3], C. HUANG[3], N. URIBE[3], O. HELBACK[3], Y. GAO[4], M. HEWITT[5], S. C. SEEMAN[5], S. DANIEL[6], L. NG[6], C. M. PAGAN[7], C. VAN VELTHOVEN[4], Z. YAO[4], J. WATERS[1], H. ZENG[8];
[1]Imaging, [2]Mol. Biol., [3]Lab. Animal Services, [4]Informatics & Data Sci., [5]Integrated Cell Physiol., [6]Data and Technol., [7]Res. Operations, [8]Brain Sci. Mgmt., Allen Inst. for Brain Sci., Seattle, WA

Abstract: Identifying the spatial distribution of distinct cell types within the developing mouse brain is essential for unraveling its remarkable complexity, elucidating the dynamic processes of cellular migration, and unveiling concurrent molecular transitions on an unprecedented scale. To initiate the establishment of a comprehensive in situ transcriptomic atlas of the entire developing mouse brain, we began collecting male and female mouse brains at various developmental stages. Leveraging MERFISH, we aimed to capture the spatial distribution of transcriptomically defined cell types. Employing state-of-the-art computational methods, both in-house and externally developed, we successfully constructed whole-brain atlases for male and female mice at different time points. These atlases were established following a series of steps: (1) cell segmentation, (2) identification of spatial domains, (3) filtering out low-quality cell based on several quality-control metrics, and (4) assigning cell-type identities by mapping the MERFISH dataset onto the Allen single-cell RNA-seq reference taxonomy. This developing whole brain atlas enables direct comparison of cell-type composition across different time points and facilitates region-by-region comparisons, capturing subtle differences over time and between sexes. Ultimately, our goal is to advance understanding of how distinct cell types are spatially allocated within the brain and the molecular mechanisms driving these processes. Continuation of brain collections at various time points will further pave the way towards achieving this objective.

Disclosures: R. Mathieu: None. **M. Kunst:** None. **L. Ching:** None. **J. Quon:** None. **D. McMillen:** None. **J. Campos:** None. **N. Martin:** None. **J. Nagra:** None. **P. Olsen:** None. **N. Valera Cuevas:** None. **M. VanNess:** None. **A. Ruiz:** None. **J. Ariza Torres:** None. **S. Barta:** None. **E. Liang:** None. **N. Uribe:** None. **O. Helback:** None. **Y. Gao:** None. **M. Hewitt:** None. **S.C. Seeman:** None. **S. Daniel:** None. **L. Ng:** None. **C.M. Pagan:** None. **C. van Velthoven:** None. **Z. Yao:** None. **J. Waters:** None. **H. Zeng:** None.

Poster

PSTR254: Genomic and Transcriptomic Techniques I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR254.17/Y28

Topic: A.08. Development of Neural Systems

Support: 1R21MH134020-01
NARSAD Young Investigator

Title: A single-cell transcriptomic atlas of Pet1(Fev) neuron development across postnatal time.

Authors: *B. W. OKATY, N. STURROCK, S. M. DYMECKI;
Genet., Harvard Med. Sch., Boston, MA

Abstract: The cell type-specific capacity to synthesize and release the monoamine neurotransmitter serotonin (5-Hydroxytryptamine; 5-HT) in the mature central nervous system is restricted to a relatively small subpopulation of brainstem neurons that express the transcription factor *Fev*, also known as *Pet1*. Our lab and others have shown that mature *Pet1*-expressing neurons can be classified into distinct molecularly-defined neuron subtypes that are phenotypically and functionally heterogeneous, displaying diverse neurochemical co-transmitter and receptor phenotypes, innervation profiles, electrophysiological properties, and anatomical distributions of cell bodies. How and when the mature molecular organization of *Pet1* neuron subtypes emerges over the course of development is poorly understood. To address these questions, we applied single-cell RNA sequencing (scRNAseq) to acutely dissociated *Pet1* neurons harvested from transgenic mice (in which neurons with a history of *Pet1* expression are fluorescently labeled) at progressive stages of postnatal development - postnatal day 0 (P0), P5, P15, and P30. We were able to link developmental single cell transcriptomes to rhombomere of origin, brainstem anatomy, and adult-defined *Pet1* neuron subtypes by integrating this data with our previously published scRNAseq studies of rhombomerically fate-mapped and anatomically microdissected *Pet1* neurons collected from P30-P90 mice (Okaty, et al. 2015; Okaty, et al. 2020). We identified thousands of differentially expressed genes, with the top two principal components correlating with developmental gene variance followed by neuron subtype-specific gene variance, respectively. Despite displaying dramatic developmental transcriptomic dynamics, we were able to reliably classify *Pet1* neurons harvested from as early as P0 mice into adult-defined *Pet1* neuron subtypes on the basis of stably expressed marker genes, suggesting that the mature subtype organization of the *Pet1* system is specified embryonically. Our *Pet1* neuron subtype-specific developmental analyses revealed that different *Pet1* neuron subtypes mature at different rates, including with respect to expression of genes related to neurotransmitter phenotype, such as *Tph2* and *Gad2*, encoding biosynthetic enzymes for 5-HT and GABA, respectively. Only a minority of *Pet1* neuron subtypes expressed mature levels of *Tph2* transcript at P0, while the majority showed significant up-regulation of *Tph2* transcripts over postnatal development, some as late as P15 and P30. We offer this data as an interactive web application for exploring the molecular underpinnings of *Pet1* neuron postnatal maturation.

Disclosures: B.W. Okaty: None. **N. Sturrock:** None. **S.M. Dymecki:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.01/Y29

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

Support: NWO-groot (175.2017.008)
Horizon Europe (101095717)
Simons Foundation (NC-GB-IBL-00002672-16)
NIH (U19NS123716)
Wellcome Trust (223144)

Title: Developing an open-source pipeline for functional ultrasound imaging

Authors: S. FLORESCU[1], A. LANDEMARD[2], M. KRUMIN[2], *M. CARANDINI[2], P. KRUIZINGA[1];
[1]Erasmus Med. Ctr., Rotterdam, Netherlands; [2]Univ. Col. London, London, United Kingdom

Abstract: Introduction. Functional ultrasound imaging (fUSi) is an emerging neuroimaging technique that measures changes in cerebral blood flow as a proxy for neural activity. The advantages of fUSi compared to fMRI include affordability, high spatiotemporal resolution and ease of use. The main components for this technique are a) an ultrasound scanner with a probe that transmits and receives thousands of frames per second, b) a powerful PC with high-speed PC-ultrasound data-link and c) software to acquire, save, and process the fUSi data in real-time. All these components can be purchased commercially, but to facilitate adoption in research labs it would be ideal if the software component was free, reconfigurable, and open source, allowing it to be tailored to specific research needs. Methods. We are therefore developing EchoFrame, an innovative open-source reconfigurable processing pipeline, facilitating the execution of data transfer, image reconstruction, and display, which are crucial for real-time fUSi. The core of the EchoFrame pipeline is implemented in C/C++ and CUDA and example Matlab code is provided for integration with a standard ultrasound scanner (Verasonics) and with a toolbox for behavioral neuroscience experiments (RigBox, Bhagat et al, 2020). For optimal performance, EchoFrame requires a CUDA-enabled GPU with at least 12 GB VRAM memory, and a fast NVMe SSD for data storage. Results. We tested an alpha version of EchoFrame in conjunction with a Verasonics acquisition system by conducting fUSi experiments in awake, head-fixed mice. We presented visual stimuli at different azimuths on three screens facing the mouse and recorded the power doppler signal using EchoFrame. We observed retinotopically-organized activations in the visual cortices and superior colliculi, and the data quality was comparable to that obtained with a commercial system. Conclusions. EchoFrame provides an open-source solution for those who want to set up fUSi in their lab to study brain wide activity through the imaging of cerebral blood flow. EchoFrame is in the final stages of development and we plan to release it soon on GitHub.

We expect it to aid in the advancement of fUSi and to empower the neuroscience community to fully exploit the potential of fUSi.

Disclosures: S. Florescu: None. **A. Landemard:** None. **M. Krumin:** None. **M. Carandini:** None. **P. Kruizinga:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.02/Y30

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

Title: Unsupervised agglomerative clustering with semi-supervised nearest centroid classification with application to face recognition using Olivetti face dataset

Authors: *H. C. YUAN[1], M. V. CHAO[2];
[1]Independent Lab., San Marino, CA; [2]Independent Lab., Rancho Palos Verdes, CA

Abstract: This poster investigates the unsupervised agglomerative clustering algorithm applied to face recognition. In machine learning, grouping together and categorizing data can be a good initial step in understanding the data to process it further for more detailed purposes as classification. When the data is unlabeled, the goal of unsupervised machine learning is to find the similarities and dissimilarities in the data and to group similar data together. For high dimensional datasets, unsupervised clustering can be useful. Siddiqui and Suman stated for computer vision that "The foremost computationally costly portion of numerous computer vision calculations comprises of looking for the most comparative matches to high dimensional vectors also referred to as closest neighbor coordinating. Having an efficient algorithm for performing expeditious most proximate neighbor matching in immensely colossal datasets bring speed amendments of several orders of magnitude to many applications". The agglomerative clustering algorithm can be applied to data needing learning algorithms to discover patterns and structures in the data. This poster applies the unsupervised agglomerative clustering algorithm to the Olivetti face dataset of 40 unique faces with 10 poses each and where each face consists of a matrix array of 4096 data points. Unsupervised agglomerative clustering starts by treating each face as a singleton cluster. Pairs of clusters are successively merged until all clusters have been merged into one large cluster containing all the face objects. At each step of the clustering algorithm, the two face clusters that are the most similar are combined into a cluster, to become a node. This process is iterated until all data are a member of a single larger cluster (root). After unsupervised agglomerative clustering, then in a semi-supervised manner, the centroid of each face cluster is computed and identified with a label. Test face samples can be classified as being the closest to a labeled centroid. Classification performance for face recognition with unsupervised agglomerative clustering and semi-supervised nearest centroid classifier is tabulated through a Monte Carlo and is found to be reasonable for the limited face dataset.

Disclosures: H.C. Yuan: None. **M.V. Chao:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.03/

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

Support: STI 2030 - Major Projects (No. 2021ZD0200402)
National Science Foundation of China (No. 82371486)

Title: Multimodal individual brain parcellation

Authors: *Y. CUI, C. LI;
Chinese Acad. of Sci. Inst. of Automation, Beijing, China

Abstract: Accurate mapping of brain functional regions at the individual level is crucial for understanding variations in brain function and behavior, identifying brain abnormalities, and developing personalized treatments for neuropsychiatric disorders. While most research focuses on a single modality of data, integrating different data modalities may provide complementary information and improve the accuracy of individual brain parcellation. We propose two novel methods for individual brain parcellation: multimodal connectivity-based individual parcellation (MCIP, Cui et al., 2024) and a deep learning pipeline for multimodal subject-specific parcellation with task contrasts synthesis (TS-AI). MCIP optimizes within-region homogeneity, spatial continuity, and similarity to a reference atlas by fusing personal rsfMRI- and dMRI-derived connectivity. As a model-free method, MCIP can be directly applied to individual subjects without training a model, and is suitable for patients with brain abnormalities or animals. TS-AI is a model-based method that captures high-order and nonlinear relationships between individual-specific information and individual parcellation. TS-AI enables the synthesis of task contrast maps without the need for actual task fMRI scans, and a feature consistency loss is designed to mitigate overfitting risks caused by the absence of ground truth for model-based individual parcellation. To assess the performance of MCIP- and TS-AI-derived individual parcellations, test-retest reliability, parcellation-based behavior prediction performance, as well as anatomical connectivity, resting-state functional, and task activation homogeneities were evaluated. Both MCIP and TS-AI demonstrated better parcellation performance compared to the multimodal individual parcellation method proposed by Glasser et al. (2016). For MCIP, comparative investigations were further conducted between humans and macaques, revealing higher topographic variability in humans than in macaques. For TS-AI, sensitivity analysis provided insights into region-specific features influencing individual variation in functional regionalization. TS-AI also identified accelerated shrinkage in the medial temporal and frontal parcels during the progression of Alzheimer’s disease. The validations of MCIP and TS-AI highlight the importance of individualized brain mapping for understanding brain function,

behavior, and neuropsychiatric disorders, offering potential applications in precision medicine, particularly personalized diagnosis and treatment.

Disclosures: Y. Cui: None. **C. Li:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.04/Web Only

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

Title: Segmenting Brain Tumors in African Patients

Authors: *M. ADEWOLE;
Med. Artificial Intelligence Lab. (MAI Lab), Lagos, Nigeria

Abstract: Glioma patients in Sub-Saharan Africa <SSA> face a dreary prognosis, with survival rates of less than two years. Limited access to imaging and advanced-stage presentation worsens the challenge. The BraTS Challenge recently introduced a dataset from SSA, characterized by poor image quality, noise, and large tumours. This study addresses the dataset's unique challenges, including labelling imbalance and limited samples, by identifying the optimal optimizer and loss function for brain tumour segmentation. Our approach, using RMSprop and Dice Loss, achieves an average Dice Score Coefficient of 0.6783, 0.8489, and 0.8568 for Tumour Core, Enhancing Tumour, and Whole Tumour, respectively. This improvement in diagnostic accuracy will help cater to the significant patient load in SSA, ultimately enhancing patient outcomes.

Disclosures: M. Adewole: None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.05/Y31

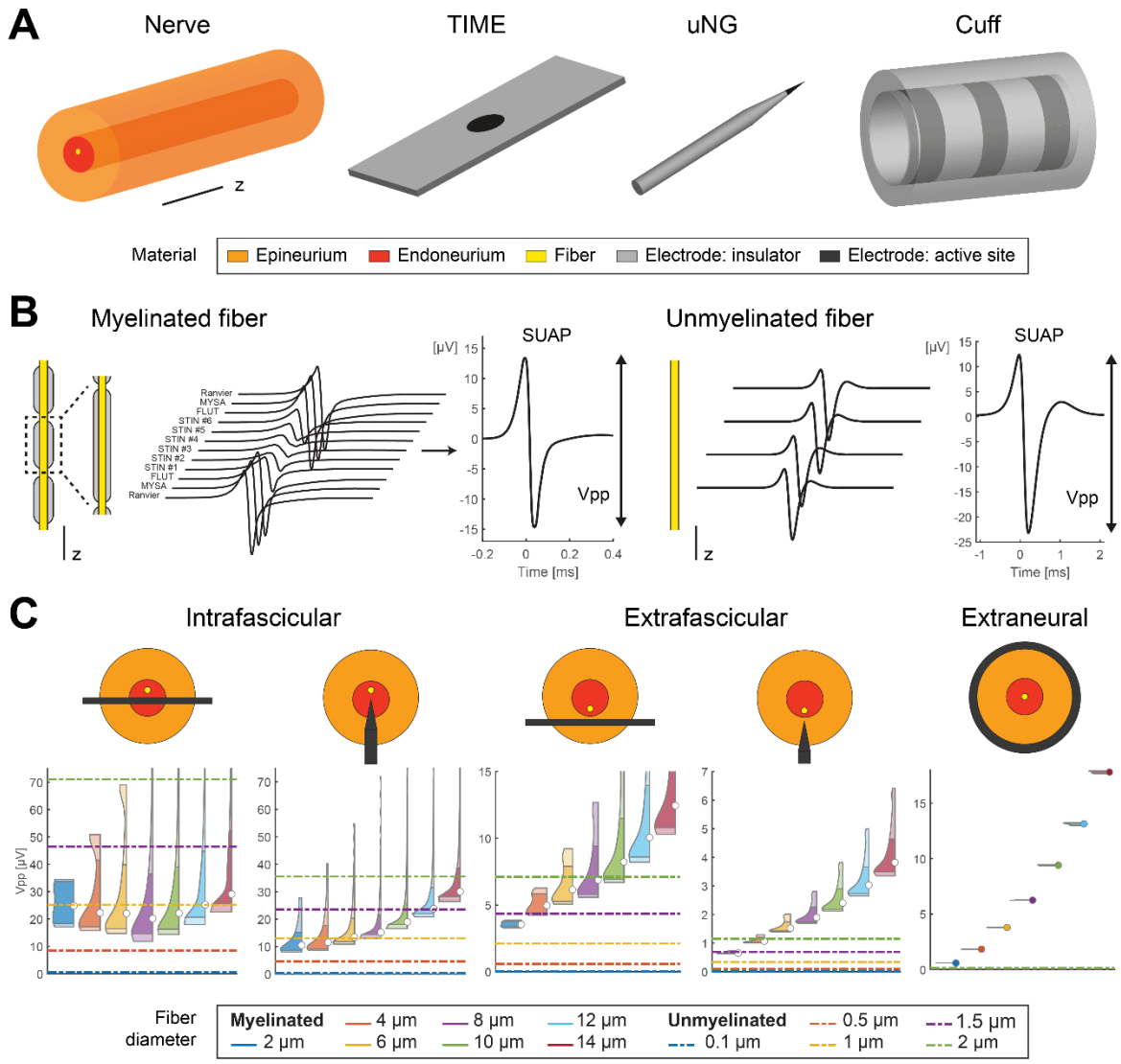
Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Project REVEAL (U54AT012307)
Bertarelli Foundation
Next Generation EU (Italy PNRR - MNESYS Project)

Title: Modeling the recording of vagal unmyelinated activity across peripheral nerve interfaces

Authors: *C. VERARDO[1], S. ROMENI[2,3], S. MICERA[2,1,3];
[1]The BioRobotics Inst. and Dept. of Excellence in Robotics and AI, Scuola Superiore Sant'Anna, Pisa, Italy; [2]Bertarelli Fndn. Chair in Translational Neural Engin., EPFL, Genève, Switzerland; [3]Modular Implantable Neuroprostheses (MINE) Lab., Univ. Vita-Salute San Raffaele & Scuola Superiore Sant'Anna, Milan, Italy

Abstract: The activity of unmyelinated fibers in the vagus nerve is linked to the functioning of the immune, metabolic, cardiovascular, and respiratory systems, making it a good candidate as a feedback signal to adapt vagus nerve stimulation (VNS) in a closed-loop fashion. While experimental and computational accounts show that cuff electrodes cannot record such activity, microneurographic (uNG) needles can record unmyelinated activity, as in the case of C-tactile afferents in somatic nerves. Whether intraneural electrodes aimed for chronic studies, such as the transverse intrafascicular multichannel electrode (TIME), may be more akin to cuff or uNG electrodes is yet an open question. To address this question, we implemented biophysical models of vagus nerve recording through a commercial cuff electrode, a TIME and a uNG needle. The hybrid modelling framework was adopted, computing fiber-wise activity in Neuron, and translating it into electrode read-out through finite element modeling in Comsol. In case of intrafascicular insertion of TIME and uNG, simulations show that the amplitudes of unmyelinated and myelinated single unit action potentials (SUAPs) are of the same order of magnitude, whereas for the extraneural insertion of the cuff simulations confirm that unmyelinated SUAPs are much smaller than myelinated ones. In the case of TIME and uNG, the amplitude separation of myelinated and unmyelinated SUAPs progressively increases with the electrode-fiber distance, and moving to extrafascicular insertion leads to an important drop of the SUAPs amplitude. Our findings support the hypothesis that the missing evidence of recordings of unmyelinated activity from intraneural electrodes is not linked to a fundamental physical limitation. Instead, other confounding factors may be at play, as surgical procedures that fail to achieve an intrafascicular insertion of the electrode, or silencing of autonomic activity due to anesthesia. The development of methods to reduce these confounding effects will be a fundamental step in formulating effective closed-loop protocols for VNS.



Disclosures: C. Verardo: None. **S. Romeni:** None. **S. Micera:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.06/Y32

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

Support: Simons Collaboration on the Global Brain
Vannevar Bush Faculty Fellowship Program of the U.S. Department of Defense

Title: Extracting latent dynamics from large-scale neural recordings through rapid training of recurrent neural network models

Authors: *F. DINC[1], U. HAPUTHANTHRI[1], A. SHAI[2], L. STORAN[1], I. D. LANDAU[3], H. TANAKA[4], M. J. SCHNITZER[5];
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Abstract: Recent advances in optical technologies allow imaging and optogenetic studies of neural activity at the scale of thousands of neurons. However, traditional approaches for modeling the dynamics of neural populations are poorly suited for capturing the dynamics of large numbers of cells. To address this gap, recent studies have adopted a dynamical systems perspective that focuses on dynamical attractors with far fewer activity dimensions than the dimensionality of the observed neural population. One approach to uncovering these attractor structures involves data-constrained recurrent neural networks (dRNNs) and aims first to replicate the observed patterns of neural activity and then to reverse engineer the learned network. However, existing algorithms for training dRNNs are inefficient and have limited scalability, making it challenging to analyze large-scale neural recordings. To address this challenge, we created a dRNN training method termed Convex Optimization of Recurrent Neural Networks (CORNN). In simulation studies with hundreds of cells, CORNN exhibited training speeds of minutes, *i.e.*, several orders-of-magnitude faster than prior RNN modeling approaches, while maintaining or enhancing modeling accuracy. We further validated CORNN on simulations with thousands of neurons, in which the target dRNNs performed tasks such as implementation of a 3-bit flip-flop or execution of a timed response. In addition, we created a non-convex (but still fast) extension for CORNN that constrains the trained dRNNs to have low-rank connection matrices. This capability facilitates the identification of low-rank latent circuits, *i.e.*, low-dimensional dynamical systems that can accurately approximate the experimentally observed neural dynamics. To showcase the applicability of these approaches to analyses of real neural recordings, we re-analyzed published Ca^{2+} imaging datasets of neocortical activity across 8 cortical regions in mice performing a visual discrimination task (Ebrahimi *et al.*, *Nature* 2022). CORNN revealed neurons' functional connectivity patterns and allowed us to identify low-dimensional neural population dynamics subserving performance of the visual task. Overall, by enabling accurate training of dRNNs within minutes of processing time, CORNN constitutes a powerful tool for experimental and computational neuroscience that will provide testable predictions about latent dynamics and facilitate selection of subsets of neurons for targeted manipulations.

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Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.07/Z1

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

Support: NIDA Grant 1R90DA060338
Whitehall Foundation research grant 2022-12-017

Title: Evaluation and signal feature informed optimization of phase detection algorithms

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Abstract: Closed-loop phase-locked neuromodulation stimulates the brain at a specific phase of neural oscillations in real time. The technology is important for manipulating neural activity for experimental neuroscience, and for the treatment of neurological disorders. Many phase detection algorithms have been developed to enable phase-locked stimulation, but we lack the tools to evaluate their performance, especially when used with diverse types of biological signals. It is unclear what factors affect performance or how these algorithms can be optimized to maximize performance.

To evaluate algorithm performance, we calculated several metrics from the stimulation events produced by the algorithms. Stimulation accuracy and precision are common metrics of stimulation quality. However, they fail to reflect whether stimulation events form a consistent and rhythmic stimulation sequence that follows the phase of ongoing rhythmic brain activity. To quantify the rhythmicity of the stimulation events, we used metrics of rhythmicity such as inter-event interval variance, normalized pairwise variability index and autocorrelation and assessed how they report stimulation quality. Using these metrics, we analyzed simulated stimulation by three algorithms - Hilbert transform (HT), endpoint-correcting Hilbert transform (ecHT) and phase mapping (PM). To investigate the effects of signal type on algorithm performance, each algorithm was used on three different rhythmic biological signals.. To identify signal features that affect stimulation quality, we quantified the signal-to-noise ratio, amplitude instability and frequency instability of each signal. Linear regression and generalized linear model were then fitted between signal features and each stimulation quality metric.

Comparing across algorithms, ecHT consistently outperformed HT and PM in all stimulation quality metrics across all signal types. The performance of HT and PM notably differed when targeting different phases. Additionally, signal type significantly affected the performance of all three algorithms. We identified signal amplitude and frequency instability as major factors in performance variations across signal types. We found that the data window used for phase estimation affected algorithm performance, and the optimal window is related to the frequency instability of the signal. This insight enables optimization of algorithm performance by matching the window size to the frequency instability of the signal. Overall, our results provide a method to evaluate and optimize the performance of phase detection algorithms for each specific phase-locked neuromodulation application.

Disclosures: M. Liufu: None. **Z.M. Leveroni:** None. **S. Shridhar:** None. **N. Zhou:** None. **J.Y. Yu:** None.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.08/Z2

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

Support: NSERC Discovery Grant RGPIN-2018-05422

Title: Axon diameter estimation in an ex vivo mouse corpus callosum using 15T diffusion MR imaging with oscillating gradient spin echo pulse sequences

Authors: *M. CHISHOLM[1], B. DHAKAL[2], E. FRIESEN[3], S. HERRERA[4], M. MERCREDI[4], J. C. GORE[2], M. MARTIN[4];
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Abstract: Previous research suggests that axon degeneration could be an early feature of various neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease. Detecting early changes in axonal structure and integrity seen across diseases has the potential for developing interventions if these changes are found to precede the appearance of disruptive symptoms. Currently, the only methods able to obtain reliable measurements of axon diameters are performed *ex vivo*, via histological analysis. Therefore, there is interest in developing an *in vivo* method to measure the diameters of axons, to assess their integrity and provide diagnostic information regarding living persons. Axon diameters can be estimated using temporal diffusion spectroscopy (TDS), which measures the diffusion of water molecules as a function of time within the tissues being imaged. Oscillating Gradient Spin Echo (OGSE) pulses sequences are used to target smaller diameter axons (1-2 μm) using shorter effective diffusion times (higher frequencies), which is necessary to target most axons that compose cortical connections, which can be as small as 0.2 μm. In this project we use TDS-OGSE combined with high frequencies and a high-field strength MRI to probe axon diameters on the order of 1-2 μm in diameter in the genu substructure of one *ex vivo* 12-week-old male C57BL/6J mouse corpus callosum, to test the technique. Perfusion fixation of one mouse brain was performed, followed by isolation of the tissues of interest. Imaging was performed using a 15.2 T Bruker BioSpec MRI System equipped with a triaxial gradient system with maximum gradient strength of 1000 mT/m. Nine diffusion weighted images with OGSE, (TR of 800 ms, TE of 50 ms, FOV of $(12\ mm)^2$, matrix size of 128 x 128, apodised cosine diffusion pulses lasting 20 ms, separated by 24.25 ms) were obtained. One Region of Interest (ROI) was drawn by hand around the genu substructure on the images, and the signals were extracted and fit to the ActiveAx model using a cylindrical geometry. The mean effective axon diameter within the ROI was determined to be 1.9 ± 0.2 um (95% confidence interval). The results suggest that the use of high frequencies combined with TDS-OGSE is capable of inferring axons on the order of 1-2 μm in size. Validation of experimental results is planned using electron microscopy (EM). Future

work focusing on optimizing imaging parameters towards better uncertainties is required. Comparisons with other methods which have identified relevant degrees of freedom affecting the measured MR signal may further improve axon diameter inferences.

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Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

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Program #/Poster #: PSTR255.09/Z3

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

Title: Re-conceptualizing brain plasticity syndrome care through large language models (LLMs) assisted natural language voice colour analysis and patient copyright licensing of plural patient data for LLM training.

Authors: *P. S. PENNEFATHER, W. SUHANIC;
gDial Inc, Toronto, ON, Canada

Abstract: Chronic neuroplasticity conditions, such as Chronic Neuroplastic Pain, are associated with those living with the condition persuaded that their embodied elementary experience of the condition signals stressful danger, no matter what behaviours they enacted, or what technologies extend their condition embodiment or the embedded systemic care they receive. That powerlessness seems to have a causal role in generating maladpative neuroplastic changes in brain biology.
gDial is developing technological solutions in a quantum-computing-ready manner to authenticate and qualify any set of mixed inductive/ deductive measures concerning systemic and personal elementary experiences. Solutions are designed to allow persons to access, own, and copyright personal data in a manner useful in annotating autonomously generated documentation of their engaged creative work associated with engaged participatory sensemaking.
We present a conceptual framework specifying a technical solution for materializing data, information and knowledge concerning sensemaking around a patient's chronic neuroplastic condition and their creative autonomous pursuit of dealing with that stressful condition. The solution materializes data, beliefs, thoughts, expressions and actions related to their condition in a manner that allows them to copyright creative work associated with their healthcare sensemaking.
Our solution builds on wide availability of large language model (LLM) transformers that will generate machine readable matrices that are also expressed in natural language predicted by those matrices. We believe that this solution can be used to normalize the wide plurality of similar but different neuroplastic conditions and support machine learning enhancement of learning system approaches to sustainable healthcare innovation.
The framework is used to structure self-authenticating copies of clinical records and patient

authored and owned analysis of their current care, in ways that can be formally licensed for ethical training of LLMs designed to assist in navigation of clinical perplexity associated with complex chronic conditions.Our approach builds on the recent discovery that machine learning driven voice interpretation and cloning is simplified by dealing with voice colour separately. The framework specifies a testable hypothesis that any patient can preform a creative and copyright-able “data-voice” specified by how they work to navigate complex care options. A secondary hypothesis is that the “data voice colour” can be characterized in a trans-disciplinary, quantum-computing-ready manner that will have economic value.

Disclosures: P.S. Pennefather: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Peter Pennefather and West Suhanic are sole controlling owners of gDial Inc set up to commercialize and license their patented IP associated with the domain that is a focus of the conceptual framework. **W. Suhanic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); See first author disclosure.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.10/Z4

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

Title: Ai closed loop optimization for vagus nerve stimulation

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Abstract: Bioelectronic therapies targeting the vagus nerve (VN) show promise in treating cardiovascular, inflammatory, and mental disorders. Since the VN extensively innervates multiple organs connected to bodily functions, VN stimulation (VNS) holds significant therapeutic potential. However, it simultaneously poses a significant challenge, because non-specific VNS causes side effects such as airway obstruction, coughing, or headache. To address these problems, technological advancements such as multi-contact electrodes and complex stimulation patterns have been designed, allowing for more flexible interventions. However, there is a cost for the increased flexibility: tuning selective and efficient multiparametric stimulations is exponentially more complex and time consuming, making comprehensive search not feasible in an in-vivo experimental setting. To this end, we developed an AI framework to

efficiently test and evaluate stimulation paradigms, electrodes and functional outcomes in anatomically accurate and functionally mapped VNS in silico models. Our framework comprises convolutional neural network (CNN) encoder/decoder architectures to quickly and efficiently predict VN responses, providing the recruitment and selectivity of the complete fiber population in a few seconds. Then, we designed a closed-loop AI optimization algorithm based on Bayesian optimization with Gaussian processes (GP-BO) to automatically and rapidly identify the best stimulation paradigms to maximize VNS therapeutic benefits and minimize side effects. We proved that GP-BO efficiently explores the VN neurostimulation space, outperforming other search strategies in a few iterations. Then, we showed that GP-BO can converge to the best set of parameters achieving target heart rate and optimizing neural B-fiber activations despite high intersubject variability. Our approach shows that a complex set of neural stimulation parameters can be optimized in real-time for optimal therapeutic benefits in VNS. By reducing the time and resources required for experimental testing, our framework facilitates a clinical translation of VNS therapies.

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Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.11/Z5

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

Support: Welch Foundation W-2057-20210327

Title: Utilizing Molecular Dynamics Simulations and Machine-Learning Enhanced Docking to Explore how Tau-fibril Disaggregating and Antioxidant Molecules Bind to and Modify Membrane-Bound Tau-Containing Oligomers

Authors: *L. Y. SEGURA[1], N. SANTOS[2], K. CHENG[3];
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Abstract: The neurotoxicity of tau fibrils induces oxidative stress and subsequent neuronal death in Alzheimer's Disease (AD). Major therapeutic approaches for AD involve compounds with tau-fibril-disaggregation and antioxidant functions. Recent evidence indicates that the smaller, less ordered membrane-bound tau-containing oligomers (mTCOs) are also neurotoxic during the early pathogenesis of AD. Additionally, hetero tau-amylin oligomers have been implicated in the cross-talk between AD and type 2 diabetes worsening pathology in individuals with both conditions. Whether the recently proposed tau-fibril-targeting compounds, epigallocatechin-3-gallate (EGCG), CNS-11, and BHT-CNS-11, also target mTCOs remains speculative. CNS-11 structurally resembles EGCG but lacks antioxidant properties, while BHT-CNS-11 has

antioxidant properties like EGCG. Using AutoDock Vina, machine learning-enhanced pose-rescoring approaches, and a Molecular Dynamics (MD) trajectory clustering approach, molecular docking of these compounds to both Cryo-EM-derived tau fibrils and different time-dependent protein conformations of in silico mTCOs from MD simulation were examined. Our mTCOs contain homogeneous tau and heterogeneous tau-amylin oligomers of different aggregation sizes, and are situated on raft-like membranes mimicking the exoplasmic and cytoplasmic areas of neuronal membranes. Like the tau fibrils, we discovered that both CNS-11 and BHT-CNS-11 consistently bind to the interchain regions of the mTCOs across different time-dependent protein conformations. In contrast, EGCG targets fibrils and mTCOs, but the binding is non-specific. Anionic phosphatidylserine and ganglioside lipids in the membranes promote the binding of compounds to mTCOs. Interestingly, our time- and residue-resolved analysis of BHT-CNS-11 binding to a dimeric tau-amylin oligomer in the presence of phosphatidylserine reveals a significant reduction of beta-sheets in tau compared to the control (no BHT-CNS-11) during the 200ns-long MD simulations. This beta-sheet dissociation result implies the potential of BHT-CNS-11 to inhibit further beta-sheet-induced aggregation of mTCOs on membrane surfaces. The combination of physics- and machine-learning-based computational approaches provides new insights into the druggability of toxic mTCOs that could be helpful for future therapeutics against AD.

Disclosures: L.Y. Segura: None. **N. Santos:** None. **K. Cheng:** None.

Poster

PSTR255: Computational Tools: Experimental

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

Support: Washington Research Foundation Postdoctoral Fellowship
Wellcome Trust
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NIH Grant U19NS123716

Title: Remote control and automation of simultaneous multi-region electrophysiology recordings

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Abstract: In vivo electrophysiology experiments, particularly those with multiple probes, face challenges in equipment management, efficiency, and reproducibility. The increasing focus on brain-wide coverage further exacerbates these challenges. To address these issues, we have developed a software platform called Pinpoint that enables remote control and automation of electrophysiology experiments. To reduce the stresses of equipment management in

electrophysiology experiments, our software enables experimenters to view and manage micromanipulators from a web application on their own devices separate from the rig. Pinpoint's modular components provide an interactive 3D visualization of the brain for experimenters to plan complex insertions, a communication platform for micromanipulators, and an automation pipeline for running experiments. During experiments with multiple probes, our remote platform handles most of the insertion process, including safeguards for preventing damage to the brain and collisions between probes. To automate the insertion process, our platform solves three challenges for experimenters: coordinating the movements of multiple probes to their entry coordinates, driving probes to their target depth at safe speeds, and coordinating the removal of multiple probes from the brain. Pinpoint takes planned insertions and automatically controls micromanipulators to maneuver the probes to their respective entry coordinates, then once through the dura, Pinpoint ensures that the probes are driven according to a set of rules designed to minimize tissue damage and improve the signal-to-noise ratio of the recording. In the future, integration of cameras and computer vision will allow our software to automatically insert probes through the dura surface, as well as detect obstructions and failed penetrations. Together, these open-source tools for remotely managing and automating multi-probe insertions enable the next generation of reproducible, high-efficiency, brain-wide electrophysiology data collection.

Disclosures: K.J. Yang: None. **D. Birman:** None. **N.A. Steinmetz:** None.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.13/Z7

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

Support: IVADO
FRQNT
NSERC

Title: Integration of expert knowledge in neurostimulation optimization

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Abstract: The efficacy of neurostimulation interventions is highly dependant on finding optimal stimulation parameter values, individually tailored to each user. Given the multitude of stimulation parameters requiring adjustment and the recent development of high-density microelectrode arrays, the number of possible combinations can easily reach the thousands or millions. Other challenges in finding optimal parameters include noise in the response evoked by

the stimulation and substantial intra-subject variability. These factors render manual testing of all potential parameter combinations prior to intervention infeasible. In recent years, there has been a growing interest in the use of Bayesian optimization (BO) to automatically identify optimal stimulation parameters by performing an intelligent search across all possible combinations. In various neurostimulation applications, BO significantly reduced the time required to find efficient parameter combinations compared to a human-performed search. BO's strength lies, in part, in its ability to outperform manual search without requiring extensive training data or explicit knowledge about the optimization problem. However, experts often have beliefs about stimulation parameter efficacy, representing a valuable source of information to help guide the search. For example, a clinician can hypothesize that although a stimulation frequency above 100Hz can evoke a desired motor response, a frequency of 130Hz is the most efficient for a majority of patients. Unfortunately, in its standard implementation, BO's ability to leverage such insights is limited. Hvarfner et al. (2022) proposed πBO, a solution that seamlessly integrates expert knowledge in BO but lacks robustness to misleading beliefs. We developed a modified version of πBO, called α-πBO, which efficiently leverages useful insights while exhibiting robustness to misleading information. We applied α-πBO to find the electrode that maximizes the electromyographic response evoked by intra-cortical stimulation on a 96-electrodes array in 4 anesthetized non-human primates. When the electrode hypothesized best was part of the 10 electrodes closest to the optimal one, α-πBO achieved an accuracy of 81 ± 3.5% after only 23 tests, compared to 71 ± 3.5% for traditional BO and 63 ± 3.6% for expert belief alone. When provided misleading information, α-πBO exhibited robustness with a marginal decline of 1.4 ± 2.9% compared to BO after 23 tests. We believe this human-AI synergy can elevate BO to unprecedented levels of performance, inspiring neuroscience experts to embrace its potential for driving new applications in the field. Funding: IVADO, FRQNT, NSERC

Disclosures: R. Guay Hottin: None. **L. Kardassevitch:** None. **G. Lajoie:** None. **M. Bonizzato:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cofounder of 12576830 Canada Inc., a startup company working on cortical stimulation and submitted a related patent application (PCT/CA2020/051047).

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.14/Z8

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

Support: Simons Center for the Social Brain at MIT
Yang-Tan Autism Center
Yang-Tan Center for Molecular Therapeutics
McGovern Institute

Title: Monkeybox: a portable and modular incage training system for macaque monkeys

Authors: *F. AZEVEDO[1], X. YANG[2], M. JIANG[3], J. SHARMA[4], M. J. LEE[5], T. INBAR[6], M. VISCONTI[7], L. WANG[8], Z. LU[9], G. FENG[4], R. DESIMONE[10];
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Abstract: Behavioral training of macaque monkeys in cognitive tasks enables researchers to investigate brain function and disorders using a model that closely approximates human cognitive processes. Traditional methods of training are labor-intensive, requiring daily transfer of animals to specialized training facilities and prolonged sessions that can last several hours and span months to years. This not only strains resources but also limits the number of animals that can be effectively studied, often leading to conclusions drawn from minimal samples that may not represent broader populations. To circumvent this problem, we developed a portable, modular, wireless, and scalable system that facilitates autonomous positive reinforcement training of monkeys in sophisticated cognitive tasks directly from their home cages, minimizing human supervision and involvement. With 8 different module configurations, the monkeys can interact with MonkeyBox using touchscreen, buttons, joysticks, levers and/or touch bars. Using our system, we were able to detect significant behavioral differences between a group of wild type macaque monkeys (n=8) and monkeys with a mutation in the gene SHANK-3 (n=7), a gene associated with autism spectrum disorder.

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Poster

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

Support: Schmidt Futures Foundation SF 857
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NSF 2134955
NIH K12GM139185
NSF 2034037
NIH R01MH120295

Title: A feedback-driven IoT microfluidic, electrophysiology, and imaging platform for brain organoid studies

Authors: *K. VOITIUK[1,2], S. T. SEILER[3,2], M. PESSOA DE MELO[4,2], J. GENG[4,2], S. HERNANDEZ[4,2], H. E. SCHWEIGER[5,2], J. L. SEVETSON[5,2], T. VAN DER MOLEN[7,8], D. F. PARKS[3,2], A. ROBBINS[4,2], S. TORRES-MONTOYA[4,2], D. EHRLICH[6,2], M. A. T. ELLIOTT[3,2], T. SHARF[3,2], D. HAUSSLER[3,2], M. A. MOSTAJO-RADJI[9], S. R. SALAMA[5,2], M. TEODORESCU[4,2];
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Abstract: The analysis of tissue cultures, particularly brain organoids, takes a high degree of coordination, measurement, and monitoring. We have developed an automated research platform enabling independent devices to achieve collaborative objectives for feedback-driven cell culture studies. Unified by an Internet of Things (IoT) architecture, our approach enables continuous, communicative interactions among various sensing and actuation devices, achieving precisely timed control of in vitro biological experiments. The framework integrates microfluidics, electrophysiology, and imaging devices to maintain cerebral cortex organoids and monitor their neuronal activity. The organoids are cultured in custom, 3D-printed chambers attached to commercial microelectrode arrays for electrophysiology monitoring. Periodic feeding is achieved using programmable microfluidic pumps. We developed computer vision fluid volume estimations of aspirated media, achieving high accuracy, and used feedback to rectify deviations in microfluidic perfusion during media feeding/aspiration cycles. We validated the system with a 7-day study of mouse cerebral cortex organoids, comparing manual and automated protocols. The automated experimental samples maintained robust neural activity throughout the experiment, comparable with the control samples. The automated system enabled hourly electrophysiology recordings that revealed dramatic temporal changes in neuron firing rates not observed in once-a-day recordings.

Disclosures: K. Voitiuk: None. **S.T. Seiler:** Other; Co-founder of OrganOmics, a company that may be affected by the research reported in the enclosed paper.. **M. Pessoa de Melo:** None. **J. Geng:** None. **S. Hernandez:** None. **H.E. Schweiger:** None. **J.L. Sevetson:** None. **T. van der Molen:** None. **D.F. Parks:** None. **A. Robbins:** None. **S. Torres-Montoya:** None. **D. Ehrlich:** None. **M.A.T. Elliott:** None. **T. Sharf:** None. **D. Haussler:** None. **M.A. Mostajo-Radji:** None. **S.R. Salama:** None. **M. Teodorescu:** None.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.16/Z10

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

Title: A closed-Loop neuroethological system for precise behavioral phenotyping in freely moving animals

Authors: ***J. LEE**[1], K. SHIN[2], D.-G. KIM[3], A. SHIN[2], Y. JEONG[2], S. PARK[4], D. KIM[2]; [1]Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of; [2]Biol. Sci., KAIST, Daejeon, Korea, Republic of; [3]ACTNOVA, Daejeon, Korea, Republic of; [4]Salk Inst. for Biol. Studies, San Diego, CA

Abstract: High-resolution markerless video techniques and AI-based analysis tools for animal behavioral studies have gained significant attention recently. However, a system capable of quantifying, classifying and detecting specific 3D action sequences in real-time for closed-loop neural manipulations has been lacking. Using 50 male C57BL/6J mice, we developed AVATAR, an AI-based system that automatically virtualizes 3D motions from the detection of 9 body parts. 2D images were acquired from 5 cameras, and a 3D reconstruction algorithm was applied to generate action skeletons at 20 fps. The system could quantify unrestricted mouse movements as 3D coordinates with a confidence interval of ±5 mm, detecting 53,397 unique motion units. We developed an LSTM neural network classifier (89.91% accuracy) that categorized exploratory behavior into 5 ethograms (walking, sniffing, rearing, immobility, grooming) using an 18,000-frame training set. AVATAR provides an integrated closed-loop neuroethological platform for high-resolution precise behavioral phenotyping of freely moving animals and real-time neural circuit manipulation triggered by specific actions. This enables mapping of behavior-neural circuit mechanisms, facilitating advancements in fields such as computational neuroethology and precision neuropsychiatry.

Disclosures: J. Lee: None. **K. Shin:** None. **D. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACTNOVA Inc.. **A. Shin:** None. **Y. Jeong:** None. **S. Park:** None. **D. Kim:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ACTNOVA Inc..

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.17/Z11

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

Support: Nemours Children's Health

Title: Validation of machine-learning based automatic diagnostic testing for cerebral palsy

Authors: *J. R. HICKS[1], K. ROBINSON[2], R. E. AKINS[2];
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Abstract: Background:Spastic Cerebral Palsy (CP), found in approximately 1 in 345 births, can severely limit quality of life by affecting neuromuscular function. The median diagnostic age is 19 months, but diagnostic confidence remains low below five years of age, and many children with CP miss an ideal interventional window before 12 months of age. New diagnostics that can better assess CP risk at an earlier age are needed. Approaches using 5-methylcytosine methylation(CpG) identification using the Illumina platform have shown promise. Previous literature shows that CpGs tend to be significantly differentially methylated between individuals affected by spastic CP and idiopathic controls in isolated peripheral blood cells, and that these differences can be used in the classification of the disease state. It is not clear if similar, potentially diagnostic DNA methylation pattern differences exist in neuromuscular tissues.
Objective:Given that Cerebral Palsy is a disorder affecting muscle, we expect that a differential CpG methylation signal will be present in muscle. This study seeks to identify the CP-specific CpG methylation signal in muscle tissue.
Methods:96 participants with spastic cerebral palsy(n=49) or idiopathic conditions(n=47) were enrolled after IRB-approved informed consent and assent. Muscle samples were collected during surgery, DNA was isolated, and Illumina MethylationEpic assays were performed. After data pre-processing, M values were calculated and filtered to identify six differentially methylated CpG loci previously identified in blood. M values were used to train and test a bootstrapped SVM model and Median F1 score is reported. Results:The 6 CpGs previously identified in whole blood samples were able to classify spastic cerebral palsy with a median F1 of 0.8182 (the harmonic mean of statistical precision and recall with 1 meaning perfect classification). This test outperformed random guessing in the classification of spastic cerebral palsy from control.
Conclusions:
DNA methylation signals identified in whole blood samples were able to classify CP muscle samples with good performance. This analysis shows that a subset of potentially diagnostic CpGs is consistent between tissues and between participants and suggest that these CpGs might be useful for broad diagnosis of spastic cerebral palsy. Future work seeks to eliminate confounding variation due to tissue heterogeneity, and to validate the CpG signal in a longitudinal study to confirm diagnostic accuracy across a wide span of ages.

Disclosures: J.R. Hicks: None. **K. Robinson:** None. **R.E. Akins:** None.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.18/Z12

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

Support: NIH DP2MH136494
FA9550-22-1-0078
FA9550-23-1-0701
NIH T32 – 5T32DC016853
DGE-1842166

Title: Artificial Brains for Artificial Intelligence: Dendritic Integration Inspired Neural Networks

Authors: *L. CACCIAPUOTI[1], H. KHAN[2], S. XIAO[2], K. JAYANT[2];
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Abstract: Artificial neural networks (ANNs) are capable of complex feature extraction and classification with applications in robotics, natural language processing, and data science. Yet, many ANNs have several key limitations; notably, current neural network architectures require enormous training datasets and are computationally inefficient. It has been posited that biophysical computations in single neurons of the brain can inspire computationally more efficient algorithms and neural network architectures. Recently, research on dendrites, including work from our lab, suggests that each biological neuron has a hidden subcellular computational power that is more complex than the conventional perceptron, providing a promising substrate for more efficient ANNs. To this end, we propose the Interconnected Dendritic Network (IDN), a new type of ANN that takes close inspiration from cellular and subcellular networks of pyramidal neurons. Each neuron in an IDN has a set of dendrites that receive inputs; these dendrites are subdivided into branches of multiple orders to mimic dendritic organization in biological neurons closely. We further employ a family of physiologically-inspired activation functions to characterize dendritic input-output transformations. Instead of discrete layers, neurons are arranged in an n-dimensional space following topographical connectivity rules, forming a recurrent network. The network comprises excitatory and inhibitory neurons acting in unison to regulate network activity. Learning happens by altering synaptic weights based on Hebbian plasticity, approximating synaptic weight distributions observed in biological networks. The IDN reached over 95% accuracy on digit classification after training on only 400 data points and utilizing 3.6% of computational operations compared to a traditional dense model performing the same task. Further, we show that such computational efficiency critically depends on the structure of the dendritic network. Thus, we present a model that performs in low-data applications, is computationally efficient, and utilizes biophysically grounded mechanisms to mitigate the limitations of current ANNs.

Disclosures: L. Cacciapuoti: None. **H. Khan:** None. **S. Xiao:** None. **K. Jayant:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.19/Z13

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

Title: Development of an advanced, interpretable, and validated, machine learning platform, for the discovery of novel acetylcholinesterase inhibitors

Authors: *N. BHATTARAI, M. K. SCHULTE;
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Abstract: The development of advanced algorithms, comprehensive exploration of molecular descriptor/feature spaces, model interpretability, and cross-species validation collectively facilitate rapid, accurate, and informed artificial intelligence-driven drug discovery. In this study, we specifically applied these approaches to the identification of novel acetylcholinesterase Inhibitors (AChEIs). Rapid identification of acetylcholinesterase inhibition can aid in the identification of new therapeutic agents as well as the identification of potential toxic effects of drug molecules. This study used four major Machine Learning (ML) variants—aggregated (ensemble + classical), AutoML (tpot), graph-based, and fine-tuned BERT large language model (LLM) ChemBERTA. All models were trained using a curated ChEMBL dataset of AChEIs, employing an IC50 activity cutoff of 1 uM for classification. Models underwent extensive cross-validation, training across 11 different feature sets, consistent hyperparameter optimization, and evaluation using six different species datasets including independent human dataset. Interpretation methods included identifying important features with LIME, fragment/atomic contributions for a prediction, and determining optimal thresholds/hyperparameters. Experimental evaluation of selected compounds will be carried out using modified Ellman's method. The study identified top performing models, evaluated by Area Under Curve of Receiver Operating Characteristics(AUC ROC) score, including ensemble extra tree classifier with rdkit features (AUC ROC=0.92) among aggregrate, AutoML (tpot) extra tree classifier with rdkit features (AUC ROC=0.92), graph convolution network (AUC ROC=0.93) among graph based model, and fine-tuned ChemBERTA(ROC AUC=0.91). Extensive validation and hyperparameter optimization demonstrated the superiority of aggregate and AutoML (tpot) models in predicting AChEIs selective for human compared to other species. Promising hits were obtained through screening FDA-approved drugs, natural products from COCONUT, and ZINC's purchasable 250k compounds, followed by docking studies. The study showed a prediction threshold of 0.8 is optimal for prediction of positives(>89% accuracy) and pinpointed important features and key structural predictors. In conclusion, the study identified robust machine learning models for rapid detection of acetylcholinesterase inhibition, particularly rdkit feature-based aggregated and AutoML models, which could effectively discriminate human AChE inhibitors from other species.

Disclosures: N. Bhattarai: None. **M.K. Schulte:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.20/Z14

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

Title: A three-dimensional eye movement measurement system using a monocular camera for fish and other animal species.

Authors: *T. UENO[1], Y. SHINJI[2], S. TADOKORO[3], T. YAMANAKA[3], Y. HIRATA[4,5,6,7]; [1]Chubu Univ., Kasugai, Japan; [2]Dept. of Computer Sci., Grad. Sch. of Engin.,, Chubu Univ., Kasugai, Japan; [3]Robotic Sci. and Technol., Chubu Univ., Kasugai, Japan; [4]Robotic Sci. and Technol., Chubu Univ. Col. of Engin., Aichi, Japan; [5]Artificial Intelligence and Robotics, Chubu University Graduate School of Engineering, Kasugai, Japan; [6]Center for Mathematical Science and Artificial Intelligence, Chubu University, Kasugai, Japan; [7]Academy of Emerging Sciences, Chubu University, Kasugai, Japan

Abstract: Various brain regions have been demonstrated to be involved in eye movement control, with different neuronal populations responsible for eye rotation around specific axes: yaw, pitch, and roll. Thus, each type of eye movement around a given rotational axis reflects the activity of distinct brain regions and neuronal populations. In this study we developed a 3D eye movement measurement system using an IR camera and AI-based feature tracking technology. We focused on goldfish eyes because they are a well-established model in neuroscience for studying oculomotor control and learning. In our experiment, an IR camera was positioned behind the fish, outside its visual field, to capture its eye reflected on a hot mirror. For an AI-based feature tracking, marker-less motion capture toolbox, DeepLabCut (DLC, Mathis et al) was employed. In DLC, we annotated 8 tracking points nearly evenly spaced outside pupil border in 19 training images. We used these 8 tracking points in each recorded image to calculate the pupil center (*P*), the eye's rotation center (*M*), the rotation radius (*R*), and a 3D vector connecting *M* and *P*. The yaw and pitch angles of the eye were measured as the yaw and pitch rotation angles of this vector. To measure the eye's roll angle, each image was transformed using a rotation matrix composed of the measured yaw and pitch angles to align the eye's roll axis with the camera axis. The average rotation angles of the 8 transformed tracking points were then calculated as the eye's roll angle. To validate the developed method, we created a realistic 3D model of a goldfish eye using Blender (Blender Animation Studio). The model was rotated sinusoidally (amplitude: 20 deg, frequency: 0.5 Hz) around each axis for 15 s, recording the eye movement as an animation. Our method was then applied to this video, and we compared the measured eye rotation angles with those given in Blender. The results showed maximum errors of 0.17, 0.06 and 0.2 deg for yaw, pitch, and roll rotations, respectively. Subsequently, we applied the method to real goldfish eye video data recorded over 15 min during 3D optokinetic response paradigms. This experiment used two orthogonally positioned IR cameras to measure 3D eye movements using Vison Development Module in LabVIEW (National Instruments) while a small marker was placed on the fish cornea to facilitate accurate object tracking. Comparisons between the results from LabVIEW using two cameras tracking the eye marker and those from our developed method using one camera showed significant improvement in the latter regarding accuracy, stability, and false rate. In principle the developed method is applicable to other animal species including humans, monkeys and mice.

Disclosures: T. Ueno: None. **Y. Shinji:** None. **S. Tadokoro:** None. **T. Yamanaka:** None. **Y. Hirata:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.21/Z15

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

Support: GYORSÍTÓSÁV-2021-00004
GINOP_PLUSZ-00143
GYORSÍTÓSÁV-2022-00064

Title: 3d acousto-optical real time motion correction for in-vivo measurements with voltage sensitive dyes

Authors: *K. ÓCSAI[1,2], P. L. FEYEN[4,6], A. JÁSZ[2], B. KERTÉSZ[3], Z. SZADAI[2,7], J. W. HERMS[8,5,9], B. ROZSA[10,2];
[1]Dept. of Mathematical Algebra and Geometry, Inst. of Mathematics, Budapest Univ. of Technol. and Econ., Budapest, Hungary; [2]BrainVisionCenter, Budapest, Hungary; [3]BrainVisionCenter, budapest, Hungary; [4]German Ctr. for Neurodegenerative Dis. (DZNE), Munich, Germany; [5]German Ctr. for Neurodegenerative Dis. (DZNE), Münich, Germany; [6]Ctr. for Neuropathology and Prion Res., Ludwig-Maximilians-University, Münich, Germany; [7]Inst. of Exptl. Med., Budapest, Hungary; [8]Neuropathology, Ludwig-Maximilians-University Munich, Munich, Germany; [9]Ctr. for Neuropathology and Prion Res., Münich, Germany; [10]Inst. Exptl. Med., Budapest, Hungary

Abstract: In-vivo measurements are inherently hampered by the motion of behaving animals, particularly in case of using voltage sensitive dyes when the signal is acquired from small regions of interest to get the maximal temporal precision. Here we present an FPGA-based, clearly acousto-optical solution 3D online motion correction for signal extraction on high temporal resolution of 100 kHz on a volume of 900x900x400 um up to 0-100 Hz of motion with a residual motion under 1 um at proper signal-to-noise ratio. The correction can be combined with all scanning modes, including point-scan, squared and ribbon-based scanning types for population and dendritic scanning, as well as raster and volume scan types (i.e. Z-Stack). Motion correction can also be combined with photostimulation to ensure precise targeting of optogenetic stimulation. Additionally, imaging and/or photostimulation can be triggered to avoid phototoxicity during long measurements when motion compensation needs to be continuous, while imaging and stimulation should be limited to the selected behaving period. The use of the closed-loop online motion compensation has been validated with recordings with voltage sensitive dyes. The use of the closed-loop online motion compensation has been validated with recordings 1) in the cortex of awake mice, via ~3kHz voltage imaging of Parvalbumin neurons using the Jedi2P sensor, and acquisition of 4D datasets (3 space + time) 2) in V1 pyramidal cells during behavioral tasks.

Disclosures: K. Ócsai: None. **P.L. Feyen:** None. **A. Jász:** None. **B. Kertész:** None. **Z. Szadai:** None. **J.W. Herms:** None. **B. Rozsa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Femtonics.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.22/Z16

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

Support: DoD Vannevar Bush Faculty Fellowship

Title: Active learning accelerates cell sorting for analyses of large-scale neural calcium imaging datasets

Authors: *Y. JIANG[1], H. AKENGIN[2], J. ZHOU[3], F. DINC[4], M. J. SCHNITZER[5];
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Abstract: Recently developed Ca^{2+} imaging methods enable concurrent recordings of ~10,000-1,000,000 neurons in behaving mammals. However, essentially all cell extractions algorithms in common usage require some degree of curation of the putative cells found in the Ca^{2+} movie data, to distinguish correctly identified cells from false-positives. Given the massive Ca^{2+} imaging datasets provided by the new imaging technologies, such cell sorting can no longer be feasibly done through human visual inspection of all cell candidates. To facilitate this critical task in Ca^{2+} imaging experiments, we introduce ActSort, a semi-supervised cell-sorting algorithm designed to accelerate the sorting process. ActSort leverages domain-expert engineered features, novel active learning paradigms, and a user-trained cell classifier to facilitate efficient sorting with minimal human feedback and computing resources. The ActSort software is optimized for use on laptop computers and has a user-friendly design. A key feature of ActSort is its use of a discriminative-confidence active learning algorithm (DCAL), which selects for human annotation candidate cells that lie near the estimated decision boundary between true- and false-positive cells and for which the cell classifier has low confidence. After a round of human annotation, ActSort re-trains the cell classifier and provides predictions for all unlabeled cell candidates, to inform the choice of further annotations or to create the final full set of automated cell labels. To benchmark ActSort, we performed extensive annotations of neurons extracted from large-scale Ca^{2+} videos. In total, four human experts annotated 40,000 candidate cells for our validation studies. We evaluated ActSort against traditional annotation strategies, in which we performed annotations that were either randomly ordered or in the ranked order provided by the cell extraction algorithm. We also assessed the capabilities of ActSort to generalize across
imaging sessions, experimental conditions, and mice other than those from which the ActSort training data were acquired. The results showed that ActSort reduces the time burden of human sorting from hours to minutes, while also increasing cell-sorting accuracy by mitigating the biases of individual annotators. Overall, ActSort requires human curation of only ~5% of putative cells extracted from a typical Ca^{2+} movie, thereby removing a major bottleneck in

systems neuroscience research by facilitating rapid pre-processing of the largest Ca^{2+} imaging datasets.

Disclosures: Y. Jiang: None. **H. Akengin:** None. **J. Zhou:** None. **F. Dinc:** None. **M.J. Schnitzer:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.23/Z17

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

Title: Maxillary Prothesis Including Fiducial Markers for Stereotactic Navigation in Humans

Authors: B. SHARAF[1], ***J. J. SHIN**[2], S. HUSSEIN[2], K. SCHEITLER[3], H. SHIN[4], K. H. LEE[2]; [1]Mayo Clin. Dept. of Surgery, Div. of Plastic Surgery, Rochester, MN; [2]Mayo Clin., Rochester, MN; [3]Mayo Clin., Rochester, MN, ; [4]Neurologic Surgery, Mayo Clin., Rochester, MN

Abstract: Maxillary Prothesis Including Fiducial Markers for Stereotactic Navigation in Humans
Jee Won Jennifer Shin[1], Sara Hussein, MD[1], Guillermo Kenji Pons Monnier, Hojin Shin, PhD, Yoonbae Oh, PhD, Megan Loghry, Nirusha Lachman, PhD, Victoria Sears, MS, Jonathan Morris, MD, Basel Sharaf, MD[2], Kendall Lee, MD, PhD[2]1.Co-first author2.Co-last authorIntroduction: An accurate alignment of the intraoperative and preoperative medical imaging data is integral to deciding the success of stereotactic surgery. There is a demand for developing stereotactic navigation that is noninvasive and affordable without compromising the accuracy. We have developed a maxillary prothesis in which the teeth can serve as the fiducials. Given the continuity of the maxilla with the skull base and the rest of the cranium, the maxillary prosthesis provides a reproducible and solid platform that improves accuracy of registration as compared with systems that use fiducial markers attached to the skin.
Method: Two dry-run experiments of stereotactic registration for accuracy measurement using the 3D printed skull with the maxillary prothesis were performed; a dental prosthesis that was designed to fit the maxillary dental pattern of the skull was scanned and printed three-dimensionally and registered using the O-arm imaging for registration using the Stealth^{TM} Navigation technology (Medtronic). Above mentioned methods were followed for the planning of and a gross total resection of a left frontal meningioma in a patient.
Result:
Accuracy from the two dry run experiments and tumor resection was 1.6 mm, 1.2mm, 1.4mm, respectively, with the prothesis alone, which was improved to 0.9mm, 0.5 mm, and 0.9mm reaching submillimetric registration accuracy after forehead tracing. After successful registration, a meningioma measuring 3cm x 2.5cm x 1.8 cm that was encasing a draining vein was resected without complications.
Discussions: This confirms a successful development and application of the maxillary prothesis

for stereotactic navigation in neurosurgery. Herein, we introduce a novel stereotactic navigation device in humans; the maxillary prosthesis can provide a platform for integrating virtual reality (VR) and/or Augmented reality (AR) applications for intracranial and craniofacial surgery procedures.

Disclosures: B. Sharaf: None. **J.J. Shin:** None. **S. Hussein:** None. **K. Scheitler:** None. **H. Shin:** A. Employment/Salary (full or part-time):; mayo clinic. **K.H. Lee:** A. Employment/Salary (full or part-time):; mayo clinic.

Poster

PSTR255: Computational Tools: Experimental

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.24/Z18

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

Support: NSF DBI 2015317

Title: Real-time, pulse-based actuation of braided pneumatic actuator muscles

Authors: *J. S. MCNEAL[1], M. ELZEIN[2], A. J. HUNT[2];
[1]Portland State Univ., Portland, OR; [2]Mechanical and Materials Engin., Portland State Univ., Portland, OR

Abstract: Braided pneumatic actuators (BPAs) have been used as proxies for muscles in robotics as their form factor and contractile activation make them attractive for biomimetics research. However, there are many challenges to overcome for successful adaptation in more systems, including real-time activation by synthetic nervous systems. This work aims to develop a methodology for activating BPAs that allows them to be directly controlled by action potentials. Our model starts with a simple neural oscillator that sends output signals to spiking neurons that we use to drive an antagonistic muscle pair on a lab robot. The neural model consists of two cross-inhibiting non-spiking neurons that form a simple central pattern generator (CPG). Each of the non-spiking neurons attaches to a spiking neuron tuned to operate at average measured frequencies of alpha motoneurons in cats. Software on the test robot interprets each spike as the onset of a square wave with variable latency and a fixed (8 ms) pulse time. The output signals drive pulses at a maximum of 50 Hz, which is the upper limit of the equipment used for testing but is on par with measured values from the literature. Although tested in a highly simplified configuration, our preliminary results support the idea that this approach is usable for an entire limb. Fast pulses with short durations not only reduced overall vibrations but also improved overall system controllability compared with longer, less frequent pulse schema. The developed setup produces CPG-driven BPA muscle activations in real time at frequencies on par with those of alpha motor neurons. Future work will explore embedding this approach in task-specific deployments like balance control.

Disclosures: J.S. McNeal: None. **M. Elzein:** None. **A.J. Hunt:** None.

Poster

PSTR255: Computational Tools: Experimental

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Program #/Poster #: PSTR255.25/Z19

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

Support: 1DP2MH129986

Title: Minicam+: an open-source multi-camera behavioral imaging platform

Authors: *Y. LIU[1,2], D. AHARONI[2];
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Abstract: Neural and behavioral imaging are the two pillars of many neuroscience experiments. While there are a variety of open-source tools such as the UCLA Miniscope to record neural data, there are limited options for open-source tools to record behavioral data. As a result, neuroscientists often resort to using either USB webcams or closed-source cameras for experimental setups. Consequently, synchronization between tools can be difficult and a considerable amount of effort often goes into post-processing of recorded videos due to lack of programmability. To address these issues, we introduce the MiniCam+, an open-source multi-camera behavioral imaging platform, as a complementary piece to freely behaving animal experiments. At the heart of MiniCam+ is an NVIDIA Jetson module that accepts up to 6 MIPI (Mobile Industry Processor Interface) CSI-2 (Camera Serial Interface) video feeds, enabling simultaneous, multi-view behavioral recording. Through a custom graphical user interface, users can configure cameras and preview live streams. The MiniCam+ platform supports real-time, hardware accelerated video encoding (H.265) and streams real-time data over ethernet for processing and closed-loop applications. Lens correction for single cameras and spatial calibration for multi-camera setup are also built into this platform. The recordings produced by the MiniCam+ can be easily fed into downstream machine learning pipelines for multi-camera pose estimation and other behavior quantification approaches.

Disclosures: Y. Liu: None. **D. Aharoni:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

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Program #/Poster #: PSTR255.26/Z20

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

Support: NRF Grant 2022M3E5E8018285

Title: Classification of Heartbeat-Related Spectral Perturbation of EEG During Interoception and Exteroception States

Authors: *E. KIM[1], W. LEE[1], J. PARK[2], H.-J. PARK[1,3,4,5];
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Abstract: Interoceptive awareness, the ability to perceive and interpret internal bodily signals like heartbeats, elicits specific brain responses detectable through electroencephalography (EEG). These responses, known as heartbeat-evoked potentials (HEP), are synchronized with the R-peaks of an electrocardiogram (ECG). A notable challenge in studying HEP is the removal of ECG artifacts, as HEP results from averaging EEG epochs timed by R-peaks, complicating selective artifact removal. To address this, our study introduces Heartbeat-Related Spectral Perturbation (HRSP), which analyzes the time-frequency characteristics of EEG signals aligned with each R-peak to identify biomarkers of interoceptive awareness. To evaluate the capacity of HRSP as a biomarker for interoceptive states, we approached the current problem regarding classification. We employed a Convolutional Neural Network (CNN) tailored for time-frequency maps of HRSP to classify different states of interoceptive awareness. Our analysis utilized a robust, publicly available dataset from 17 participants, structured in blocks alternating between attention to internal heartbeats and external white noise. This design allowed for a comprehensive examination of both interoceptive and exteroceptive states.Initial analyses were dedicated to optimizing parameters such as HRSP averaging and exploring the significance of different frequency bands. Averaging five epochs of HRSP showed a sufficient classification accuracy higher than a single epoch classification. The theta and alpha frequency bands emerged as crucial in distinguishing the two brain states, with the theta band in the central and parietal lobes proving particularly significant for identifying heightened interoceptive awareness. These findings suggest that HRSP is deeply connected with interoceptive attention rather than merely representing electrical heartbeat signals. Incorporating these features into our model achieved a classification accuracy of approximately 95% across various evaluations.Our results indicate that the neural foundations of HRSP are influenced by focused interoceptive attention and that actively attending to one's own heartbeats enhances this awareness, potentially offering new clinical applications. This study also highlights the value of HRSP in understanding the neural mechanisms underlying interoceptive brain states.

Disclosures: E. Kim: None. **W. Lee:** None. **J. Park:** None. **H. Park:** None.

Poster

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Program #/Poster #: PSTR255.27/Z21

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

Title: Stereo Investigator AI: Applying deep learning methods for automatic cell counts in a new unbiased 3D stereology software package

Authors: *J. CASTRO, A. WILSON, N. ROUSSEL, B. EASTWOOD, P. ANGSTMAN, J. GLASER;
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Abstract: The manual aspects of stereology have been a barrier to the wider utilization of design-based stereology, the gold standard for unbiased cell counting. Stereo Investigator AI combines a systematic random sampling methodology with deep learning techniques for cell identification to perform true 3D stereological analysis. This is a novel method compared to other automated cell detectors that analyze 2D images collapsed from 3D volumes, which are inaccurate and biased.
Using convolutional neuronal networks Stereo Investigator AI replicates human observer judgments in cell identification by using trained classifiers we have developed that consider cell shape, size, internal structure, and location. We have analyzed and curated extensive volumetric datasets with matching pre-trained detectors. Thanks to this large knowledge base, the platform is ready for high throughput detection of various stains such as NeuN, Iba1, and TH, and diverse experimental preparations like confocal and widefield fluorescence imaging of different brain regions. New stains and experimental preparations can be readily added with only limited training data thanks to advanced transfer learning strategies whereas detectors operating on compatible domains are selected using rigorous differential entropy scoring.
In this poster, we are showing performance metrics like precision and recall of Stereo Investigator AI when compared to an expert human annotator. Our results show that results generated by the software are unbiased, accurate, repeatable, and comparable to that of an expert human annotator, across multimodal imaging and diverse neuronal and glial populations.

Disclosures: J. Castro: A. Employment/Salary (full or part-time):; MBF Bioscience. **A. Wilson:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N. Roussel:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **J. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.28/Z22

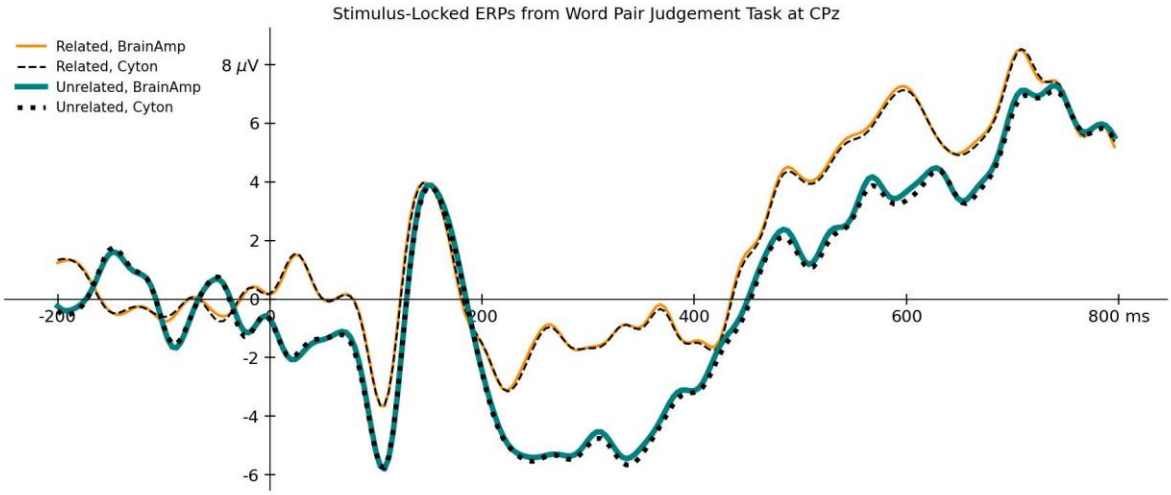
Topic: I.06. Computation, Modeling, and Simulation

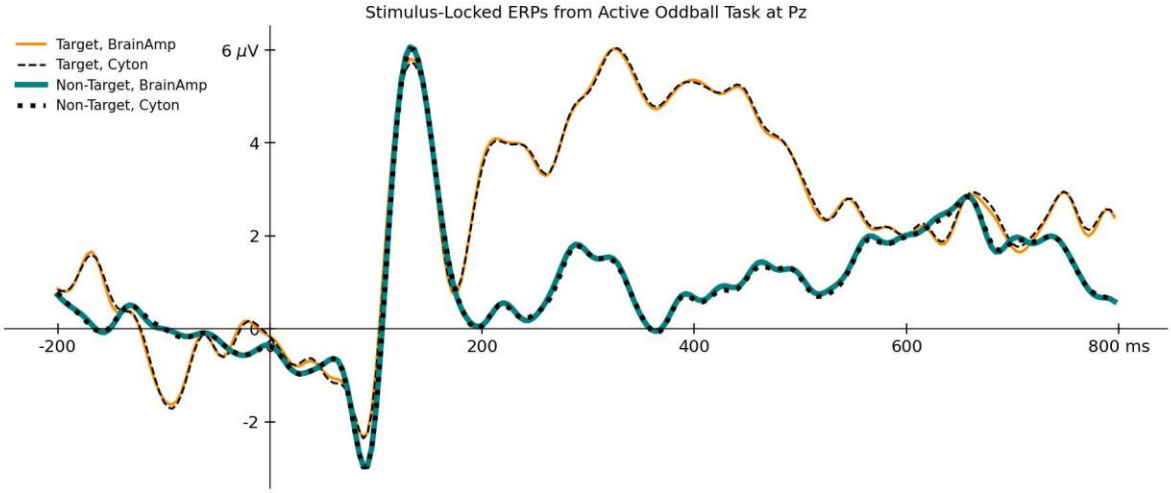
Support: HDSI DataPlanet Fellowship
UCSD Sanford Compassion and Empathy Fellowship

Title: Comparing simultaneously recorded ERPs from high and low-cost EEG systems

Authors: *A. D'AMICO;
Cognitive Sci. & Halıcıoğlu Data Sci. Inst., UC San Diego, La Jolla, CA

Abstract: EEG recordings can be prohibitively expensive for researchers. Consumer-grade amplifiers have started becoming more popular, but their quality remains relatively unknown. In this study, we compare the low-cost, consumer-grade OpenBCI Cyton to the high-cost, state-of-the-art BrainVision BrainAmp in an ERP study of an active visual oddball task and a word pair judgment task. Data were collected with both amplifiers simultaneously from the same electrodes. For the visual oddball task, there was no statistically significant difference in the target ($p > 0.9$) or non-target conditions ($p > 0.9$) when compared across amplifiers. Likewise, the word pair judgment task showed no statistically significant difference in the related ($p > 0.9$) or unrelated ($p > 0.7$) conditions. We also found that across all single-trialed waveforms, the average Pearson's correlation between the amplifiers was 0.994. We conclude that the Cyton could be a suitable low-cost replacement for traditional EEG systems, one that could lower the barrier of entry for the study of human cognition, although with caveats and limitations which we discuss.





Disclosures: A. D'Amico: A. Employment/Salary (full or part-time):; UCSD Department of Cognitive Science, UCSD HDSI, UCSD Sanford Institute for Empathy and Compassion.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.29/Z23

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

Support: NIH Grant EY014924
NIH Grant NS116623

Title: Measuring the influence of pre-stimulus bias on visual cortical coding with neurofeedback

Authors: *S. MURALIDHARAN[1], R. XIA[2], E. TREPKA[1], T. MOORE[3];
[1]Stanford Univ., Stanford, CA; [2]Neurosci., Stanford Univ., Redwood City, CA; [3]Neurobio., Howard Hughes Med. Inst. - Stanford Univ., Stanford, CA

Abstract: A major challenge of studying cognitive processes (e.g., attention and decision-making) is that there are unobservable states that must be inferred from indirect behavioral measures. A potential solution to this problem is to decode cognitive processes directly from neuronal activity, ideally in real-time (e.g., [1]). We have designed a real-time system that leverages and combines a recently developed online spike-sorting [2] and traditional threshold crossing methods with newly available high-density, high-channel count, silicon probes specifically designed for use in nonhuman primates (NHPs), namely NHP Neuropixels probes (IMEC). In this system, extracellular recordings from 100s of neurons in behaving NHPs are made with Neuropixels probes, and online spike detection or threshold crossing of individual neurons is achieved. Networking infrastructure connects a recording machine, decoding machine, and stimulus display computer to adjust stimuli in real time based on decoding of neural states. During an initial training period, an NHP performs a task while real-time threshold crossing is performed. Subsequently, a decoder (e.g. logistic regression) is trained on this activity to predict neural states in real-time. The decoded neural states are then used to modify or update behavioral conditions (e.g., adjust a visual stimulus display viewed by the NHP). We use this system to test the effect of ambient, pre-stimulus bias on neuronal responses to visual stimuli. Prior work suggests that spontaneous activity is linked to the underlying connectivity of cortical networks and shapes computations[3] Monkeys perform a fixation task with a grating drifting in one of two directions. We train a binary decoder to predict the direction of drift from neuronal responses. Next, we trigger the display of a stimulus based on above-threshold predictions of one of the binary states during the pre-stimulus activity. Thus far, we find that decoder performance and population activity is greater for trials on which the stimulus shown is congruent with the pre-stimulus bias, compared to trials on which the stimulus is incongruent.
[1] Piexoto et al. Nature 2021[2] Pachitariu M, Steinmetz N, Kadir S, Carandini M, Kenneth D. H. Kilosort: realtime spike-sorting for extracellular electrophysiology with hundreds of channels. bioRxiv. 2016:061481. doi: 10.1101/061481[3] Ringach DL. Spontaneous and driven cortical activity: implications for computation. Curr Opin Neurobiol. 2009 Aug;19(4):439-44. doi: 10.1016/j.conb.2009.07.005. Epub 2009 Aug 3. PMID: 19647992; PMCID: PMC3319344.

Disclosures: S. Muralidharan: None. **R. Xia:** None. **E. Trepka:** None. **T. Moore:** None.

Poster

PSTR255: Computational Tools: Experimental

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR255.30/Z24

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

Support: PAPIIT program: IN204023
PAPCA-2022-FESI-UNAM program
CONAHCYT CF-2023-I-654

Title: Using an artificial intelligence algorithm for detect experimental facial palsy and its neuronal correlation in antero-lateral motor cortex

Authors: *E. PERRUSQUIA[1,2], D. VILLEDA ARIAS[2], I. O. PEREZ-MARTINEZ[3];
[1]UNAM, Tepotzotlan, Mexico; [2]UNAM, Facultad de Medicina, Cdmx, Mexico; [3]Lab. of Neurobio. of Oral Sensations, Cuautitlán Mexico, Mexico

Abstract: INTRODUCTION: Facial palsy is defined as the total or partial loss of function of one or several structures innervated by the facial nerve. Its main characteristic is muscle weakening in the affected area; changes have also been observed in central nervous system (CNS), mainly in motor regions. However, the experimental study of facial paralysis in rodents has certain limitations such as the cost of the equipment, the preparation of the study subjects and the neuronal in CNS correlation with the condition. OBJECTIVE: Generate a new technique for the identification and study the facial palsy in rodents that allows overcoming existing limitations. C57BL/6 mice, with reversible and irreversible facial palsy, were used on a head-fixation system. Videorecording of whiskers and faces were used. At the same time, electrophysiological recordings were made in the antero-lateral motor cortex (motor region, ALM). RESULTS: The irreversible facial paralysis model shows a permanent loss in whisker movement. On the other hand, reversible model had an apparent recovery of movements on day 15 after injury. A facial palsy identification algorithm (FPIA) was designed with the use of artificial intelligence and the video recording of the mice's faces. FPIA allowed us to discriminate the moments in which facial paralysis is present in the reversible and irreversible model. FPIA was used to correlate ALM neuronal activity with facial palsy condition, demonstrating electrophysiological changes in this region. CONCLUSION: FPIA can identify different models of facial paralysis effectively and can be used to study the neuronal activity in CNS.

Disclosures: E. Perrusquia: None. **D. Villeda Arias:** None. **I.O. Perez-Martinez:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.01/Z25

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

Support: NSF GRFP DGE-2036197
NIH/NIMH grant DP2MH132944
NIH/NINDS grant T32NS115699

Gatsby Charitable Foundation GAT3708
NINDS grant R00NS104215

Title: Emergent developmental phenotypes of neural networks through a meta-learning inspired approach in human cerebral organoids

Authors: *C. J. LIU[1,2], J. T. WHITELEY[1,2], C. D. MAKINSON[1,2];
[1]Neurol., Columbia Univ. Irving Med. Ctr., New York, NY; [2]Neurosci., Zuckerman Inst., New York, NY

Abstract: Understanding the principles that govern the development of the complex human brain is a fundamental goal in biology. Recent advances in human pluripotent stem cell (hPSC) technologies have made hPSC-derived cell models powerful tools for understanding human brain development and for neurological disease modeling. One critical application of this technology is the generation of three-dimensional brain cell cultures, or organoids. Neural organoids can reproduce key features of the perinatal human brain and are currently the closest cellular model to native human brain tissue available. Using this system, we study the emergent dynamics of developing human organoids by leveraging a novel, meta-learning approach applied to calcium imaging data. Traditional event detection approaches require human input to specify parameters, leading to less objective event classification. Through a meta-learning inspired approach, a neural network dynamically identifies the best set of parameters to classify various event characteristics at different stages of neural development with minimal human input. Additionally, this model continuously improves selection criterion as more example data is introduced. With this computational model, we were able to identify and classify specific calcium events and track the evolution of these events across development. From this analysis, we determined that as organoids become more active but display a less diverse repertoire of event types as they continuously mature in culture which contrasts to measures of complexity extracted from multi-unit or LFP measures. Less mature networks as seen in D114-119 organoids exhibit slightly less spontaneously occurring calcium events (10.17% fewer) as compared to older ones (D248-297). Interestingly, the less mature organoids exhibit a 50% higher normalized entropy as compared to the more mature ones, indicating a greater diversity of event types in early developing networks. Through the use of a novel machine learning approach applied to calcium imaging data, these results help to elucidate the electrophysiological behaviors that develop from hPSC-derived neurons in brain organoids.

Disclosures: C.J. Liu: None. **J.T. Whiteley:** None. **C.D. Makinson:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.02/Z26

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.

Title: Mouse brain histological slice interpolation using adjacent slice-to-slice registration

Authors: *S. PILUSO[1], H. CAREY[2], C. VERASZTO[1], J. BJAALIE[2], H. MARKRAM[1], D. KELLER[1];
[1]Blue Brain Project, École Polytechnique Fédérale de Lausanne, Geneva, Switzerland; [2]Inst. of Basic Med. Sci., Univ. of Oslo, Oslo, Norway

Abstract: When studying the mouse brain, the spatial distribution of different markers can be of great interest. Whether this is the distribution of proteins, cell types, or markers of pathology, the regions and patterns in which these markers are found can reveal much about the structure and function of the brain. While methods such as lightsheet microscopy are able to image the whole brain in 3D, they are largely time consuming and costly. Most studies at present are still done by slicing the mouse brain into thin sections, before treating and imaging the sections individually. Commonly, only a subset of the sections will be labelled for a particular marker. When this subset is anchored to a reference template using a registration method such as DeepAtlas, QuickNII (RRID:SCR_016854), DeepSlice, or giRAff, the missing data leaves large gaps between the sections. Interpolation methods are therefore required to fill in the missing space between the sections. Since the interpolated data must accurately depict brain anatomy at the microscopic scale, the interpolation of these slices still represents a challenging task. Here, we propose a new interpolation method which uses the anatomy of the mouse brain to ensure that interpolated data conforms to neuroanatomical boundaries. First, we take a reference template and warp each coronal cross-section to the adjacent coronal cross-section iteratively. Performing this registration for every coronal cross-section produces a volume of deformations at the whole brain scale. When we take a sectioned histological dataset that has been anchored to the reference template, this volume of deformations allows us to interpolate data while respecting neuroanatomical boundaries, producing a smooth interpolation of brain derived data. We assessed interpolation quality on a ground truth dataset using the Normalized Mutual Information similarity criterion. Further, we started to apply this method to the *in situ* hybridization database from the Allen Institute, paving the way for the production of 3D gene expression and cell atlases in one of the most used mouse brain atlases, the common coordinate framework.

Disclosures: S. Piluso: None. **H. Carey:** None. **C. Veraszto:** None. **J. Bjaalie:** None. **H. Markram:** None. **D. Keller:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.03/Z27

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

Support: Whitehall Foundation grant
NIH K08NS109315
Larry L Hillblom Foundation Startup grant

Title: A graphical user interface that integrates real-time marker-less pose detection and experimental interventions

Authors: *J. CHEN[1], D. LIPKIN[2], N. JEONG LEE[2], A. ARAC[3];
[1]Dept. of Neurology, Dept. of Statistics and Data Sci., [3]Neurol., [2]UCLA, Los Angeles, CA

Abstract: A graphical user interface that integrates real-time marker-less pose detection and experimental interventions
Jun Yu Chen, David Lipkin, Nicolas YH Jeong Lee, Ahmet Arac
Department of Neurology, David Geffen School of Medicine, UCLA, Los Angeles, CA
In recent years, marker-less pose detection has emerged in neuroscience research as a new methodology, due to its productivity and reliability in auto-labeling behaviors. However, integrating these methods with interventions such as optogenetic manipulations that function in real-time (minimal latency) has been challenging. To address this, we developed DeepBehavior-Realtime, a comprehensive all-in-one GUI (Graphical User Interface) pipeline featuring real-time object detection, customized labeling, model training modules, and interface to control parameters for optogenetic manipulations. Utilizing the PyQt5 package, our system supports NI DAQ boards, FLIR cameras, and employs OpenCV along with TensorFlow and PyTorch for object detection. In benchmark testing, our GUI proves particularly effective in detecting specific mouse movements, such as paw actions and grasping, enabling precise optogenetic intervention based on predefined probability thresholds. The system also automatically organizes videos, regions of interest (ROI), frames, and relevant metrics into designated folders for streamlined data management. Notably, our GUI is adaptable for various experimental needs beyond our initial setup, offering an intuitive, code-free solution for the broader scientific community. It allows for the detection of any object or feature of interest with adequate training sets. The labeling and training modules are intuitive, requiring only mouse clicks to select, drag, and draw ROIs on images. Training is simplified by allowing users to select pre-trained models such as MobileNet for fine-tuning, with easy customization of training parameters. This user-friendly interface significantly enhances experimental efficiency by reducing the redundancy of manual labeling and automating the process of optogenetic intervention. The broad applicability and user-friendly design of our GUI support researchers in integrating sophisticated object detection methods into their experiments, particularly for extensive behavioral studies that require substantial post-experiment labeling and complex setups involving optogenetic manipulation. This capability is pivotal for conducting long-term and large-scale experiments efficiently, streamlining processes that traditionally involve intricate and labor-intensive configurations for the neuroscience community.

Disclosures: J. Chen: None. **A. Arac:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.04/Z28

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

Support: National Sciences Foundation (grant number 1734735)

Title: Identifying networks within an fMRI multivariate searchlight analysis

Authors: *M. SHARMA, M. N. COUTANCHE;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: There is great interest in understanding how different brain regions represent information across space and time. Information-based searchlight analyses systematically examine the information encoded within clusters of voxels across the brain. Significant searchlights from this analysis will typically contain information that can be used to decode conditions of interest, but significant discriminability can be achieved in a variety of ways. We have developed and report on a new analysis method that can identify sub-networks of searchlights. Notably, unlike methods that collapse trials by condition, such as Representational Similarity Analysis (RSA), our method uses temporal changes in information to group searchlights. We have tested this method on fMRI BOLD data collected as 20 participants viewed words, faces, shapes, and numbers. We first conducted a searchlight analysis with a 4-way Gaussian Naive Bayes (GNB) classifier. The accuracy vector from each significant searchlights was then submitted to a multi-subject Independent Component Analysis (ICA) to group searchlights based on their decoding timeseries. The ICA identified seven components of searchlights, which reflect the brain areas commonly associated with the processing of faces, words, shapes and numbers. For instance, two of the components drew strongly on the face-processing system, including the Fusiform Face Area. These results demonstrate that this method can identify sub-networks of searchlights across the brain.

Disclosures: M. Sharma: None. **M.N. Coutanche:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.05/Z29

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

Title: Robust identification of neural manifolds using pi-VAE

Authors: *Z. WU, X. WEI;
Dept. of Neurosci., Univ. of Texas at Austin, Austin, TX

Abstract: One important modern approach for understanding neural population activity is through the inference of the “neural manifold”: a low-dimensional representation that captures the essential structure of the high-dimensional response patterns. While neural manifolds are often inferred through unsupervised methods (such as PCA and fac- tor analysis), recent work proposed that semi-supervised approaches that leverage both neural activity and behavioral variables can be advantageous in extracting neural mani- folds. Two notable methods are Poisson identifiable variational auto-encoder (pi-VAE; Zhou & Wei, NeurIPS, 2020), and CEBRA (Schneider et al, Nature, 2023). It was reported that CEBRA outperformed pi-VAE in recovering the latent structure in simulated data. We have investigated this issue and found that the inferior performance of pi-VAE reported by Schneider et al (2023) was largely due to the numerical instability of pi-VAE. We then refine the pi-VAE method to achieve robust model inference by either optimization based on multiple random seeds or a modified learning objective during model fitting. Application of our refined method on synthetic dataset reveal that pi-VAE achieves stable identification of the neural manifold, and outperforms CEBRA in previously used benchmark setting.
We further apply our refined pi-VAE method to neural population recordings from macaque motor cortex during a center-out-reaching task. We found interpretable latent structures emerge automatically from fitted pi-VAE model. In the sub-space spanned by the first two latent dimensions, neural states corresponding to different reaching directions are separated and their geometrical structure resembles the geometry of the target locations. The rest of two latent dimensions represent the temporal dynamics of the neural activity in the reaching behavior, independent from the specific target directions. Importantly, the neural manifolds extracted by pi-VAE are consistent across experimental sessions and the two monkeys. Our results suggest that pi-VAE can achieve robust recovery of neural manifolds. Finally, we discuss the advantages and disadvantages of the aforementioned semi-supervised methods, and make recommendations for how to use them in practice.

Disclosures: Z. Wu: None. **X. Wei:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.06/Z30

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

Title: Spike-sorting pipeline: integrating kilosort and human curation for enhanced single-unit isolation

Authors: *S. HUANG[1], Q. XU[2], V. DINH[2], A. DUBEY[3], B. PESARAN[4];
[1]Bioengineering, [3]Ctr. For Neural Sci., [4]Neurosurg., [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Accurate identifying and characterizing spiking patterns of single neurons with high accuracy is essential to discovering the network mechanisms of the brain. Spike sorting is an

important but challenging problem in separating the activities of isolated neurons. Electrophysiology experiments traditionally performed with single or few contact probes have now been replaced with high-density probe techniques, including Neuropixels that allow dense sampling of the extracellular fields. Kilosort, an automatic spike-sorting algorithm designed specifically for Neuropixel probes, is a valuable tool for sorting large-scale spiking recordings. However, in our experience, Kilosort suffers two major drawbacks when applied to recordings in the non-human primate (NHP): 1) incorrect classification of multiunit activity and noise and 2) signal drift due to the probe slowly moving with respect to brain tissue. Here, we present a procedure and tools to enable manual curation of outputs of Kilosort applied to recording the NHP.
We recorded neural activity acutely using Neuropixel 1.0 NHP probes from the motor, posterior parietal, and prefrontal cortices of two macaque monkeys across multiple (5 to 10) days while the animals performed behavioral tasks. Each day, we obtained multiple recordings of 7 to 15 minutes each for five hours. For each recording session, Kilosort-2.5 identified approximately 300-900 putative units. Inspecting waveform and spiking statistics, we found that approximately 20% of units required manual refinement, showing the necessity of human oversight to optimize spike-sorting accuracy. We developed a GUI that enables human curation of Kilosort output based on principal component features, spike waveforms, and spiking patterns on a behavioral task. We also developed a separate merging GUI to evaluate drift across recordings and merge single-unit activity across recordings. Our work sheds light on the limitations of using Kilosort-2.5 to accurately identify single units and compensate for drift in neural recordings obtained in NHP and presents procedures for manual curation to address these limitations.

Disclosures: S. Huang: None. **Q. Xu:** None. **V. Dinh:** None. **A. Dubey:** None. **B. Pesaran:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.07/Z31

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

Support: NIH 1U19NS107613
IARPA MICrONS D16PC0003
Chan Zuckerberg Initiative 2018-183188

Title: Computational approach for barcode demixing and cell separation on BARseq images

Authors: *S. CHEN[1], A. PASARKAR[1], J. LOPER[2], M. RUE[3], A. ZHANG[3], X. CHEN[3], L. PANINSKI[1];
[1]Columbia Univ., New York, NY; [2]Univ. of Michigan, Ann Arbor, MI; [3]Allen Inst., Seattle, WA

Abstract: Mapping neuronal projections and characterizing morphology across diverse cell types at a brain-wide scale are critical for unveiling brain functions. Beyond transcriptomic profiles, these characteristics of neurons offer crucial insights into cellular heterogeneity and potential connectivity. BARseq is a high-throughput neuronal barcoding technique based on in situ sequencing, proven to effectively capture brain-wide projection patterns at single-neuron resolution and low cost.
However, two main computational challenges hinder a better characterization of neuronal morphology from these experimental images. First, neurons are labeled by random nucleotide sequences through virus infection, and thus the barcode is not known pre-experiment; we must identify this information from the data. Second, identifying the dendritic and distant axonal structures is challenging due to their lower signal intensity compared to soma.
To address these computational challenges, we present a computational method leveraging the matrix factorization approach to iteratively identify the structure of neurons from BARseq data. We first use maskR-CNN to segment somas, which provides the barcode information from these somas. The learned barcodes are then used to refine the structure of these neurons. We then identify the one-hot structure from the residual images, where signals from cells missed from the first round are present. The newly learned barcodes are merged with the initial set to optimize the reconstruction of the original image. We iterate this process until the fine structure of the neurons are captured.
Our method effectively identifies most of the barcodes present in the images, capturing the fine structures of individual neurons and axonal signals that simple segmentation approaches could not identify. Combined with the in situ gene expression profiles of these cells that can also be captured from such experiments, we can better understand the cellular heterogeneity and circuit structures underlying the brain functions and connectivity.

Disclosures: S. Chen: None. **A. Pasarkar:** None. **J. Loper:** None. **M. Rue:** None. **A. Zhang:** None. **X. Chen:** None. **L. Paninski:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.08/Z32

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

Support: U01

Title: Assessing the effects of MK-801 administration in dynamic brain connectivity using functional ultrasound imaging

Authors: *E. HAKOPIAN[1], A. E. STEPANIAN[2], W. CHOI[3], C. LIU[4], D. J. LEE[5], V. N. CHRISTOPOULOS[6];
[1]Neurosci., Univ. of California, Riverside, Burbank, CA; [2]Neurosci., Univ. of California,

Riverside, Riverside, CA; [3]Biomed. Engin., [4]Clin. Neurolog. Surgery, Urology and Surgery, [5]Neurolog. Surgery, USC, Los Angeles, CA; [6]Bioengineering, UC Riverside, Riverside, CA

Abstract: N-methyl-D-aspartate receptor (NMDAR) hypofunction has been proposed as a mechanism underlying the cognitive deficits observed in neuropsychiatric disorders such as schizophrenia. Dizocilpine (also known as MK-801) is a potent and selective NMDA antagonist and has been used to model NMDA hypofunction in rodents. Despite its widespread use as a research model, little is known about the effects of MK-801 on functional connectivity between brain regions important to memory and learning. Functional ultrasound imaging (fUSI) is an emerging neuroimaging technology that represents a new platform with high sensitivity, spatial coverage and spatiotemporal resolution. Based on power Doppler imaging, fUSI measures changes in cerebral blood volume (CBV) by detecting backscattered echoes from red blood cells moving within its field of view. Recently, we showed that acute administration of MK-801 causes a strong reduction in CBV in areas within and outside brain circuits that involve in memory and learning, including the hippocampus, medial prefrontal cortex (mPFC), thalamus, hypothalamus, pallidum, and striatum. In this study, we explore the effects of MK-801 administration on the functional connectivity between these regions. To do so, we acquired baseline CBV recordings for 5 minutes followed by an intraperitoneal injection of MK-801 (1.5 mg/kg) in a group of 8 isoflurane-anesthetized mice. Drug effects were compared to saline-vehicle injection in another group of 8 animals. By recording power Doppler activity for 45 min post injection, we aimed to understand the drug effect on the dynamic functional connectivity between the 6 regions of interest (ROI). We need to point out that the power Doppler time series from the 6 ROIs are non-stationary with strong autocorrelation structure. Thus, we first render the time series stationary and non-correlated before assessing functional connectivity. This preprocessing is called “prewhitening” and is accomplished by fitting an AutoRegressive Integrative Moving Average (ARIMA) model. The results showed that MK-801 causes a strong reduction in the dynamic functional connectivity among the 6 ROIs. These results reveal: 1) the importance of prewhitening power Doppler time series recordings before functional connectivity analysis and 2) MK-801 does not only reduce blood perfusion within and outside brain circuits that involve in learning and memory, but it also reduces the functional connectivity between them as a function of time.

Disclosures: E. Hakopian: None. **A.E. Stepanian:** None. **W. Choi:** None. **C. Liu:** None. **D.J. Lee:** None. **V.N. Christopoulos:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.09/Z33

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

Support: NIDCD-R00-DC018051
T32ES007271

Title: Component-encoding models provide interpretable and predictive models of neural computation in the human brain

Authors: *D. SKRILL[1], D. BOEBINGER[2], C. M. GARCIA[3], K. V. NOURSKI[4], M. A. HOWARD III[5], T. WYCHOWSKI[6], W. H. PILCHER[7], S. V. NORMAN-HAIGNERE[8]; [1]Univ. of Rochester Med. Ctr., Rochester, NY; [2]Univ. of Rochester, Rochester, NY; [3]Neurosurg., Univ. of Iowa, Iowa City, IA; [4]The Univ. of Iowa, Iowa City, IA; [5]Dept. of Neurosurgey, Univ. of Iowa Hosp. and Clinics, Iowa City, IA; [6]Neurol., Univ. of Rochester, Sch. of Med. and Dent., Rochester, NY; [7]Div. Neuroendocrinol, Univ. Rochester Sch. Med., Rochester, NY; [8]Biostatistics & Neurosci., Univ. of Rochester, Rochester, NY

Abstract: A central goal of sensory neuroscience is to build parsimonious computational models that can both predict neural responses to natural stimuli and reveal interpretable functional organization in the brain. Statistical “component” models can learn interpretable, low-dimensional structure across different brain regions and subjects, but cannot generate predictions for new stimuli or generalize across different experiments because they lack an explicit “encoding model” that links these components to the stimuli that drive them. To overcome these limitations, we develop "component-encoding models" (CEMs) which approximate neural responses as a weighted sum of a small number of component response dimensions, each approximated by a stimulus-computable encoding model derived from a predictive deep neural network. We show using simulations, fMRI data, and human intracranial recordings that our CEM framework can infer a small number of interpretable response dimensions across different experiments with non-overlapping stimuli and subjects (unlike standard components) while maintaining and even improving the prediction accuracy of standard encoding models.

Disclosures: D. Skrill: None. **D. Boebinger:** None. **C.M. Garcia:** None. **K.V. Nourski:** None. **M.A. Howard:** None. **T. Wychowski:** None. **W.H. Pilcher:** None. **S.V. Norman-Haignere:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.10/Z34

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

Support: R24MH114785
R01MH126684
R24MH114799

Title: Bossdb: advancing neuroscience with a robust ecosystem for storing, accessing, and analyzing high-resolution images, annotations, and connectivity data

Authors: *N. GUITTARI, D. XENES, H. MARTINEZ, J. MATELSKY, N. STOCK, T. GION, P. K. RIVLIN, E. C. JOHNSON, R. T. HIDER, Jr., W. R. GRAY RONCAL, B. A. WESTER; Johns Hopkins Applied Physics Lab., Laurel, MD

Abstract: The NIH BRAIN Initiative Informatics Program was created to establish data archives, define consensus data standards, and develop computational tools for analysis and visualization, all to support the various data communities within the neuroscience research domain. As a part of this, the Brain Observatory Storage Service and Database (BossDB) data archive and ecosystem were developed to support the storage, access, and analysis for high-resolution neuroimaging datasets, particularly those derived from Electron Microscopy (EM) and X-ray Microtomography (XRM). For BossDB and other archives, managing the complex volume of data requires the adoption of comprehensive standards for neurodata images, metadata, and annotations that facilitate the exchange of findings within and across research teams and data resources, ensuring researchers can compare and build on each other's work. In adherence to Findable, Accessible, Interoperable, and Reusable (FAIR) practices, BossDB data and tools are openly available to promote secondary analysis, enhance the reliability of findings, foster a collaborative environment, and enable comparative analyses across expanding datasets from various species and modalities. The platform has enhanced its data ingestion process by implementing scalable, containerized tools for essential data preprocessing tasks, such as downsampling and meshing, which have significantly increased data throughput and processing speeds.

Herein we present on the BossDB integration with community-developed image, metadata, and annotation standards, as well as use of community frameworks and tools such as Neuroglancer for 2D and 3D data visualization, NeuVue for proofreading and annotation, NeuPrint and DotMotif for annotation and graph querying, and the Connectome Annotation Versioning Engine (CAVE) platform that supports segmentation proofreading, links annotations to spatial coordinates, and aligns them with corresponding segments, effectively mirroring how annotations relate to metadata.

Through these development and community tool integration efforts, BossDB aims to support connectomic research at multiple parts of the data and research pipeline, from image processing to the detailed analysis of connectome networks.

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Poster

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Program #/Poster #: PSTR256.11/Z35

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

Support: Summers Grant

Title: Machine Learning-Guided Selection of Evolutionarily Conserved Cis-Regulatory Elements in Mammalian Cortex

Authors: *A. WANG[1], A. R. PFENNING[2], B. N. PHAN[3], M. LEONE[1], J. HE[4], W. R. STAUFFER[5], H. SESTILI[1];
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Abstract: Cell type-specificity is a defining characteristic of neural gene expression across species, governing functions as fundamental as cognition. As such, the importance of controlling and understanding cell-type specific activity in neurons has risen with advances in sequencing technology resolution and knowledge of neuropsychiatric disorders. Despite this, the process of screening for regulatory elements governing this specificity remains time-consuming and expensive. To address this, we develop a machine learning pipeline, SNAIL (Specific Nuclear Anchored Independent Labeling) to computationally perform this screen for cis-regulatory elements, capable of achieving cell-type specific activity in vivo.Distal regulatory elements, such as enhancers, are of particular interest in establishing gene expression patterns. More specifically, enhancer-driven expression can be targeted to cell types defined at multiple resolutions including major neuronal classes, well-defined neuronal class subtypes, or specific cortical layer subtypes. By computationally screening for enhancers with cell-type specific activity, we gain the ability to control gene expression at a high resolution, which holds implications for gene therapies and other experimental work that rely on high-resolution manipulation of neural cell populations at will.To this end, we used scRNA-Seq to identify and distinguish cell types within cortical brain regions across human, mouse, rat, and rhesus macaque monkeys. We mapped the enhancer landscape of these neuron types and subtypes using single nucleus ATAC sequencing, and used these to train our Convolutional Neural Networks that integrates genomic and epigenetic conservation across our four species to learn the underlying sequence sufficient for cell type-specific gene expression. In order to predict whether an open chromatin peak was specific to a cell type of interest versus other excitatory, inhibitory, or glial cells. In training models in 21 cortical cell types, we observed that most of our models exceeded an auROC of 0.75, auPRC of 0.7, and F1 score of 0.7. After initial screening of cortex enhancers, we considered the level of evolutionary conservation of the candidates in selecting the top 3 enhancer candidates for each cell type in order to select candidates with high machine learning scores that display evolutionary conservation, as enhancers derived from evolutionarily conserved genome sequences have been shown to target cell type-specific populations in multiple species. Preliminary results indicate cell type-specific activity as well as strong evolutionary conservation in the selected candidates.

Disclosures: A. Wang: None. **A.R. Pfenning:** A. Employment/Salary (full or part-time):; Founder of Snail Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Scientific Advisory Board of Avista Therapeutics. **B.N. Phan:** None. **M. Leone:** None. **J. He:** None. **W.R. Stauffer:** None. **H. Sestili:** None.

Poster

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Program #/Poster #: PSTR256.12/Z36

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

Support: NIH UM1MH130981

Title: Multi-level partitioning and reconstruction for building an atlas of the primate brain

Authors: *M. HUANG[1], S. C. SEEMAN[2], J. L. CLOSE[3], M. HEWITT[2], R. SANCHEZ[4], S. MUFTI[1], E. LEIN[4], L. NG[2];
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Abstract: Creating a comprehensive cellular atlas of the primate brain involves significant challenges due to the constrained imaging area available with current spatial transcriptomics technologies. To address this, we have devised a method to partition the brain into manageable sections. Specifically, we coronally sectioned the brain into 4 mm thick slabs, which were further subdivided into 1 cm² blocks aligned with anatomical boundaries. These blocks were then sliced into 10 µm sections suitable for imaging using the commercial MERSCOPE platform, supplemented by various histological stains. For each slab and block, we captured images of their respective faces. To reconstruct the original brain structure from these partitioned sections, we employed a multi-level registration approach: (1) registering spatial transcriptomic data to corresponding block face images captured after each sectioning event, (2) aligning sequential block face images across all sections, (3) registering block face images to their respective slabs, and (4) reconstructing the full brain by aligning all slabs. The resultant data will be integrated with existing reference atlases and common coordinate frameworks to facilitate comparisons across different studies and modalities. For data visualization, we utilize Neuroglancer, a WebGL-based GUI viewer that allows detailed exploration of the volumetric data.

Disclosures: M. Huang: None. **S.C. Seeman:** None. **J.L. Close:** None. **M. Hewitt:** None. **R. Sanchez:** None. **S. Mufti:** None. **E. Lein:** None. **L. Ng:** None.

Poster

PSTR256: Computational Tools: Analytical I

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Program #/Poster #: PSTR256.13/Z37

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

Support: NIH NINDS Grant 1RF1NS133972-01

Title: Enhancing device and contact placement via information capacity optimization

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Abstract: Electrode placement strategies have predominantly been guided by avoidance of vascular structures and direct targeting of regions of interest (ROIs). Optimization of ROI coverage with multi-device arrangements is a critical development to improve surgical planning and resultant data quality. In this study, Shannon-Hartley information capacity is applied to neural sources organized in a gray matter cortical structure, which has well-defined cell-type distribution and columnar orientation that directly drives the possible information patterns observable in an individual. Employing the principles of Shannon-Hartley in conjunction with a quasi-static dipole assumption, we assess the total information capacity of simulated sensors operating within a defined source space. The resultant information capacity score is intended to serve as a metric for neuroscientists and neurosurgeons to refine electrode coverage, augmenting the signal-to-noise ratio (SNR) and the diversity of detectable neural sources. The methodology encompasses the integration of patient-specific anatomical data and implant details to model the surgical plan. Segmented magnetic resonance imaging (MRI) facilitates the accurate reconstruction of the patient's ROI into simplified cortical surfaces. We apply finite element modeling (FEM) to simulate common subdural and intracortical devices, which generates lead field tensors. These sensitivity maps are utilized across various source models, incorporating parameters of cortical column diameter, current density, and ROI-weighted information density, to form quantitative comparisons of different electrode trajectories and contact patterns. Together, the simulated anatomical ROI and devices enable an adaptive search for optimal information capacity coverage. In this study, an auditory cortex ROI is investigated using stereotactic electroencephalogram (sEEG), directional and scalable array (DiSc), and electrocorticography (ECoG). Given orientation and proximity limits, a search algorithm is performed to find optimal device organization options. In considering montaging where multiple devices observe the same signal improve SNR, the relative positioning of devices becomes more important and further diversifies the parameter space. Overall, this process results in a quantitative score for proposed devices and contact placement relative to a maximum achievable score thus offering a strategic tool for improving electrophysiological data quality.

Disclosures: J.A. Willis: None. **J.P. Seymour:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.14/Z38

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

Support: National Science Foundation AI Institute in Dynamic Systems (grant number 2112085)

Title: Deep generative networks as a computational approach for global non-linear control modeling in the nematode C. elegans

Authors: *D. VOINA;
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Abstract: A long-standing premise in theoretical neuroscience holds that the complex interactions between neurons or, at a different systems level, between networks in the nervous system can be expressed in terms of an underlying system of nonlinear dynamics. A classical approach has been dynamical systems theory, whereby biophysical processes in the brain can be naturally formalized in terms of differential equations (ODEs) that may be capable of offering an interpretable account of how different biochemical and physiological processes in the brain evolve to produce the complex repertoire of network dynamics. In parallel with these traditional approaches, popular machine learning architectures with vastly more parameters (recurrent neural networks - RNNs, variational autoencoders - VAEs) have been successfully implemented to study underlying nonlinear neural dynamics despite their lack of interpretability and direct analogy to biological systems. In this study, we combine the power of deep learning methods like VAEs with the interpretability and parsimony of ODEs to study the network dynamics given by Calcium activity in the nematode C. elegans during movement. VAEs extract important features of the data and generate time series of the coefficients for a system of ODEs (including the control parameter) describing the first two principal components of the neural data. To discover the ODE, we use the SINDy (system identification for nonlinear dynamics) framework, whereby the coefficients output from the VAE are combined with a pre-selected library polynomial of basis functions, along with sparsity regularization for the coefficients, to ensure parsimony of the system. The result is an ODE with control that switches between phases of the nematode behavior and otherwise has constant coefficients throughout. The resulting parsimonious system matches important features of the data: two stable states corresponding to the forward and backward crawling behavior, along with switching trajectories that describe turning. This work describes a new method to characterize complex nonlinear dynamics with control that combines the power of deep learning tools and the interpretability of differential equations, with the potential to generalize to other organisms and behaviors.

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Poster

PSTR256: Computational Tools: Analytical I

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Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.15/Web Only

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

Support: T32 5T32HD098066-03

Title: Enhancing Understanding of Newborn Brain Imaging: A Variational Auto-Encoder Approach for Functional MRI Analysis

Authors: A. KESHWANI[1,2], *J. KIM[3], C. LIMPEROPOULOS[4];
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Abstract: Sophisticated brain representation techniques may allow us to extract more information from fMRI data presented in complex cortical space by projecting them into a more understandable representation space, potentially boosting our understanding of brain disorders. However, many conventional neuroimaging methods assume linearity in fMRI data, which has been proven to be not entirely true. Nonlinear techniques, like deep-learning methods, frequently necessitate large fMRI datasets, which are not available in the neonatal neuroimaging field and have prolonged training periods due to fMRI's complexity, i.e., 3D + 1D dimension. This study aims to address this critical gap. Recently, we proposed a novel deep-learning-based brain representation tool that projects the fMRI patterns of adults to a new data space via a VAE (Variational Autoencoder). In brief, a VAE takes noisy fMRI as input and outputs a cleansed version that retains the input's useful information. Yet, it is unknown whether the VAE model architecture optimized on adult's fMRI data can be useful in representing brain dynamics of newborns. Therefore, we investigated model performance changes over varying key VAE hyperparameters: # of latent variables (4, 8, 16, 32, 64, 128, 256, 512, and 1024), # of channels (quarter, half, and double of original model), # of convolutional layers (4, 5, and 6), and beta values (0, 0.1, 1, and 5, weights of representation power over reconstruction performance). This experiment used public newborn fMRI data from developing Human Connectome Project consisting of >200 newborns. We found that several hyperparameters that performed optimally in the adult's fMRI data also did so in the neonatal cohort. For example, beta value of 0.1 (compared to 1) and halved channels showed decreased reconstruction loss. It was also found that 256 latent variables and 5 layers were optimal with these parameters. Finally, with 10-fold cross-validated support vector machine, encoded latent variables of the optimal VAE model showed significant prediction power of their age (mean squared error <2wks) and their biological sex (accuracy =62%). In this study, we demonstrated that the VAE model that was optimized based on brain dynamics of adults was largely applicable to the brain activity of newborns. We believe this novel VAE-based brain representation technique can help other researchers who study fMRIs of pediatric populations by making their analysis more comparable to findings from older age groups such as adults. Furthermore, improving brain representation can increase the accuracy of classifying disorders and diseases like Alzheimer's and epilepsy early in the development of the brain.

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Poster

PSTR256: Computational Tools: Analytical I

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Program #/Poster #: PSTR256.16/AA1

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

Support: NS125863
5R01NS125863-03
T32NS041234

Title: Using Supercomputers and Reverse-Engineering Techniques to Create Accurate Computer Models of Human Tibialis Anterior Motoneuron Pools

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Abstract: High density-surface electromyography (HD-sEMG) allows for the ability to record from large populations of simultaneously active motoneurons (MNs) in humans via. the motor unit. Advancements in this area resulted in a vast literature that characterizes human MN firing behaviors in various conditions, muscles, and motor tasks. However, internal mechanisms underlying human motor control remain elusive due to a lack of techniques. Differences in MN firing patterns may be explained by signal integration from descending commands stemming from various unresolved sources. Variances in intrinsic properties will also fundamentally change a MN pool's firing behavior. Reverse-engineering (RE) techniques proposed by Chardon et al. (2023) may be the next step in elucidating potential mechanisms. This work attempts to RE experimentally collected HD-sEMG data collected from tibialis anterior (TA) at submaximal isometric contractions. To achieve this, computer models representing a MN pool received 12,285 combinations of excitation, inhibition, and neuromodulation. These combinations also include manipulations in afterhypolarization (AHP) time constant and voltage-threshold for persistent inward current (PIC) activation. Estimation of inputs was achieved after iterative recalibrations of excitatory input to the models' dendrites until model output (cumulative spike train (CST)) "matches" with experimentally collected CST. A good match is defined as having a mean squared error < 0.16. Nearly 5 million independent simulations were executed with most combinations achieving a good match to experimental CST. Experimental and model datasets are compared through feature extraction. These features include (de)recruitment times, activation duration, recruitment range, peak firing frequencies, duration ratios, and measures of PIC contributions (deltaF and braceheight). Our working hypothesis is that human TA motor unit firing patterns are due to shorter AHP as well as a reduced voltage threshold for PIC activation compared to animal data these models were originally designed after. So far, reducing AHP is adequate to create realistic firing output while only a narrow range of voltage thresholds produce realistic firing behaviors. Analyses are currently underway to further restrict combinations to define a good solution set for this TA model. This work is part of a larger collaborative project involving supercomputers at the Laboratory Computing Research Center at Argonne National Laboratory and at the National Energy Research Scientific Computing Center at Lawrence Berkeley National Laboratory.

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Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.17/AA2

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

Title: From bench to model: Exploring a MultiModal Markov Chain Monte Carlo (MM-MCMC) applied to Renshaw cell data from mice.

Authors: *F. L. A. PLACE[1], M. K. CHARDON[2], C. WANG[3], F. J. ALVAREZ[4], C. HECKMAN[5];
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Abstract: Ionic-based models are essential to understanding the voltages produced by neurons. The parameters of these models are, however, difficult to find because coupling multiple ion channels into a single model forms a system of ordinary differential equations with underdetermined coefficients. We have recently shown that a new parameter search technique, MM-MCMC, can quickly find solutions to these systems (Wang et al., 2022). Specifically, we have demonstrated that MM-MCMC can replicate the spiking output of a model of the stomatogastric ganglion neuron, provided by Alonso-Marder (Alonso and Marder, 2019), by varying solely the conductances of its eight ionic channels. This thus informed our hypothesis: MM-MCMC can find appropriate conductances, of ionic-based models, to reproduce laboratory data. In the present work we expand upon our previous work by testing our hypothesis on real-world data measured from the Renshaw cells of mice provided by the Alvarez laboratory at Emory University. We developed two in-silico experiments using to determine if varying conductances alone can replicate this experimental data. Each model sought to mimic the critical characteristics contained within the data over an array of applied currents. Experiment 1: We asked the experimentalist community to give us the most likely channels present in the Renshaw cell. The first iteration of the model attempted to fit the data by varying conductance for a few selected channels: general sodium, potassium delayed rectifier, potassium-based afterhyperpolarization (K-AHP) channel, and leak. Experiment 2: We implemented all channels from Channelpedia (repository of channels), expanding our parameter space to sixteen channels with Hodgkin-Huxley representations. We further varied the bounds of the search space to allow for better channel selection. Experiment 1 proved inadequate in describing the data. We presumed the addition of more channels could resolve these issues. With an improved cost function, greater conductance bounds, and a more extensive ion channel selection, experiment 2 failed as well to describe the data accurately. The results of the numerical experiments indicate

that a parameter space solely limited to conductances is inadequate to fully characterize the model. The next step in the work is to establish a model utilizing generic channels, allowing for variation in activation rate, half activation, activation slope, deactivation rate, half deactivation rate, and deactivation slope for each channel.

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Poster

PSTR256: Computational Tools: Analytical I

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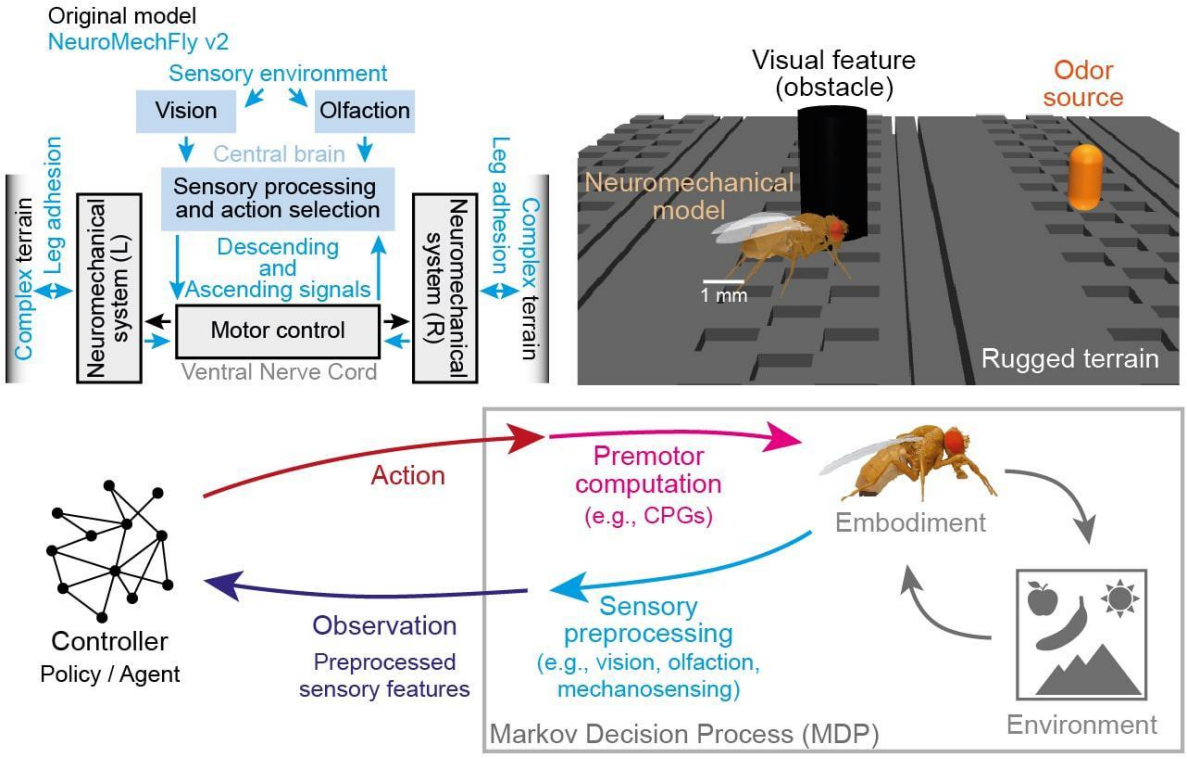
Google PhD Fellowship (PGO)

Title: NeuroMechFly v2, simulating embodied sensorimotor control in adult Drosophila

Authors: *S. WANG-CHEN, V. STIMPFLING, T. LAM, P. OZDIL, L. GENOUD, F. HURTAK, P. RAMDYA;
EPFL, Lausanne, Switzerland

Abstract: Discovering principles underlying the control of animal behavior requires a tight dialogue between experiments and computational models, particularly physics-embedded neuromechanical models. Until now, such models, including NeuroMechFly for the adult fly, *Drosophila melanogaster*, have primarily been used to investigate low-level motor control. Thus, we lack a means for studying hierarchical sensorimotor control at different levels of abstraction and across the brain and motor system. Here, we present NeuroMechFly v2, a framework that expands *Drosophila* modeling by enabling visual and olfactory sensing, ascending motor feedback, and complex terrains that can be navigated using leg adhesion. To illustrate its capabilities, we construct a biologically-inspired walking controller to perform path integration (i.e., estimating one's position based on idiothetic cues) and head stabilization with ascending motor feedback. Then, we use reinforcement learning to train an end-to-end hierarchical controller with vision, olfaction, descending commands, ascending motor feedback, and low-level motor control to perform a navigation task in closed loop. Finally, we show how one can incorporate greater biorealism by interfacing NeuroMechFly with other simulating systems. We start by implementing a *Drosophila* odor-taxis strategy to follow a complex odor plume, obtained using a fluid dynamics simulator. Similarly, we couple NeuroMechFly with a

connectome-constrained neural network model of the fly visual system to infer activities of visual neurons as the fly moves in space, and to perform vision-guided fly following. With this framework, NeuroMechFly can be used to accelerate the discovery of explanatory models of the nervous system and to develop machine learning models for autonomous artificial agents and robots. It also serves as an educational resource for embodied sensorimotor control and bio-inspired robotics. We release our simulation framework as the FlyGym package, accompanied by detailed documentation and tutorials, available at https://neuromechfly.org.



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Poster

PSTR256: Computational Tools: Analytical I

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

Support: SNSF Project Grant 175667
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CONACYT 709993

Title: Mechanisms of goal-directed reaching in Drosophila

Authors: *T. LAM, F. HURTAK, V. LOBATO RIOS, P. RAMDYA;
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Abstract: The study of motor control encompasses cyclically repeating actions—walking and grooming—as well as more static goal-directed behaviors like reaching. Reaching is important for many behaviors across species including object grasping, active sensing of the environment, and social interactions. It encompasses several processing stages including the use of sensory organs to identify a target, descending motor commands to steer the reaching limb, and finally motor execution. Although progress is being made in interpreting the roles of various brain areas in mammalian reaching, a complete understanding of the full sensorimotor loop encompassing sensing, descending commands, motor control, and ascending motor feedback, remains largely inaccessible in those systems. By contrast, the adult fly, *Drosophila melanogaster* offers an opportunity for holistic studies of motor behaviors: it has a small, genetically accessible brain for which connectomes are becoming available.
Previously described as "fending" or "warding off" in the context of courtship, we and others have observed that stationary "observer" female flies reach their middle leg toward other "target" females moving nearby. This reaching follows the expected path of the target fly demonstrating that it is a complex, goal-directed motor behavior requiring the full feedback loop rather than a simple reflexive action. We are investigating the kinematic strategy of reaching, testing its reliance on olfaction and visual features of the "target" fly. As well, we have designed a robotic system that enables the automated presentation of dummy flies to an "observer" fly to systematically examine important sensory features that drive and guide reaching behaviors.
As well, we aim to identify neural substrates for reaching movements. We have designed a high-throughput system for measuring reaching simultaneously across dozens of animals while silencing specific neurons and brain regions. To visualize neural dynamics during reaching, we have also designed a two-photon imaging setup that allows us to tether the observer fly while allowing the target fly to walk freely through an arena. Using this system, we have confirmed that tethered observer flies also generate reaching behaviors. This experimental framework will allow us to define, from sensing to motor behavior, the neural basis for a goal-directed reaching behavior, advancing our understanding of biological motor control and potentially informing the design of artificial and robotic control.

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Poster

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

Support: NIH/NIGMS GM068524
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Title: Cannabidiol treatment induces broadband spectral electrophysiological changes in boys with low-functioning autism spectrum disorder

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Abstract: Oral cannabidiol (CBD) treatment has been suggested to alleviate negative symptoms of autism spectrum disorder (ASD). While many CBD preparations have been studied in randomized clinical trials involving ASD, none have used preparations approved by the U.S. Food and Drug Administration, nor have they focused on low-functioning children with ASD. Previous studies have identified several candidate electrophysiological biomarkers for the cognitive and behavioral disabilities in ASD, with one emerging biomarker being aperiodic neural activity. Here we examined whether periodic and/or aperiodic electroencephalography (EEG) features are predictive of any symptomatic changes in ASD following pharmacological CBD intervention. To do this, we leveraged resting-state EEG from children with low-functioning ASD (27 boys, aged 7-14 years) using a double-blind, placebo-controlled, crossover Phase III Clinical Trial study. Participants were given 8 weeks of oral administration of FDA-approved CBD (Epidiolex®, 20 mg/kg/day). Using linear mixed effect models, we found that normalized CBD blood plasma levels had little association with any of the cognitive behavioral outcome measures. In contrast, we found that EEG activity did vary with CBD blood plasma levels, evidenced by a “flatter” aperiodic exponent and increased aperiodic-adjusted beta (12-30 Hz) power. Our results suggest that this CBD preparation and administration schedule does not exert clinical benefits related to cognitive behavioral symptoms in a low-functioning ASD children population. However, it may nonetheless increase broadband electrical brain activity in a manner typically observed during healthy neurodevelopment. Our findings support the use of resting-state, aperiodic activity measures as a candidate biomarker to track the clinical impact of CBD treatment on brain electrical activity within a neurodevelopmental context.

Disclosures: C. Cazares: None. **A. Hutton:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.21/AA6

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

Title: Assessing electrocardiogram waveform interference in simulated electroencephalogram signals

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Abstract: Neural electrophysiological signals, such as those recorded using non-invasive electroencephalography (EEG), contain both periodic and aperiodic components. Aperiodic activity is captured in the power spectrum from the spectral exponent. This aperiodic feature has been robustly correlated with human aging, demonstrating spectral flattening across the lifespan. A parallel line of research has also demonstrated age-related changes in the spectral exponent of cardiac signals as captured by the electrocardiogram (ECG). Critically, cardiac artifacts are common in neural EEG signals. This has called into question if age-related changes in aperiodic EEG activity are driven by true neural activity or by contamination from the heart ECG signal. Additionally, the extent to which cardiac ECG signals influence neural aperiodic activity and the degree to which beat-to-beat cardiac signals shape neural aperiodic patterns remain unclear. Here, we present an expansion of neurodsp (an open-source Python tool that simulates electrophysiological activity) to simulate ECG time series. To achieve bio-realistic simulations, we employ a novel ECG parameterization algorithm that analyzes and quantifies waveform shape features of each cardiac component (P, Q, R, S, and T waves) from actual ECG data. Using these parameters, we recreate a simulated age-specific ECG. These bio-realistic ECGs are then integrated into aperiodic neural time series generated by neurodsp. By manipulating the detailed waveform features in the simulated ECG to reflect age-related changes, such as the amplitude of the R-wave or the sharpness of the T-wave, we can explore how varying these ECG characteristics affects the simulated neural power spectra.

Disclosures: M.C. Fitzgerald: None. **E.L. Kosik:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.22/AA7

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

Support: R00MH126161

Title: Causal Role of Aperiodic Neural Activity in Human Working Memory

Authors: *Q. VAN ENGEN[1], B. VOYTEK[2], J. RIDDLE[3];
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Abstract: Working memory is a core cognitive function in which information is actively maintained in mind for a brief period of time. It is characterized by its fidelity as well as its limited capacity. The vast majority of research relating electrical brain activity to working memory processes have focused on narrow frequency bands of oscillatory activity. These oscillations arise when neuronal signals are synchronized. Such synchrony is the exception,

however; most neuronal activity manifests as asynchronous, aperiodic activity that is often treated as noise. Aperiodic activity is characterized in the frequency domain as having higher power in the low-frequency range, compared to the high-frequency range (referred to as a 1/f power distribution). Relative changes in power over frequencies are quantified by the slope of this distribution. Instead of it being treated as noise, recent analytical advances have revealed that aperiodic activity is systematically altered in several diseases, and dynamically changes with cognitive demands such as sustained attention, working memory, and mathematical learning. While there is growing physiological evidence that aperiodic activity at least partially captures the ratio of local neuronal excitatory and inhibitory inputs through the slope, this has yet to be casually tested in humans. Here, we introduce a novel, noninvasive neurostimulation protocol: transcranial random aperiodic stimulation (tRAS). Leveraging excitation / inhibition theories of working memory maintenance — and using a randomized, double-blind, placebo-controlled, crossover neurostimulation protocol — we causally modify aperiodic neural activity in order to test its functional role in human visual working memory fidelity. Specifically, a delayed continuous report task is used in combination with a color wheel response. The working memory load is individually manipulated by determining individual capacities before stimulation is applied. Three tRAS conditions are created by manipulating the slope of the decay in power to be steeper or flatter than the average endogenous aperiodic exponent. We hypothesize that baseline aperiodic slope influences the efficacy of tRAS, and its subsequent influence on working memory capacity. These results help us understand the complex neural dynamics underlying working memory.

Disclosures: Q. van Engen: None. **B. Voytek:** None. **J. Riddle:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.23/AA8

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

Title: Oscillatory Eigenvectors

Authors: *R. HAMMONDS[1], B. VOYTEK[2];
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Abstract: Neural signals from multi-electrode arrays or multiple experimental trials are often analyzed using principal component analysis (PCA). Of particular interest are cases when eigenvectors or principal components are oscillatory, indicating a circulant covariance matrix. In such cases, the eigenvalues or eigenspectrum are equivalent to a single power spectral density (PSD). This work a) provides a measure of how circulant a matrix is and b) given a circulant matrix, demonstrates eigenspectra use cases.A circulant matrix is confirmed by ensuring the covariance matrix is diagonalizable given Fourier modes as eigenvectors. This measure takes the sum of squares along the diagonal of putative eigenvalues, divided by the sum of all squares.

After the covariance matrix is confirmed to be circulant, the eigenspectra may be decomposed, clustered, or filtered, and inverted back to signal space via singular value decomposition (SVD).Eigenspectra may be interpreted through spectral parametrization, e.g., disentangling periodic and aperiodic components, which is often performed on averaged spectra. Relatedly, denoising of the input signals may be performed by keeping only the top-k eigenvalues, known as low-rank approximations, or by clustering subsets of eigenvalues. These methods involve decomposing eigenspectra or removing low variance eigenvalues. Furthermore, manipulated or decomposed eigenspectra may be inverted back to the signal space by using eigenvalues and eigenvectors in singular value decomposition (SVD). The circulant measure and approach is validated in simulations and then applied to local field potentials from neuropixels recordings of a visual coding task, publicly available from the Allen institute.

Disclosures: R. Hammonds: None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.24/AA9

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

Title: Distinct time-domain modifications to alpha bursting account for alpha power changes in working memory

Authors: *Y. LEE[1], A. BENDER[2], B. VOYTEK[1,3,4,5];
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Abstract: Synchronous bursts of neural activity in the frequency range of 8-12 Hz manifest as alpha oscillations in the human visual cortex. These alpha oscillations play a role in visual information processing such as working memory maintenance and attentional control. Computational modeling has suggested that alpha has two modes: (1) strong and sustained oscillatory activity that suppresses excitatory gain and (2) weak and short bursts of oscillatory activity that increases gain (Peterson & Voytek, 2017). Different modifications of alpha activity can lead to decreases in alpha power in the frequency domain, and previous studies of alpha power fail to examine which modification of alpha activity best accounts for alpha power reductions in working memory. By parameterizing alpha oscillatory activity in the time domain, our goal is to ascertain whether reductions in burst duration, burst probability, or burst amplitude drive the differences in alpha power observed in visual working memory. We use an open dataset of brain activity recorded with electroencephalography (EEG) from 45 participants performing a working memory color change detection task. Adam and colleagues previously showed decreased alpha power in contralateral electrodes relative to that in ipsilateral electrodes (Adam,

Robison, & Vogel, 2018). We use the bycycle package (Cole & Voytek, 2019) to analyze alpha waveform shape in the time domain. We hypothesized that these contralateral alpha power reductions reflect dominance of the strong, sustained oscillatory alpha mode to protect the visual information held in working memory. Thus, we predicted that both alpha burst length and alpha burst amplitude decrease in contralateral electrodes relative to ipsilateral electrodes. Alpha burst amplitude was significantly lower in contralateral electrodes than ipsilateral electrodes (W=85, g=-0.354, p=0.016), while alpha burst length was not significantly different between contralateral and ipsilateral electrodes (W=132, g=-0.347, p=0.271). While these results are inconsistent with a parsimonious framing of excitatory and inhibitory alpha modes, they highlight how alpha power changes can be accounted for by distinct changes in alpha waveforms from the time domain. Furthermore, determining which alpha waveform changes underpin alpha power changes is vital to identifying the underlying neurophysiological mechanisms in cognition and their aberrations across disease states.

Disclosures: Y. Lee: None. **A. Bender:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.25/AA10

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

Title: Stimulus-evoked changes in aperiodic electrophysiological activity in macaque visual cortex

Authors: *A. RUIZ D'ARGENCE[1], M. W. PRESTON, Jr.[2], B. VOYTEK[1];
[1]Cognitive Sci., UCSD, La Jolla, CA; [2]Neurosciences, UCSD, La Jolla, CA

Abstract: Sensorial changes in our environment produce stimulus-evoked responses in electroencephalography (EEG) and local field potential (LFP) recordings. The role of transient event-related potentials (ERP) and neural oscillations in cognitive processes such as vision and attention have long been studied; however, recent human intracranial EEG studies have highlighted the presence of dynamic shifts in non-oscillatory, aperiodic activity in response to visual stimulation. Despite burgeoning interest in the functional role of aperiodic neural activity, the biophysical mechanisms have not been fully characterized. Non-human primate (NHP) models allow us to investigate the cellular underpinnings of LFP activity; however, anatomical differences in visual cortices between human and NHP models might cause a difference in stimulus-evoked visual processing. We aim to: 1) establish whether LFPs in the primate visual cortex exhibit event-related changes in aperiodic activity, and 2) characterize the biophysical mechanisms of these aperiodic shifts. We hypothesize that the presentation of visual stimuli will cause event-related changes in aperiodic activity. Specifically, due to the potential link between aperiodic activity and the nature and strength of postsynaptic currents, we hypothesize that visual stimuli will cause a flattening of the LFP power spectrum, reflecting excitatory drive. To

investigate this hypothesis, we leveraged an openly available dataset of LFP recordings from two macaques implanted with 1024 electrodes across the primary visual cortex (V1) and supplementary visual area V4 while they viewed a visual checkerboard (Chen et al., Scientific Data, 2022). We performed time-resolved spectral decomposition and parameterization of LFP responses, and observed dynamic fluctuations in aperiodic activity in response to visual stimulation. We show that the aperiodic exponent and offset of the LFP power spectra both increase upon stimulus presentation and show a slow decay after onset. These changes manifest as a broadband upward shift and steepening of the power spectra. These effects were widespread across recording electrodes in V1 and V4. Furthermore, we show that these spectral changes are correlated with local multiunit activity across time, supporting previous findings linking broadband spectral power to local spiking activity. These findings suggest that aperiodic activity is functionally relevant for visual encoding and linked to the underlying biophysics. Primate visual cortex provides a model for studying the cellular mechanisms of stimulus-evoked aperiodic shift, though further investigation is still needed.

Disclosures: A. Ruiz D'Argence: None. **M.W. Preston:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.26/AA11

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

Support: CIRM UC San Diego Training Fellowship

Title: Not all-or-nothing: intracellular action potential waveform varies systematically with causal stimulation and extracellular oscillatory state

Authors: *B. MARTIN-BURGOS[1], P. D. RIVIERE[2], R. HAMMONDS[3], A. L. JUAVINETT[4], B. VOYTEK[5];
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Abstract: Individual neurons' action potentials (APs) have a dynamic waveform, the shape of which differs by neuron subtype and species. In analyses of single-unit data, however, this waveform is reduced to a binary "all-or-nothing" event, with systems and cognitive neuroscience focusing on the number and timing of spike events. This analytical convenience has, in turn, resulted in theories of neural coding that assume binarity: rate, temporal, phase, and population coding all assume binary spiking. Despite this assumption, a considerable body of evidence shows that AP waveforms exhibit systematic, within-neuron variation that might contribute additional information beyond the rate or timing of discharge that is often ignored. To address this, we have developed an AP waveform parameterization approach that quantifies the fine-

scale features of extracellular and intracellular spike waveforms. Using this parameterization on patch-clamp recordings of APs, we find within-neuron correlations between features such that, for example, individual APs that decay faster result in subsequent spikes occurring more quickly. Leveraging this parameterization, we analyzed a dataset of simultaneously recorded patch-clamp APs and local-field potentials (LFPs) in rats. This allows us to examine, for the first time, the causal influence of the LFP on AP shape. We observed within-neuron differences in spike waveform features in relation to LFP gamma oscillatory states that have been overlooked using traditional analyses. Further, to study the effect of causal stimulation, we analyzed a patch-clamp dataset where APs were recorded while injecting varying currents (constant, ramp, pink noise). We found that the stimulation type influenced AP waveform, and that features of the pink noise correlated with the shape of APs. Our results suggest that AP waveforms show systematic variation with causal stimulation and LFP state. While these findings provide preliminary evidence for the importance of studying within-neuron variance in AP waveform alongside traditional spiking metrics to enhance our understanding of neural coding and cognition, they also have significant implications regarding the inference of single-unit identity using spike sorting algorithms that assume systematic waveform stability.

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Poster

PSTR256: Computational Tools: Analytical I

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Program #/Poster #: PSTR256.27/AA12

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

Support: NSF GRFP (to DC)
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NIH National Institute of General Medical Sciences grant R01GM134363-01 (to B.V.)

Title: Neural timescales are dynamic, not intrinsic, and reflect gradations of task abstraction

Authors: *D. CELLIER[1], J. RIDDLE[2], B. VOYTEK[3];
[1]UCSD, La Jolla, CA; [2]Psychiatry, Florida State Univ., Tallahassee, FL; [3]Cognitive Sci., UCSD, La Jolla, CA

Abstract: Navigating everyday environments requires that the brain multiplex information processing over many different timescales. Variety in the timescales of the environment are therefore mirrored in large-scale anatomical and functional gradients across cortex. Association cortices such as the prefrontal cortex exhibit neural activity which remains self-similar over long timespans. Conversely, sensory cortices exhibit short neural timescales, with temporally variable neural activity. However, empirical evidence for a hierarchy of neural timescales across cortex

was primarily driven by analyses of resting-state data. This calls into question whether neural timescales reflect a static, intrinsic network property of the brain, or alternatively, reflect a range of dynamic temporal capacities for a given cortical region.
To assess whether the human brain exhibits dynamic changes in timescales, we quantified pre-to-post stimulus changes using electroencephalography (EEG) during a hierarchical cognitive control task which manipulated the level of contextual control required for participants to complete the task, defined as task abstraction. We then modeled the distinct contribution of both oscillatory and non-oscillatory (aperiodic) activity to the EEG data and to behavior. This allowed us to disambiguate whether changes in neural timescales were due to shifts in the slope of aperiodic activity, changes in oscillatory power, or changes in the peak frequency of neural oscillations.
We found that neural timescales are not static, but lengthened upon stimulus onset. Furthermore, neural timescale length was longest at prefrontal sites in task conditions that required the highest level of task abstraction. Non-oscillatory, aperiodic activity exhibited marked slowing in prefrontal sites that coincided with a decrease in alpha-band oscillatory power. These changes occurred across all conditions but were greatest during the highest level of task abstraction. This suggests that neural timescales are not only a dynamic feature of the cerebral cortex, but also capture task-specific reorganization of functional network dynamics when a greater degree of contextual information must be integrated.

Disclosures: D. Cellier: None. **J. Riddle:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.28/AA13

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

Title: Methodological considerations for estimating timescales in electrophysiological data

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Abstract: The timescales of neuronal activity allow us to approximate the amount of time that information is held in a given brain region. Neuronal timescales exhibit robust gradients across the cortex, mirroring other anatomical, functional, and cognitive hierarchies. Moreover, both aperiodic and periodic contributions to neural activity can be analyzed in light of neural timescales, reflecting independent changes in fast or slow activity. However, with multiple methods for measuring timescales from electroencephalographic (EEG) data, it can be challenging to know exactly which one is best, and under what conditions. One such method is to compute the autocorrelation function (ACF), which correlates the original signal to every time-lagged version of itself. The rate of decay in this self-similarity function is used to estimate how

similar to itself a time series is, or, in other words, how much “memory” a signal has. However, empirical studies on neural timescales sample from the ACF in a variety of ways, leading to inconsistencies across studies. Timescales can also be estimated from the power spectral density (PSD) function resulting from transforming the time-domain data into the frequency domain using the Fourier transform. Changes in the timescales of aperiodic activity manifest in the "knee" of the PSD (the frequency where the PSD bends), whereas it is reflected in shifts in the center frequency of oscillatory peaks. While the ACF and PSD methods are theoretically mathematically comparable, they may have different practical advantages in quantifying neural timescales. Here, we quantify timescales using both approaches, comparing their outputs and tradeoffs in the separation of periodic and aperiodic activity, as well as their ease of interpretability. We leverage a public dataset containing intracranial EEG data from patients performing a visual working memory task (Johnson et al., 2018) and compare those to simulated data, transforming both datasets using similar analysis protocols. Results suggest that a benefit of estimating timescales in the spectral domain is the ease with which the contribution of periodic and aperiodic activity can be separated. ACF estimation, on the other hand, might be more straightforwardly interpretable. Furthermore, we observe nested timescales that vary across cortical ROI, with the frontal cortex exhibiting two timescales more commonly than other ROIs. These findings suggest that the method of timescale estimation has the potential to greatly affect results observed, or the interpretations of those results, and should therefore be carefully tailored to the research context.

Disclosures: Q. van Engen: None. **D. Cellier:** None. **R. Hammonds:** None. **B. Voytek:** None.

Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.29/AA14

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

Title: Modeling the independence and convergence of top-down frontal anticipation signals and bottom-up auditory drive in human auditory cortex

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Abstract: Our sensory environment is rich with predictable stimuli that we can observe and use to respond to the world around us. To accomplish this, bottom-up sensory information must be integrated with top-down anticipatory signaling. While it is unclear how this is instantiated across distributed neural populations, many models of attention are predicated on the rhythmic modulation of activity in anticipation of upcoming stimuli, such as through oscillatory entrainment. In such models, neural oscillations in the local field potential (LFP) entrain to rhythmic stimuli in slow (< 8 Hz) frequencies which can, in turn, couple with the amplitude of

neural activity in the beta (15-25 Hz) range in apparent cross-frequency coupling. Cross-frequency coupling is argued to reflect a convergence between “bottom-up” sensory inputs and “top-down” attentional modulation of neural excitability in sensory cortices. Here, we propose a computational model of the neural basis of dynamic attention for rhythmic auditory stimuli. In this model, top-down gain is modeled as spikes clustered in windows of narrowing Gaussian probability, reflecting the learning and anticipation of expected stimulus timing. Bottom-up auditory input is modeled as exponentially increasing, then plateauing, intervals of neural responses based on cochlear nerve impulses. These spike trains are then convolved with excitatory postsynaptic AMPA currents to simulate the hypothesized LFP, creating complex, nonlinear time-series. These model time-series are then compared, on a trial-by-trial basis, to invasive human electrophysiological recordings from nine patients performing a rhythmic auditory temporal discrimination task. We observe that the bottom-up model is most strongly correlated with activity in early auditory cortex while the top-down model has a broader cortical distribution. A mixed, bottom-up-plus-top-down model shows maximal convergence in secondary auditory cortex. Furthermore, we demonstrate how this model can provide a plausible explanation for observations of oscillatory entrainment and apparent cross-frequency coupling interactions associated with dynamic attention, without any explicit modeling of coupled oscillations, due to the sharp temporal features of the simulated LFP. This modeling approach and its validation in iEEG provides a simple, yet powerful, method to unify observations of dynamic attention, oscillatory entrainment, and cross-frequency coupling under a single model of anticipatory auditory perception.

Disclosures: S.E. Smith: None. **B. Voytek:** None.

Poster

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

Support: This work was funded by the Bundesministerium für Bildung und Forschung BMBF, Förderkennzeichen: 01ZZ2016

Title: Optimizing targeted spinal cord stimulation to improve lower limb motor function in a digital twin population

Authors: *V. GEMAR[1], L. WOLFART[2], A. ALASHQAR[1], Z. HU[1], J. GARCIA ORDONEZ[3], B. ESKOVIER[4], T. NEWTON[5], W. VAN GEIT[5], E. NEUFELD[5], N. KUSTER[5,6], A. ROWALD[1];
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for Res. on Information Technologies in Society (IT'IS), Zurich, Switzerland; [6]Dept. of Information Technol. and Electrical Engin., Swiss Federal Inst. of Technol. (ETH), Zurich, Switzerland

Abstract: Epidural spinal cord stimulation (SCS) targeting the dorsal roots of the lumbosacral spinal cord facilitates lower limb motor function after paralysis. However, the effectiveness of SCS across diverse patient anatomies remains uncertain, particularly in how tailored clinical decisions—such as electrode lead selection, neurosurgical positioning, current distribution, stimulation waveform, and frequency—affect dorsal root targeting. Here, we present a comprehensive computational evaluation and optimization of SCS parameters using a series of digital twin models of the lumbosacral spinal cord with regard to their efficacy of activating different axon fibers in the dorsal and ventral roots as well as the dorsal column. First, we analyzed the recruitment order of axon fibers across more than 180 electrode positions within the epidural space for each digital twin model, illuminating the mechanisms of action across different anatomical variations and electrode positions. Second, we assessed the dorsal root targeting efficacy of three commonly implanted electrode leads at various neurosurgical positions, revealing a need for tailored clinical decision making of SCS parameters. Third, we introduced a computational optimization methodology for identifying Pareto-optimal sets of stimulation parameters to maximize dorsal root selectivity, specifically for roots innervating key locomotor muscles. Finally, we present practical clinical decision making guidelines to determine stimulation parameters in settings that lack the computational resources or expertise necessary to perform detailed simulations, paving the way for widespread clinical adoption of targeted SCS to improve recovery of lower limb motor function.

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Poster

PSTR256: Computational Tools: Analytical I

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Title: Exploring the Mechanisms of Sacral Neuromodulation through computational modelling

Authors: *Z. HU, V. GEMAR, A. ALASHQAR, A. ROWALD;
ProModell Group, Chair of Digital Hlth., Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Abstract: Sacral Neuromodulation (SNM) has shown promise for the treatment of disorders related to pelvic floor, bladder, and bowel control. SNM typically involves inserting a needle electrode lead into the S3 foramen to provide electrical stimulation, while alternative stimulation protocols including epidural and transcutaneous spinal cord stimulation (SCS) are being actively investigated. Despite its clinical benefits, the precise mechanisms of action underlying SNM remain unclear, especially given its applications across diverse conditions with varying pathophysiology. Here, we present a comprehensive hybrid computational model combining a volume conductor of the entire lower human trunk with multi-compartment models of afferent and efferent axon fibers in the spinal roots and nerves, extending through the foramina into the lumbosacral plexus. We tested a series of different SNM stimulation protocols, including the application of needle electrodes in all sacral foramina as well as epidural and transcutaneous SCS electrodes applied over the entirety of the thoracic, lumbar, and sacral spine. Our findings reveal notable differences in neural activation sites, the recruitment order of axon fibers related to individual spinal segments, and the selectivity between afferent and efferent axon fibers, contingent upon stimulation parameters. We provide guidelines on how to harmonize the recruitment of neural structures across different stimulation protocols by modification of stimulation parameters, including electrode location, current distribution across active sites and stimulation waveform. Our results provide insights into the mechanisms underlying SNM paving the way for tailored therapeutic strategies.

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Poster

PSTR256: Computational Tools: Analytical I

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Title: An end-to-end deep learning pipeline for 3D mapping of local and laminar neuronal activity in tera-voxel light sheet microscopy data

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Switzerland; [7]SV, EPFL, Geneva, Switzerland; [8]Biol. Sci., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [9]Bioengineering, Stanford Univ., Stanford, CA; [10]Stanford, Stanford, CA; [11]Swiss Federal Inst. of Technol., Geneve, Switzerland

Abstract: Mapping neuronal subpopulation-specific activity is critical to understanding brain network dynamics underlying behavior and cognition. Light sheet fluorescence microscopy (LSFM) and tissue clearing have enabled high-fidelity imaging in intact tissue that produces large, high-resolution 3D datasets. However, current computational pipelines are either 2D-based or based on traditional algorithms requiring parameter tuning. Current tools also rely on registration to brain atlases for statistical analyses, via an ROI-based approach, requiring a priori knowledge to choose ROIs and aggregating cell features within brain regions, and obscuring the heterogeneity within regions; yet many pathologies are thought to exert salient laminar effects. Recently, we developed an end-to-end pipeline (ACE: AI-based Cartography of Ensembles). ACE incorporates state-of-the-art (SOTA) deep learning (DL) segmentation models and threshold-free cluster enhancement (TFCE) cluster-wise permutation algorithms that enable an unbiased and generalizable mapping of 3D neuronal local activity or morphological changes in tera-voxel scale LSFM data. ACE segmentation models were trained on optically cleared brains from 18 healthy transgenic mice (TRAP2-Ai9) with the cFos promoter (~45,600 3D patches for training). Here, we evaluated the models against the SOTA cell-detection and segmentation algorithms Cellfinder and Ilastik using an out-of-distribution unseen dataset with completely different image characteristics, and signal-to-noise and contrast-to-noise ratios w.r.t training data. We then applied ACE to chart local and laminar neuronal ensembles across the whole brain during walking (locomotion). ACE demonstrated consistently superior segmentation performance (on all evaluation metrics) compared to Ilastik (an average improvement in Dice of 0.27, $p<0.0001$) and detection performance compared to Cellfinder (an average improvement in F1 score of 0.45, $p<0.0001$). ACE cluster-wise analysis shed light on several sub-regional and laminar neuronal ensembles activated during movement, including clusters confined to the secondary motor area layer 2/3, lateral hypothalamic regions (an ROI recently shown to play a crucial role in recovery after lateral hemisection spinal cord injuries in rodent), or those spanning different layers of primary motor cortex. We present ACE, a novel 3D pipeline for LSFM data, that enables high-fidelity unbiased and generalizable 3D mapping of local and laminar neuronal ensembles across the entire brain, independent of pre-defined atlas regions.

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Poster

PSTR256: Computational Tools: Analytical I

Location: MCP Hall A

Time: Monday, October 7, 2024, 1:00 PM - 5:00 PM

Program #/Poster #: PSTR256.33/AA18

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

Support: R21EY026434
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Title: A biophysical model of GCaMP for improved spike inference

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Abstract: With its high signal-to-noise ratio and fast kinetics, jGCaMP8f holds exceptional promise for allowing extraction of neuronal spike times from optical recordings with millisecond temporal precision. However, current methods for inferring spike times from fluorescence fail to produce improved time resolution over older, slower GCaMPs. Using controlled ex vivo recordings of cerebellar granule cell boutons, we determined that this suboptimal performance arises from a failure to account for the nonlinear slowing of responses by jGCaMP8f during periods of high activity. To understand and compensate for this hysteretic process, we built a biophysical model of spike-to-fluorescence transformation by GCaMP, with parameters constrained by stopped-flow calcium step responses. Using the biophysical model as the basis for an unsupervised, generative Bayesian inference approach, we demonstrate 3 millisecond spike time resolution in the Janelia (HHMI) ground truth dataset. To take advantage of the speed of machine learning-based inference, we used the biophysical model to generate synthetic GCaMP data to train a neural network-based spike decoder. The Bayesian and synthetic data-trained approaches outperform current best-in-class inference approaches including CASCADE (7 ms) and MLSpike (18 ms) and ENS2 on all tested cell types. Thus, we leveraged our mechanistic understanding of how GCaMP converts spikes into light to improve both supervised and generative methods for spike inference.

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